

FOLIA HISTOCHEMICA ET CYTOBIOLOGICA

Official Journal of the Polish Histochemical and Cytochemical Society

A scientific quarterly devoted to problems of histochemistry, cytochemistry
and cell & tissue biology

- Editor-in Chief:** Lech Chyczewski
Co-editors: Z. Darzynkiewicz (New York)
A. Hinek (Toronto)
L.M. Montuenga (Navarra)
M.Z. Ratajczak (Kraków/Louisville)
J.P. Robinson (West Lafayette)
- Editorial Board:** E. Bacsy (Budapest)
B. Bilińska (Kraków)
Z. Bielańska-Osuchowska (Warszawa)
S. Carreau (Caen)
B. Chwirot (Bydgoszcz)
M. Dietel (Berlin)
J. Długosz (Białystok)
J. Kawiak (Warszawa)
W.M. Kilariski (Kraków)
A. Koj (Kraków)
J. A. Litwin (Kraków)
A. Łukaszyk (Poznań)
J. Nikliński (Białystok)
J. Niweliński (Kraków)
C.J.F. van Noorden (Amsterdam)
M. Olszewska (Łódź)
M. van der Ploeg (Leiden)
D. W. Scheuermann (Antwerpen)
J. Stachura (Kraków)
S. Wolczyński (Białystok)
A. W. Vorbrodtt (New York)
M. Zabel (Wrocław)
J. Żeromski (Poznań)
- Managing Editor:** Ł. Minarowski
Editors: M.M. Stepaniuk
A. Sulewska
- Editorial Office:** Dept of Clinical Molecular Biology
Medical University of Białystok
Waszyngtona Str. 13, 15-269 Białystok, Poland
tel./fax.: +48 85 748 59 88
e-mail: fhc@umwb.edu.pl
<http://www.fhc.umwb.edu.pl>

Folia Histochemica et Cytobiologica (pISSN 0239-8508, eISSN 1897-5631) is published quarterly, one volume a year, by the Polish Histochemical and Cytochemical Society and Association for Research and Improvement of Diagnosis and Treatment of Cancer, at KON-Tekst Publishing House, Kraków.



Indexed in: Index Medicus/MEDLINE, Excerpta Medica/EMBASE, Chemical Abstracts/CAS, SCI Expanded, SciSearch, Biochemistry & Biophysics Citation Index, ISI Alerting Services, Biosis Previews Index Copernicus, Biological Abstracts, SCOPUS, Research Alert, ProQuest, EBSCO, DOAJ

POLISH HISTOCHEMICAL ET CYTOCHEMICAL SOCIETY STATEMENT OF FOLIA HISTOCHEMICA ET CYTOBIOLOGICA EDITORIAL POLICY

Folia Histochemica et Cytobiologica is an international, English-language journal devoted to the rapidly developing fields of histochemistry, cytochemistry, cell biology, cell and tissue biology.

The Folia Histochemica et Cytobiologica publishes papers that meet the needs and intellectual interests of medical professionals, basic scientists, college and university teachers and students. Prospective authors should read most recent issues of FHC to determine the appropriateness of a possible contribution. However, such an examination does not provide an infallible guide because editorial policy is always under review. Technical correctness is necessary, but it is not the only condition for acceptance. Clarity of exposition and potential interest of the readers are important considerations; it is the reader, not the author, who must receive the benefit of the doubt.

Folia Histochemica et Cytobiologica publishes review articles, original articles, short communications and proceedings of scientific congresses/symposia. Fields of particular interests include development and application of modern techniques in histochemistry and cell biology, cell biology and pathology, cell-microenvironment interactions, tissue organization and pathology.

Manuscripts announcing new theoretical or experimental results, or manuscripts questioning well-established and successful theories, are highly desirable and are a subject for evaluation by specialists. Manuscripts describing original research that clarifies past misunderstandings or allows a broader view of a subject are acceptable. Manuscripts that demonstrate new relations between apparently unrelated areas of fields of interests are appropriate. Manuscripts that show new ways of understanding, demonstrating, or deriving familiar results are also acceptable. Such manuscripts must provide some original cytobiological insight and not just a clever derivation.

Regularly, review or tutorial articles are published, often of a length greater than that of the average article. Most of these articles are a subject of a review; authors planning such articles are asked to consult with the editors at an early stage.

Most readers of a particular article will be specialists in the subject matter presented; the context within which the paper is presented should be established in the order given in Instructions for Authors. Manuscripts must be technically correct and must take proper cognizance of previous work on the same subject regardless of where it may have appeared. Such referencing is especially important for reminders of once well known ideas, proofs, or techniques that may have again become useful to readers. It is the responsibility of the author to provide adequate references; editors and referees will not do the literature search that should have been done by the authors. The references are a matter of review though.

Contributions considered include: Regular Articles (Papers), Short Communications, Review Articles, Conference Proceeding, Book Reviews and Technical Notes, which describe new laboratory methods or substantial improvements of the existing techniques. Regular articles should be about five journal pages or less in length. Short communications are usually confined to the discussion of a single concept and should be about two journal pages in length. Review articles are confined to a broad discussion and should contain the most recent knowledge about the subject.

Instructions concerning the preparation of manuscripts are given in the Information for Authors. Care in following those instructions will permit editors and referees to devote more time to thoughtful evaluation of contributions and will ultimately lead to a better, more interesting Journal.

Folia Histochemica et Cytobiologica by Polish Histochemical and Cytochemical Society is licensed under a Creative Commons Attribution 2.5 Poland License.

Copying information, in part or in whole by any means is prohibited without a written permission from the owner.



INFORMATION FOR SUBSCRIBERS

Annual subscription rates:	Personal	€ 75.00
	Institutional	€ 90.00

Orders and payment. Orders should be sent to the Editorial Office. Payment may be done either by bank transfer (acc. no. 41 1240 5211 1978 0000 4928 2461, IBAN: PL 41 1240 5211 1978 0000 4928 2461, SWIFT: PKOPPLPW, bank name: PKO S.A. Białystok, bank address: Rynek Kosciuszki 7, Białystok, Poland), credit card or money transfer (in euros to Association for Research and Improvement of Diagnosis and Treatment of Cancer, Waszyngtona Str. 13 Apt. 439, 15-269 Białystok, Poland).

Please send the money transfer confirmation to the Editorial Office. Subscription can also be ordered from RUCH SA, ARS POLONA, DHN, GarmondPress and KOLPORTER S.A. Back issues are available from the Editorial Office at € 10.00 per copy.

We acknowledge the support of the Ministry of Science and Higher Education of the Polish Republic for printing of this Supplement to Folia Histochemica et Cytobiologica.

Publisher editor: Wojciech Marcinek, KON Tekst, Kraków, www.kon-tekst.pl

Electronic version: Versita

Printing: Leyko, Kraków



ABSTRACT BOOK

**XIII International Congress of
Histochemistry and Cytochemistry**

ICHC2008

Imaging of Cell Dynamics

23rd-27th August 2008

Medical University of Gdansk, Poland



Executive organizer

OLYMPUS

Your Vision, Our Future

Contents

Lectures

1

- L000** Metabolic mapping
3 Cornelis Van Noorden
- L001** Connectomics in the developing nervous system
3 Jeff W. Lichtman
- L002** Histochemical approaches to visualizing brain feeding pathways
3 James Blevins, Brendan Thatcher, Gregory Morton, Diana Williams, Michael Schwartz, Denis Baskin
- L003** Illuminating the secretory pathway
4 Rainer Pepperkok
- L004** Imaging of signalling across the plasma membrane
4 Theodoros W.J. Gadella Jr., Joachim Goedhart, Merel Adjobo-Hermans, Laura van Weeren
- L005** Imaging and perturbation of receptor endocytosis
4 Carsten Schultz
- L006** APPL1 and the FSH-induced IP3 pathway for calcium mobilization in human granulosa cells
4 Joseph E. Mazurkiewicz, Richard M. Thomas, Cheryl A. Nechamen, Alfredo Ulloa-Aguirre, Kerri Kluetzman, James A. Dias
- L007** Immunofunctional multiple signal detection by quantum dot and spectrum analysis
5 Johbu Itoh, Yoshiko Itoh, Robert Y. Osamura
- L008** Sensitive and stable immunohistochemical detection using combined tyramide signal amplification and quantum dots
5 Kevin Roth
- L009** Imaging the cytoskeleton in cell-matrix dynamics
5 Hinke Multhaupt, Atsuko Yoneda, Eok-Soo Oh, John Couchman
- L010** Cryptic translocations demystified: BAC-FISH assays resolve complex karyotypes in failed human reproduction and cancer
5 Heinz-Ulli Weier
- L011** Updated non-radioactive *in situ* hybridization in cell biology and physiology
6 Takehiko Koj
- L012** Cytochemical studies of signaling pathways linking nutritional status to fertility
6 Gwen Childs, Noor Akhter, Zachary Brodrick, Anessa Haney
- L013** Laser capture microdissection: a contemporary tool for molecular pathology
6 James L. Wittliff, Sarah A. Andres, D. Alan Kerr II
- L014** Application of laser assisted microdissection and liquid-based cytology for molecular genetic analysis on surgical specimens
7 Norimichi Nemoto, Yohko Nakanishi, Toshimi Seki, Fumi Fuchinoue, Eriko Murakami, Noriko Watanabe, Toshiyuki Ishige, Kentarou Kikuchi, Keishin Sunagawa, Sumie Ohni
- L015** Ancillary techniques in the diagnosis and prognosis of bladder cancer
7 Donna Hansel
- L016** Development of peritoneal dissemination of ovarian clear cell adenocarcinoma can vary with sialidase expression
7 Hiroyuki Nomura, Fumio Kataoka, Hiroshi Tsuda, Yutaka Tamada, Atsushi Suzuki, Nobuyuki Susumu, Daisuke Aoki
- L017** Molecular analysis of lung carcinogenesis with new technical tools
8 Luis M. Montuenga, Isabel Zudaire, Maria J. Pajares, David Blanco, Alberto Orta, Ignacio Fernandez-Garcia, Thomas Pengo, Arrate Munoz, Carlos Ortiz de Solorzano, Ruben Pio
- L018** Genetic background of undifferentiated thyroid carcinomas
8 Ryohei Katoh, Tetsuo Kondo, Tadao Nakazawa, Nakamura Nobuki, Tomonori Kawasaki, Tetsu Yamane, Kunio Mochizuki
- L019** Phospho-beta-catenin and microtubule reorganization in polarized cell migration
8 Michinari Hamaguchi
- L020** Evaluation of mitochondrial disorders
8 Giovanni Meola
- L021** Histochemical and immunohistological approach to neuromuscular disorders
9 Orlando Paciello
- L022** Mitochondrial pathogenesis of collagen VI myopathies: from the animal model to the clinical trial
9 Nadir M. Maraldi, Paolo Bernardi, Paolo Bonaldo, Luciano Merlini
- L023** Evaluation of sarcoglycans in muscular diseases of ureter: an immunohistochemical study
9 F. Trimarchi, G. Santoro, A. Consolo, A. Duca, A. Micali, A. Pisani, S. Arena, D. Puzzolo
- L024** Muscle-specific integrins in masseter muscle fibers of chimpanzees: an immunohistochemical study
10 A. Favalaro, G. Speranza, S. Rezza, V. Gatta, G. Vaccarino, L. Stuppia, F. Festa, G. Anastasi
- L025** Input of some feeding-regulating neurons revealed by a genetically encoded tracer
10 Haruaki Kageyama, Fumiko Takenoya, Seiji Shioda
- L026** Hypothalamic control of energy homeostasis: the importance of a balanced autonomic nervous system
10 Andries Kalsbeek, Felix Kreier, Eric Fliers, Ruud Buijs
- L027** Regulation of hypophysiotropic thyrotropin-releasing hormone and corticotropin-releasing hormone-synthesizing neurons by feeding related signals
11 Csaba Fekete, Ronald M. Lechan
- L028** Neuron network of feeding-regulating neurons in the hypothalamus
11 Fumiko Takenoya, Haruaki Kageyama, Seiji Shioda
- L029** Histology leads the way; what the *in situ* distribution of the manose receptor and its ligands tells us about the function of this receptor in homeostasis and immunity
11 Luisa Martinez-Pomares
- L030** Synthetic glycoconjugates as probes for flow cytometry and beyond
11 Nicolai Bovin
- L031** Quality control of glycoprotein folding: a glyco-cytochemical perspective
11 Jürgen Roth
- L032** An intrinsic feedback mechanism that modulates peripheral pain in the skin
12 Joseph E. Mazurkiewicz
- L033** Multi-laminar epidermal substrates for sensory transduction and integration: a potential contributor to neuropathic pain
12 Frank Rice
- L034** Neuro-immune interactions in skin and their relevance for cutaneous stress-responses and inflammatory disease in skin
12 Eva Peters
- L035** Sensing the environment: Regulation of local and global homeostasis by the skin neuroendocrine system
12 Andrzej Slominski
- L036** Advances in automated cell identification and classification
13 J. Paul Robinson
- L037** Contribution of histochemistry in pituitary development and pathology
13 Robert Y. Osamura
- L038** LacZ as an *in vivo* gene reporter for real-time magnetic resonance imaging
13 Niclas E. Bengtsson, Gary Brown, Glenn A. Walter, Edward W. Scott
- L039** Malignant and normal stem cells and detection of clonal hematopoiesis in hematologic disorders
13 Jaroslaw Maciejewski
- L040** Myogenic stem cells
14 Thomas Braun

- L041** Neurogenesis in the adult brain: genetic dissection
14 Robert K. Filipkowski, Leszek Kaczmarek
- L042** Identification of very small embryonic like (VSEL) stem cells in adult tissues
14 Mariusz Z. Ratajczak
- L043** Biology, transplantation and tissue regeneration potential in human umbilical cord blood
14 Arnon Nagler
- L044** "Decoding The Dot": The ImageStream system as a novel supportive tool for flow cytometric analysis
15 Ewa Zuba-Surma, Magdalena Kucia, Janina Ratajczak, Wan Wu, Mariusz Ratajczak
- L045** A glioblastoma-specific kinase expression profile
15 Philip De Witt Hamer, Shahryar Mir, Cornelis Van Noorden, Tom Wurdinger
- L046** Systematic analysis of the healthy and diseased brain using human antibody based proteomics
15 Jan Mulder
- L047** Analysis of proteolysis in a three-dimensional tumor-microenvironment architecture
16 Kamiar Moin, Mansoureh Sameni, Quanwen Li, Julie Doseescu, Hitchintan Kaur, Raymond Mattingly, Bonnie Sloane
- L048** Expression and clinical relevance of zinc-finger proteins in human cancer cells
16 James L. Wittliff, Traci L. Kruer
- L049** Seeing more with light microscopy
16 Tetsuro Takamatsu
- L050** Vibrational (FTIR and Raman) microspectroscopies and spectral imaging in cells and tissues: biomedical applications
16 Michel Manfait, Olivier Piot, Elodie Ly, Ganesh Sockalingum, Ali Tfayli, Florence Draux, Abdel Beljebbar
- L051** Fast dynamics of the cytoskeleton in 3D
17 Ulrike Engel, Margaret Clarke, Günther Gerisch
- L052** Novel techniques for biomedical imaging in microscopy
17 Bernhard Zimmermann
- L053** Simultaneous imaging of intracellular calcium dynamics and membrane potentials in the heart revealed by dual-view rapid scanning confocal microscopy
17 Hideo Tanaka, Katsuji Fujiwara, Tetsuro Takamatsu
- L054** Revisiting the insulin factory: What has cellular tomography added to our understanding of the pancreatic beta cell
17 Brad Marsh
- L055** High-resolution 3D imaging of larval zebrafish: from brain structure to function
18 Pertti Panula, Maria Sundvik, Yu-Chia Chen, Madhusmita Priyadarshini, Juha Kolehmainen, Ville Sallinen
- L056** 3D architecture of membrane undercoat and spatial specificity of actin binding proteins revealed by immuno-freeze-etching and cryo-electron microscopy
18 Jiro Usukura, Takashi Watanabe, Nobuhiro Morone, Kozo Kaibuchi
- L057** Regulation of caveolin- trafficking and formation of caveolae
18 Carlos Enrich, Laia Cubells, Thomas Grewal
- L058** Neutral lipid storage: dynamics and regulation
18 Christoph Thiele
- L059** Analysis of lipid supramolecular structures by electron microscopy
18 Toyoshi Fujimoto
- L060** Cellular cholesterol distribution and trafficking
19 Elina Ikonen
- L061** Decorin and biglycan signaling in inflammation and fibrosis
19 Liliana Schaefer
- L062** Matrikines, the products of extracellular macromolecules.
19 Yanusz Wegrowski, Stephane Brezillon, Francois-Xavier Maquart
- L063** Regulation of matrix metalloproteinases in inflammation: effects on cell adhesion and migration
19 Jelena Gavrilovic
- L064** Desmosealin and other elements of the epidermal extracellular matrix
20 Marek Haftek
- L065** Cell-matrix adhesion, cytoskeleton and the activated fibroblast
20 John Couchman, James Whiteford, Hinke Multhaupt, Atsuko Yoneda
- L066** Advantages of laser microdissection in histochemistry, molecular biology and pathology
20 Susumu Takekoshi, Noboru Egashira, Kajiya Hanako, Shinobu Umemura, R. Yoshiyuki Osamura
- L067** A decade of non-invasive visible light imaging: What have we accomplished and what are the current directions?
20 Peter Lassota
- L068** Quantitative colocalization analysis of confocal immunofluorescence microscopy images
21 Vadim Zinchuk, Olga Grossenbacher-Zinchuk
- L069** Endothelial-neural progenitor cell interactions in the neurovascular niche: implications for recovery from CNS injury & tissue engineering
21 Joseph A. Madri, Erin Lavik
- L070** New fluorescent tools give new insight in cell biology
21 Carsten Schultz
- L071** Cellular actions of angiogenesis inhibitors on the vasculature of tumors
21 Donald M. McDonald
- L072** Computational time-lapse analysis of vascular patterning, *in vivo*
22 Charles Little, Andras Czirok, Brenda Rongish, Evan Zamir, Cheng Cui, Rusty Lansford
- L073** Novel imaging strategies to assess myocardial angiogenesis
22 Albert Sinusas
- L074** Noninvasive *in vivo* molecular imaging of peripheral angiogenesis
22 Lawrence W. Dobrucki
- L075** Vascular oxidative stress: relative importance of pro- and anti-oxidative enzymes
23 Huige Li, Ulrich Förstermann
- L076** Multiple roles of caveolin in tumor and post-ischemic angiogenesis
23 Olivier Feron
- L077** Nitric oxide as a marker of vascular endothelial function
23 Leszek Kalinowski
- L078** The structure and dynamic properties of mammalian chromosomes – linking nuclear structure and function
23 Dean Jackson, Apolinar Maya-Mendoza, Chi Tang, Dorota Feret
- L079** The modification and dynamics of histones in human cells as revealed by a panel of specific monoclonal antibodies
24 Hiroshi Kimura, Yoko Hayashi-Takanaka, Naohito Nozaki
- L080** Immunocytochemical detection of DNA damage: activation of ATM and Chk2, and phosphorylation of histone H2AX and tumor suppressor protein p53 on Ser15
24 Zbigniew Darzynkiewicz
- L081** Heterochromatin protein 1 and repair of DNA damage – a new job for the old fellow?
24 Jurek Dobrucki
- L082** Visualizing the fate of nuclear proteins during apoptosis
25 Carlo Pellicciari, Maria Grazia Bottone, Anna Ivana Scovassi, Marco Biggiogera
- L083** Post-translational modifications, subcellular relocalization and release in apoptotic bodies: apoptosis turns nuclear proteins into potential autoantigens
25 Jürgen Dieker, Marion Decossas, Sylviane Muller
- L084** Apoptotic nucleases – a role and regulation
25 Piotr Widlak
- L085** Advantages of microlens-enhanced dual spinning disk confocal microscopy in functional imaging of chemo- and/or mechanoreceptors in the lung
26 Jean-Pierre Timmermans, Ian De Proost, Isabel Pintelon, Inge Brouns, Dirk Adriaensen
- L086** Fluorescence imaging of neuroplastic changes induced by peripheral axotomy
26 Lars Klimaschewski
- L087** The esophagus as a source of unorthodox ideas on gastrointestinal innervation
26 Winfried Neuhuber

- L088** Immunohistochemical investigations on the plasticity of enteric neurons in the course of some infectious and non-infectious diseases of the gastrointestinal tract
26 Jerzy Kaleczyc
- L089** Neurotoxins as tools for "sculpturing" of the peripheral nervous system
27 Mariusz Majewski
- L090** Impact of light stimuli on the maturation in neonatal mice and potential effects of the abnormal expression of stromal glycoprotein Lumican
27 Guo-min Zhou, Li-yun Liu
- L091** Varying colocalization of extracellular matrix components in the perineuronal nets of cortical parvalbumin-expressing neurons revealed by quantitative analysis
27 Olga Grossenbacher-Zinchuk, Marco Celio, Vadim Zinchuk
- L092** Dynamic imaging of cancer cell invasion and plasticity
27 Peter Friedl
- L093** Imaging of cytoskeletal functions of disseminating cancer cells
28 Erik Sahai, Silvia Giampieri, Caroline Hill
- L094** Visualizing stromal cell dynamics in different tumor microenvironments by spinning disk confocal microscopy
28 Mikala Egeblad
- L095** Visualization of chemokine signaling in morphogenetic tissue movements *in vivo*
28 Darren Gilmour
- L096** Cell migration assay of glioblastoma cell using an automated screening method
28 Marco Rossi, Cinzia Giordano, Patrizia Tunici, Massimiliano Salerno, Alessandra De Robertis, Silvia Matteoni, Roberto Raggiaschi, Elisa Mori, Letizia Magnoni, Hendrick Bothmann, Serena Pellegatta, Annette Bakker
- L097** Force generation during leukocyte locomotion
28 Michael Sixt
- L098** The dynamics of immune cells *in vivo*
29 Matthias Gunzer
- L099** Endoplasmic reticulum stress and cellular toxicity of mutant huntingtin
29 Yufeng Jiang, Huiping Tan, Yinong Zhang, Weixi Wang, He Li
- L100** Exploratory study of co-transplantation of neurotrophin-3 gene modified Schwann cells and neural stem cells in the recovery of spinal cord injury
29 Yuan-shan Zeng
- L101** Neurodegenerations: from neuropathology to structural biology
29 Paweł P. Liberski
- L102** Progression of Alzheimer disease correlates with the increased neuronal DNA replication
30 Attila Tarnok, Birgit Mosch, M. Morawski, Thomas Arendt, Anja Mittag
- L103** Quetiapine facilitates oligodendroglia development and prevents mice from demyelination
30 Lan Xiao, Yanbo Zhang, Xinmin Li
- L104** Confocal microscopy of amyloid plaques in prion diseases
30 Beata Sikorska, Paweł P. Liberski, Herbert Budka, James W. Ironside
- L105** *In situ* hybridization with quantum dot detection and confocal scanning microscopy for three-dimensional visualization of protein and mRNA: comparison with electron microscopic *in situ* hybridization
30 Akira Matsuno, Johbu Itoh, Susumu Takekoshi, R. Yoshiyuki Osamura
- L106** Applications of ImageStream system for identification and characterization of murine and human stem cells
31 Ewa K. Zuba-Surma, Magdalena Kucia, David A. Basiji, William E. Ortyn, James W. Lillard, Mariusz Z. Ratajczak
- L107** New technology and new possibilities in flow cytometry with use of the Cell Lab Quanta SC MPL Beckman Coulter instrument
31 Roman Pińkowski
- L108** Publishing and publications in histochemistry: Round Table Symposium
31 Raymond Coleman
- L109** Measuring journal success: impact factor alternatives
31 Kevin Roth
- L110** Acta Histochemica et Cytochemica, an official Journal of Japan Society of Histochemistry and Cytochemistry: Introduction and recent achievement to modernize it as on-line Journal
32 Takehiko Koji
- L111** Cytometry Part A – the quantitative cell analysis journal
32 Attila Tarnok
- L112** Folia Histochemica et Cytobiologica – Official Journal of Polish Histochemical and Cytochemical Society
32 Lech Chyczewski, Łukasz Minarowski

Disorders of cell structure and function

- P1.1** Down-regulation of P2X₇ receptor expression after oxygen and glucose deprivation in rat oligodendrocyte progenitor cells
35 Wenqin Cai, Li-Yan Wang, Peng-hui Chen, Qi-yue Deng
- P1.2** Maternal hypothyroidism and its role in the placenta: a morphometric and immunohistochemical study
35 Candan Ozogul, Serap Cilaker, Basak Baykara, Cetin Pekcetin, Alper Bagriyanik, Soner Atmaca, Erdener Ozer
- P1.3** Fornix transection alters corticosteroid receptors in the hippocampus and corticotrophin-releasing hormone and vasopressin in the hypothalamic paraventricular nucleus
35 Fang Han, Hong Liu, YanHui Zhang, YuXiu Shi
- P1.4** Adrenaline and noradrenaline effects on glucose homeostasis and on vasoactive peptide expression in different organs of *Podarcis sicula* lizard
35 Maria De Falco, Claudia Barletta, Rosaria Sciarriello, Anna Sellitti, Sabrina Caliendo, Salvatore Valiante, Anna Capaldo, Flaminia Gay, Vincenza Laforgia
- P1.5** Recombinant extracellular matrix protein expressed in a limited number of cells propagates to the target organ throughout the body using its nascent tissue-targeting signal
36 Yumi Suzuki, Mikako Ito, Takeshi Okada, Shin'ichi Takeda, Takayasu Fukudome, Toshiro Yoshimura, Eric Krejci, Kinji Ohno
- P1.6** Expression of galectin-3 in nephrotic syndrome glomerulopathies in children – preliminary report
36 Michal Nowicki, Beata Kondraciuk, Danuta Ostalska-Nowicka, Bogdan Miśkowiak
- P1.7** Plastic changes in the ventral tegmental area-nucleus accumbens feeding circuitry after its partial destruction
36 Karolina Plucińska, Maciej Jankowski, Bogna Ignatowska-Janowska, Weronika Trojnar
- P1.8** Exendin-4 triggers NGF/P75NTR system and antioxidant defense in diabetic mice pancreas
37 Selda Gezginci-Oktayoglu, Sehnaz Bolkent
- P1.9** HTC hepatoma cells as a model for investigating the effects of low herbicide concentrations
37 Manuela Malatesta, Federica Perdoni, Giada Santin, Serafina Battistelli, Marco Biggiogera, Carlo Pellicciari
- P1.10** Alterations in kidney tissue following zinc supplementation to STZ-induced diabetic rats
37 Ayse Karatug, Engin Kaptan, Ozgur Mutlu, Sehnaz Bolkent, Refiye Yanardag
- P1.11** Quantitative immunofluorescence staining: a tool for detection of spatio-temporal changes of protein molecules alongside nerve fibres following nerve injury
37 Petr Dubovy
- P1.12** Comparison of collagen biomatrix and omentum effectiveness on peripheral nerve regeneration
38 Berker Cemil, Durukan Ture, Banu Cevirgen, Figen Kaymaz, Memduh Kaymaz
- P1.13** The effect of Quetiapine on the treatment of experimental acute spinal cord injury
38 Hamit Aytar, Memduh Kaymaz, Ertan Ergun, Figen Kaymaz, Aydin Pasaoglu
- P1.14** Dipeptidyl peptidase IV (DDP IV) /CD 26 in NASH patients
38 Yasemin Balaban, Petek Korkusuz, Halis Şimşek, Hale Gökcan, Gökhan Gedikoglu, Asli Pinar, Gülsen Hasçelik, Esin Aşan, Erhan Hamaloglu, Gonca Tatar
- P1.15** Expression of serotonin receptors mRNA in the frontal cortex and hippocampus of PTSD rats
39 Haitao Wang, Fang Han, Yuxiu Shi
- P1.15A** The evaluation of megacaryocyte emperopoiesis in patients with multiple myeloma
39 Dorota Lemancewicz, Janusz Dzieciol Jarosław Piszcz, Agnieszka Lebelt
- P1.16** Effects of cadmium chloride on glial cells of lizard *Podarcis sicula*
39 Rossana Favorito, Maria Consiglio Grimaldi, Ida Ferrandino

- P1.17** The protective role of Z-FA.FMK on the lung injury induced by D-GaIn/TNF-alpha in mice
39 Fusun Oztay, Selda Gezginci-Oktayoglu, Bertan Boran Bayrak, Refiye Yanardag, Sehnaz Bolkent
- P1.18** Tight junction molecules in pathogenesis of Barrett's esophagus and adenocarcinoma of the esophagus
40 Tatiana Demura, Evguenia Kogan, Ronald Moll
- P1.19** MMP-1, MMP-9, TIMP-1, VEGF, Ki-67 and apoptosis in endometrial hyperplasia in premenopausal women: what is new
40 Tatiana Demura, Juliya Zadonskaya, Natalia Litvinova, Juliya Dobrohotova, Gennadij Sukhikh
- P1.20** Immunohistochemical approach to adenomyosis
40 Evgenija Kogan, Ara Unanyan, Tatjana Demura, Olga Grechukhina
- P1.21** Early morphological and physiological changes of canine hepatocytes after alpha-amanitin exposition
41 Aleksandra Piotrowska, Jan Magdalan, Ilona Izykowska, Alina Ostrowska, Barbara Dolińska-Krajewska, Marzena Podhorska-Okolów, Piotr Dziegiel
- P1.22** The role of insulin-like growth factor-I and polyamins in developing rat small intestine
41 Omur Karabulut-Bulan, Sehnaz Bolkent
- P1.23** Wolfram expression in different tissues of rats subjected to hyperlipidic diet
41 Maria De Falco, Simona Capodanno, Anna Sellitti, Lucrezia Manente, Raffaella Crescenzo, Italia Falcone, Salvatore Valiante, Antonio De Luca, Vincenza Laforgia

Tumor cell biology

- P1.24** The effects of retinoic acid and selenium on human neuroblastoma cells in two and three dimensional cell culture models
42 Leyla Tapul, Sevilcan Tuna, Betul Bozkurt, Selcuk Erdem, Ayhan Bilir
- P1.25** Immunohistochemical study of 8-OHdG and its relationship with OGG1, G6PD, and some cancer related proteins in cutaneous melanoma
42 Daniela Murtas, Franca Piras, Luigi Minerba, Jorge Ugalde, Carlo Floris, Cristina Maxia, Paolo Demurtas, Maria Teresa Perra, Paola Sirigu
- P1.26** Relationship between cyclooxygenase 2 and survivin expression in primary pterygium
42 Paolo Demurtas, Cristina Maxia, Maria Teresa Perra, Luigi Minerba, Daniela Murtas, Diego Carpio, Romano Colombari, Franca Piras, Paola Sirigu
- P1.27** Application of FISH method in karyotype analysis of radiation induced mouse lymphomas – models of human lymphoid neoplasms
43 Joanna Piskorska, Beata Grygalewicz, Hanna Szymańska, Elżbieta Krysiak, Barbara Pieńkowska-Grela
- P1.28** Annexin A5 expression up-regulated in the uterine cervical squamous cell carcinoma
43 Fulu Gao
- P1.29** p53 and Bcl-2 expression in malignant melanomas and spitz nevi
43 Selma Yilmazer, Fatma Kaya Dagistanli, Gultekin Kaner
- P1.30** Implication of a cancer stem cell marker CD133 in the phenotypes of lung cancers
44 Natsumi Hata, Noriyo Tokuda, Yuji Kondo, Zhang Qing, Koichi Furukawa
- P1.31** Correlation between hormone receptors, HER-2 expression status and biological features of early advanced breast cancer
44 Beata Biesaga, Joanna Niemiec, Marek Ziobro, Joanna Wysocka, Anna Kruczak
- P1.32** The effects of gemcitabine and vinorelbine on inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) distribution of MCF-7 breast cancer cell line
44 N. Dilara Zeybek, Sevinc Inan, Nuran Ekerbicer, Seda Vatanserver, Jale Karakaya, Sevd F. Muftuoglu
- P1.33** Enhanced B-Raf protein expression is independent of V600E mutant status in thyroid carcinomas
44 Tetsuo Kondo, Nakazawa Tadao, Kawasaki Tomonori, Nakamura Nobuki, Yamane Tetsu, Katoh Ryohei

- P1.34** Heme oxygenase-1 expression in stromal cells protects against tumor induction and progression
45 Halina Waś, Aleksandra Sierpniowska, Małgorzata Sokołowska, Tomasz Cichoń, Ryszard Smolarczyk, Paweł Dominik, Klaudia Skrzypek, Anna Ratajska, Bożena Lackowska, Agnieszka Mazur, Krzysztof Lemke, Stanisław Szala, Józef Dulak, Alicja Józkowicz
- P1.35** Immunohistochemical localization of nucleostemin in some human tumour cells
45 Sheng-hong Liu, Ye-Qing Tong, Hong-hui Jiang, Yan Wu, Xiao-li Wang, Liu Zhi-long
- P1.36** Morphological and cellular alternations of HepG2 cells overexpressing CYP3A4 after treatment with antitumor imidazoacridinone derivative C-1311
45 Ewa Augustin, Joanna Koprowska, Monika Demusiak, Jerzy Konopa
- P1.37** Expression of luminal and basal cytokeratins in node negative breast cancer patients
46 Joanna Niemiec, Krzysztof Malecki, Andrzej Komorowski, Kaja Niesiołowska, Janusz Ryś
- P1.38** HER2 expression in neuroblastic tumors – the preliminary study
46 Ewa Izyczka-Świeszewska, Grażyna Kobierska-Gulida, Elżbieta Drożyńska, Wiesława Grajkowska, Bożena Dembowska, Sylwia Kołtan, Teresa Klepacka, Danuta Perek, Anna Balcerska
- P1.39** Histochemical and immunohistochemical techniques as a tool to classify mouse hematopoietic neoplasms – counterparts of human diseases
46 Hanna Szymańska, Joanna Piskorowska, Elżbieta Krysiak, Marek Woszczyński
- P1.40** Immunohistochemical expression of Olig2 in gliomas focused on tumor morphology and biological behavior
47 Taku Homma, Makoto Sano, Yukari Obana, Tetsuyo Maeda, Toshinori Oinuma, Masahiko Sugitani, Norimichi Nemoto
- P1.41** Carbohydrate-binding profile of mammary carcinoma and lymphoma cells
47 Olga Kurmyshkina, Eugenia Rapoport, Ekaterina Moiseeva, Galina Pazynina, Nikolai Bovin
- P1.42** Effect of MG-132 proteasome inhibitor on viability and apoptosis of Bomirski amelanotic melanoma cells
47 Justyna Sidor-Kaczmarek, Sławomir Wójcik, Mirosława Cichorek, Jan H. Spodnik, Janusz Moryś
- P1.43** Presence of intestinal nervous system neuropeptides VIP, NPY, CGRP, and GAL in carcinoma of the human large intestine
47 Janusz Godlewski, Mirosław Łakomy, Zenon Pidsudko, Waldemar Sienkiewicz
- P1.44** Quercetin as a P-glycoprotein inhibitor in resistant gastric cell line
48 Sylwia Borska, Małgorzata Drag-Zalesińska, Teresa Wysocka, Maciej Zabel, Piotr Dziegiel
- P1.45** Expression of mRNA for metallothionein isoforms in ductal breast cancer
48 Agnieszka Gomułkiewicz, Piotr Dziegiel, Marzena Podhorska-Okołów, Andrzej Wojnar, Maciej Ugorski, Maciej Zabel
- P1.46** Heat shock protein 70 (HSP 70), prostatespecific antigen (PSA), E-cadherin and racemase in the differential diagnosis and prognosis of prostate intraepithelial neoplasia and prostate cancer
48 Evguenia Kogan, N. Kovalenko, Anna Markova, D. Zdanov, O. Dobrovolskaya, A. Vinarov
- P1.47** The effect of betulinic acid on the proliferation and apoptosis induction in human sensitive and resistant cancer cell lines
49 Małgorzata Drag-Zalesińska, Teresa Wysocka, Urszula Ciesielska, Ewa Jagoda, Piotr Dziegiel, Maciej Zabel
- Histochemistry of endocrine cells**
- P1.48** Expression of beta-chemokine RANTES and its receptors in human epididymis
50 Zhen Li, Zhijian Sun, Jie Zhao, Yuanqiang Zhang
- P1.49** Calcitonin gene-related peptide: an experimental study in hypertension of rat gastric endocrine cells
50 Irena Kasacka, Halina Ostrowska
- P1.50** Atrial natriuretic peptide-immunoreactive cells in gastric mucosa of hypertensive rats
50 Irena Kasacka, Halina Ostrowska
- P1.51** The influence of moderate exposure to cadmium on the thyroid parafollicular cells of female rats
51 Barbara Piłat-Marcinkiewicz, Małgorzata M. Brzóska, Bogusław Sawicki
- P1.52** Effects of GLP-1 receptor agonist on pancreatic β cell neogenesis and islet organization in STZ treated neonatal rats
51 Fatma Kaya Dagistanli, Melek Ozturk
- P1.53** Alterations in ghrelin cells induced by short and long-term omeprazole treatment in the rat fundus mucosa
51 Sema Bolkent, Selma Yilmazer, Neslihan Türk
- P1.54** Exendin-4 exerts its insulinotropic effect through decreasing somatostatin expression in diabetic mice pancreas
51 Sehnaz Bolkent, Selda Gezginici-Oktayoglu
- P1.55** Expression of estrogen receptors in porcine granulosa cells: the effect of phytoestrogen genistein
52 Anna Nynca, Olga Kraszewska, Brian Petroff, Renata Cierieszko
- P1.56** Prolactin receptor distribution in the dorsal skin of non-hibernating and hibernating frog (*Rana ridibunda*)
52 Meliha Sengezer Inceli, Serap Sancar Bas, Engin Kaptan.
- P1.57** The use of prostaglandin E (1) for the prevention of the lung injury occurred with renal failure induced by renal ischemia-reperfusion in rats
52 Refiye Yanardag, Fusun Oztay, Beyhan Kara, Nurcan Orhan, Sehnaz Bolkent
- P1.58** The effect of season on histology and morphometry of domestic gander testis
52 Anna Leska, Ewa Banachowska, Marek Opalka, Barbara Kamińska, Luiza Dusza
- P1.59** Seasonal changes of luteinizing hormone (LH), prolactin (PRL) and thyrotropin (TSH) protein expression in the pituitary gland of domestic gander
53 Anna Leska, Monika Budżyńska, Luiza Dusza
- P1.60** Expression of the StAR protein in ovarian and testicular cells of the pig and bank vole
53 M. Słomczyńska, M. Duda, J. Galas, B. Bilińska
- P1.61** Connexin 43 expression in human and mouse testes with impaired spermatogenesis
53 Małgorzata Kotula-Balak, Anna Hejmej, Jolanta Sadowska, Barbara Bilińska
- P1.62** The distribution of chromokinesin KIF4A in human decidua during early pregnancy
54 Sati Leyla, Yasemin Seval, Ramazan Demir
- P1.63** Expression of ghrelin, its receptors and transcripts in rat thyroid and their putative functional role. An immunohistochemical and *in situ* hybridisation study
54 Marek Ruchała, Andrzej Łukaszyk, Ludmiła Rafińska, Marcin Ruciński, Piotr Sawiński, Aldona Kasprzak, Karolina Olejniczak, Jerzy Sowiński
- P1.64** Ghrelin signaling system in rat seminiferous epithelium: an immunohistochemical and hybridocytochemical study
54 Andrzej Łukaszyk, Ludmiła Rafińska, Piotr Sawiński, Aldona Kasprzak, Karolina Olejniczak, Marcin Ruciński, Marek Ruchała, Jerzy Sowiński
- P1.65** Immunohistochemical and morphological properties of mammalian pineal cells in the monolayer culture
55 Bogdan Lewczuk, Barbara Przybylska-Gornowicz, Michał Bulc
- P1.66** Immunohistochemical studies on the post-hatching development of the turkey pineal gland
55 Barbara Przybylska-Gornowicz, Bogdan Lewczuk, Michał Bulc, Magdalena Prusik
- P1.67** Expression of estrogen receptor beta (ESR2) in adult European bison (*Bison bonasus*) testis – a preliminary study
55 Elżbieta Czykier, Grzegorz Zalewski
- P1.68** Ultrastructural changes of the mitochondria and immunocytochemically localized SCC enzymes within the zona glomerulosa region of the adrenal cortex of the wild type (+/+) and mutant mice (+/- and L/L) due to sodium and potassium chloride administration
55 Chanchal Kumar Manna

Poster session 2

57

Histochemistry of immune system cells

- P2.1** Foxp3+ regulatory T cells are increased in the untreated coeliac mucosa and are expanded by gliadin in the *in vitro* cultured treated mucosa
59 Giuseppe Mazzarella, Rosita Stefanile
- P2.2** Mast cell's development and density in fetal sheep skin
59 Suzan Daglioglu, Abit Aktaş
- P2.3** Lymphocyte populations following high frequency stimulation of the subthalamic nucleus in rats
59 Beata Grembecka, Paweł Ledóchowski, Wojciech Glac, Danuta Wrona
- P2.4** Innervation of particular hematopoietic cell populations in the bone marrow of the rat
59 Zbigniew Tabarowski, Mariusz Gajda
- P2.5** Some equivocality of "markers" used for identification of infiltration cells in myositic muscles
60 Hanna Gendek-Kubiak, Ewa Gendek
- P2.6** Immune component of human umbilical cord tissue: an immunohistochemical study
60 Yesim Ugur, Aysegul Nazikoglu, Pergin Atilla, Sinan Beksac, A Nur Cakar
- P2.7** Effect of experimental *Brachyspira hyodysenteriae* infection on the concentrations of selected inflammation-associated neuropeptides (SP and galanin) and on CD2+, CD5+ and CD21+ lymphocyte subpopulations in ileum and ileal lymph nodes in the pig
60 Krzysztof Wasowicz, Anna Winnicka, Waldemar Sienkiewicz, Piotr Podlasz, Jerzy Kaleczyc, Mirosław Lakomy
- P2.8** Immunomorphological analysis of neuroblastic tumours with opsoclonus-myoclonus-ataxia syndrome
61 Ewa Iżycka-Świeszewska, Joanna Stefanowicz, Elżbieta Drożyńska, Ewa Bień, Jerzy Pienczk, Wiesława Grajkowska, Edyta Szurawska

Apoptosis and necrosis

- P2.9** Ghrelin stimulates enterocyte apoptosis and autophagy in the small intestine of pig neonates
62 Anna Kotunia, Piotr Pietrzak, Ikuo Kato, Atsukazu Kuwahara, Michał M. Godlewski, Joanna Bierła, Romuald Zabielski
- P2.10** Markers of cell proliferation, apoptosis and angiogenesis in remodeling of bronchoalveolar duct junctions
62 Vuong Tuong, Sophia Demoura, Evguenia Kogan.
- P2.11** The effects on nephropathy and apoptosis of renin-angiotensin-system blockade in the STZ-diabetic rats
62 Matem Tunçdemir, Melek Ozturk
- P2.12** Effect of lead acetate on the apoptosis and the expression of apoptosis-related gene XIAP and Smac in hippocampus of budding rates
63 Xia Huo, Xijin Xu, Dai Han.
- P2.13** Is mitosis/apoptosis index a good marker of gut mucosa rebuilding?
63 Joanna Bierła, Michał M. Godlewski, Anna Kotunia, Piotr Pietrzak, Romuald Zabielski.
- P2.14** Distribution of mitochondria in pEYFP-Mito *in vivo* electroporated mouse testis; its possible relation to the induction of germ cell apoptosis
63 Yoshitaka Hishikawa, Sucai An, Yasuaki Shibata, Yoko Sato, Takehiko Koji
- P2.15** The effect of bradykinin postconditioning on activities of antioxidant enzymes in postischemic rat brain
63 Viera Danielisova, Mirosław Gottlieb, Mirosłava Nemethova, Petra Kravcukova, Iveta Domorakova, Jozef Burda
- P2.16** Expression and immune-detection of MnSOD and CuZnSOD in the postischemic rat hippocampus after ischemic postconditioning
64 Mirosłava Nemethova, Viera Danielisova, Mirosław Gottlieb, Jozef Burda
- P2.17** The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on viability and incidence of apoptotic granulosa cells in pigs
64 Olga Kraszewska, Anna Nynca, Joanna Piasecka, Anna Robak, Barbara Wąsowska, Renata E. Ciereszko

- P2.18** Quercetin synergistically co-operates with etoposide in arresting cell cycle and inducing apoptosis of rat promyelocytic leukemia LT 12 cells
64 Monika A. Papież, Małgorzata Bzowska
- P2.19** Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) induce apoptosis by alterations of mitochondrial respiratory chain in human colon carcinoma HCT116 cell line
65 Konrad Kleszczyński, Łukasz Piszczek, Andrzej C. Składanowski.
- P2.20** Dietary sodium butyrate stimulates rebuilding of small intestinal epithelium in neonatal calves
65 Piotr Pietrzak, Anna Kotunia, Justyna Wrzeńska, Paweł Górka, Joanna Bierła, Zygmunt M. Kowalski, Michał M. Godlewski, Romuald Zabielski
- P2.21** Effects of h[Gly2]GLP-2 on apoptosis and cell proliferation in jejunal mucosa of mice administered TNF- α /Act D
65 Pelin Arda-Pirincci, Sehnaz Bolkent
- P2.22** The response of human colon cancer cells to C-1748 (4-methyl-1-nitroacridine derivative) treatment
66 Dorota Nowak-Ziatyk, Ewa Augustin, Anna Moś-Rompa, Anna Skwarska, Jerzy Konopa
- P2.23** Effect of doxazosin treatment on rat penis
66 N. Dilara Zeybek, Ozgur Oruc, Kubilay Inci, Ali Ergen, Celik Tasar, Sevda Muftuoglu
- P2.24** Apoptosis and expression of relative genes in chorionic villi and decidua of spontaneous abortion in the first trimester
66 Melek Ozturk, Tugan Bese, Fatma Kaya Dagistanli, Fazile Akyuz Gölgeci, Matem Tunçdemir, Penbe Cagatay.
- P2.25** Effects of iloprost on ischemia/reperfusion induced apoptosis in the ipsilateral rat ovary
67 Sureyya Barun, Lale Sokmensuer, Billur Demirogullari, Devrim Gursoy, Anil Dolgun, Mustafa Ozat, Tayfun Gungor, Sevda Muftuoglu.
- P2.26** The comparison of the anti-leukemic effect of selected polyphenols on rat promyelocytic leukemia LT 12 cells
67 Monika A. Papież, Małgorzata Bzowska
- P2.27** The effects of increased intraabdominal pressure onto the urinary system during gestation
67 Ibrahim Karnak, Pergin Atilla, Sevda Muftuoglu, Saniye Ekinci, F Cahit Tanyel
- P2.28** The influence of (-)-epicatechin on extent of DNA damage and apoptotic cell death in the bone marrow and spleen of rats with acute myeloid leukemia
68 Monika A. Papież, Tomasz Mikołajczyk, Wirginia Krzyściak
- P2.29** Proteins of the p53 family in psoriasis
68 Dana Kylarova
- P2.30** Cell cycle analysis of apoptotic melanotic and amelanotic melanoma cells
68 Mirosława Cichorek, Małgorzata Wachulska, Katarzyna Zielińska-Chomej
- P2.31** Morphological analysis of apoptosis in hippocampus of PTSD rats and expression of apoptosis-related genes
69 Xiao-Ming Li, Fang Han, Yuxiu Shi
- P2.32** Apoptosis-inducing factor reveals differentiated contribution to cell death in the cerebral cortex and basal ganglia during transient ischemia in the rat
69 Przemysław Kowianski, Grażyna Lietzau, Zbigniew Karwacki, Jerzy Dziewiątkowski, Sławomir Wójcik, Jan Spodnik, Janusz Moryś
- P2.33** IL-2 stimulates telomerase activity and reduces spontaneous apoptosis in human NK cells
69 Lucyna Kaszubowska, Agnieszka Dettlaff-Pokora, Jolanta Myśliwska, Andrzej Myśliwski
- P2.34** The role of apoptosis in the modulation of colon carcinogenesis in rats by oligofructose and bifidobacteria
69 Ewa Wasilewska, Barbara Wąsowska, Maria Bielecka
- P2.35** Cell proliferation and apoptosis in normal and diabetic mice testis
70 Fulu Gao, Fengming Yue, Xin Li, Siyun Niu

Fluorescence microscopy and new fluorescent markers

- P2.36** Newly developed technique for dual localization of keratin 13 and 14 by fluorescence immunohistochemistry
71 Shin-ichi Iwasaki, Hidekazu Aoyagi
- P2.37** Gene amplification of Myc and its coamplification with ERBB2 and EGFR
71 Akishi Ooi, Shioto Suzuki, Yoh Dobashi.
- P2.38** Expression of keratan sulfate on microglia in the spinal cord of amyotrophic lateral sclerosis model mouse
71 Iori Kojima, Kenji Kadomatsu
- P2.39** The function of annexins in plasma membrane repair
71 Andrea Erlbruch, Alen Piljić, Charlotta Funaya, Claude Antony, Carsten Schultz
- P2.40** Fluorescent *in situ* hybridization digital image composition using computerized image analysis procedure
72 Marylene Lejeune, Carlos Lopez, Patricia Escrivá, Ramon Bosch, Joaquin Jaen, Lluís Pons, Teresa Salvado, Tomas Alvaro
- P2.41** A natural product from marine Agelas sponges is a cell permeable pH-sensor
72 Ulf Bickmeyer, Achim Grube, Karl-Walter Klings, Matthias Köck
- P2.42** FRET probes to monitor MMP-12 activity in lung inflammation
72 Amanda Cobos-Correa, Joanna Trojanek, Marcus Mall, Carsten Schultz
- P2.43** Age-related changes to the distribution of perisynaptic Schwann cells at the murine neuromuscular junction
73 Angie Rupp, Susan K Halstead, Hugh J Willison
- P2.44** Sarcoglycans and integrins in gingival epithelium during biphosphonates treatment: immunohistochemical study
73 G. Cutroneo, E. Nastro Sinischalchi, G. Oteri, E. Magaouda, A. Allegra, C. Musolino, D. Bruschetta, F. De Ponte
- P2.45** Distribution of costameric proteins in normal human ventricular and atrial cardiac muscle
73 D. Di Mauro, R. Gaeta, A. Arco, D. Milardi, S. Lentini, M. Runci, G. Rizzo, L. Magaouda
- P2.46** Immunohistochemical localization of DCAMLK1 on rat pancreas
73 Yoshiko Itoh, Johbu Itoh
- P2.47** Cytogenetic characterization of brook trout (*Salvelinus fontinalis*): location of DNA sequences on chromosomes
74 Agnieszka Śliwińska-Jewsiewicka, Konrad Ocalewicz, Małgorzata Jankun-Woźnicka
- P2.48** A simple, rapid technique for fluorescent labeling of collagen and elastin fibers in live tissues
74 Natalia Celejewska, Krzysztof Wójcik, Ewa Biela-Witkowska, Tomasz Włodarski, Jerzy Galas, Gail McConnell, Zbigniew Darzynkiewicz, Jurek Dobrucki

Molecular methods on normal and pathological specimen

- P2.49** Expression profiling of MTA1 in adult mouse tissues
75 Yuanqiang Zhang, Wei Li, Zhen Li, Jie Zhao
- P2.50** Subcutaneous injection from birth of epigallocatechin-3-gallate, a component of green tea, limits the onset of muscular dystrophy in mdx mice: a quantitative histological, immunohistochemical and electrophysiological study
75 Yoshiko Nakae, Katsuya Hirasaka, Junpei Goto, Takeshi Nikawa, Masayuki Shono, Mizuko Yoshida, Peter J. Stoward
- P2.51** Mutational analysis of p53 in patients with long-lasting chronic hepatitis C
75 Aldona Kasprzak, Agnieszka Adamek, Wiesława Przybyszewska, Arkadiusz Czajka, Karolina Olejniczak, Jacek Juszczak, Wiesława Biczysko, Maciej Zabel
- P2.52** Cellular expression of interleukin 2 (IL-2) and of its receptor (IL-2R, CD25) in lung tumours
76 Karolina Olejniczak, Aldona Kasprzak, Wiesława Przybyszewska, Patrycja Sujka, Piotr Dzięgieł, Maciej Zabel
- P2.53** The study of the interaction between hepcidin and ferroportin
76 Xiao-Zhi Liu, Yan-Zhong Chang, Xiang-Lin Duan
- P2.54** The influence of new MDP and tuftsin analogs on cytokines levels in experimentally induced sepsis
76 Anna Wardowska, Magdalena Szaryńska, Piotr Trzonkowski, Krystyna Dzierzbicka, Andrzej Myśliwski

- P2.55** Spatial identification of the proteins from the tissue of renal cell carcinoma by imaging mass spectrometry
77 So Dug Lim, Wan-seop Kim, Sang Hwa Lee, Bom Chae Chi, Chan-Hyun Na
- P2.56** Effects of swimming on the iron absorption and utilization in rats
77 Yu-qian Liu, Yan-zhong Chang, Hai-tao Wang, Xiang-lin Duan, Jing-ze Liu
- P2.57** Decreased TLR-5 expression in the mucosal biopsies of ulcerative colitis patients
77 Aleksandra Gołąb, Piotr Wierzbicki, Krystian Adrych, Joanna Wypych, Marcin Stanisławowski, Zbigniew Kmiec
- P2.58** Promoter hypermethylation and underexpression of PLAGL1 and LATS1 tumor suppressor genes in colorectal cancer
78 Piotr Wierzbicki, Joanna Dubowska, Krystian Adrych, Dorota Kartanowicz, Sebastian Dobrowolski, Marcin Stanisławowski, Janusz Chybicki, Bartłomiej Korybalski, Janusz Godlewski, Agnieszka Mazurek, Marzena Kotwas, Agata Wrońska, Zbigniew Kmiec

Proteomics in histochemistry

- P2.59** A novel approach to understanding neurodegeneration: an analysis of single, purified inclusions
79 Federica Fulceri, Paola Lenzi, Alessandro Casini, Alessandra Cecchetti, Antonio Paparelli, Francesco Fornai

Quantification in cyto- and histochemistry

- P2.60** Locomotor reactivity to novelty correlates with the number of brain cells expressing tyrosine hydroxylase in a response to new environment and after electrical stimulation of the ventral tegmental area
80 Grażyna Jerzemowska, Karolina Plucińska, Weronika Trojnar
- P2.61** Quantitative colocalization analysis of RNA binding protein TLS and its target mRNA in mouse hippocampal neurons
80 Ritsuko Fujii, Olga Grossenbacher-Zinchuk, Yu Wang, Vadim Zinchuk, Toru Takumi
- P2.62** Quantification of Epstein-Barr virus-infected lymphocytes by flow cytometry coupled with *in situ* hybridization
80 Kanae Miyake, Kana Nishiyama, Yoshinori Ito, Yohei Yamauchi, Yukihiro Nishiyama, Hiroshi Kimura
- P2.63** Effects of image compression on area and roundness parameters in digital image analysis
80 Carlos Lopez, Marylene Lejeune, Patricia Escrivá, Ramon Bosch, Salvado Teresa, Lluís Pons, Alvaro Tomas, Joaquin Jaen
- P2.64** Proliferation index and percentage of positive nuclear area in follicular lymphoma evaluated with digital image analysis
81 Carlos Lopez, Ramon Bosch, Marylene Lejeune, Patricia Escrivá, Marta Rodriguez, Maria Teresa Salvado, Barbara Tomas, Lluís E. Pons, Tomas Alvaro, Joaquin Jaen
- P2.65** Diagnostic significance of the distribution of CD34(+) stromal cells and ASMA(+) stromal cells in the stroma of chronic pancreatitis and pancreatic cancer
81 Adam Nałęcz, Kazimierz Jaskiewicz, Robert Rzepko
- P2.66** Blood lymphocyte subpopulation after behavioral stress conditions in rats
81 Danuta Wrona
- P2.67** Substance P in the lymphoid intestinal tissue and intestinal lymph nodes of the growing and dysenteric pigs – quantitative studies
82 Waldemar Sienkiewicz, Jerzy Kaleczyc, Krzysztof Wąsowicz, Piotr Podlasz, Mirosław Łakomy
- P2.68** Human sperm chromatin evaluated by flow cytometry
82 Grace Chojnowski, Oswaldien Claassens, Bernhard Wehr, Keith Harrison, John Irving

Poster session 3

83

Stem cell biology

- P3.1** Simultaneous expression of Oct4 and genes of three germ layers in single cell-derived multipotent adult progenitor cells
85 Ji Kaihong, Liu Houqi.
- P3.2** Distribution of cardiac primitive cells with epithelial and mesenchymal markers expression: identification of a population of epicardially derived cells in the adult human heart
85 Clotilde Castaldo, Franca Di Meglio, Veronica Romano, Daria Nurzynska, Elena Piegari, Antonella De Angelis, Rita Miraglia, Ciro Bancone, Gianpaolo Romano, Stefania Montagnani
- P3.3** Role of laminin-1 and laminin-2 signalling mediated by $\alpha 6$ integrin in the CD117(+) cardiac primitive cells' proliferation, survival and migration
85 Daria Nurzynska, Franca Di Meglio, Clotilde Castaldo, Rita Miraglia, Veronica Romano, Ciro Bancone, Cristiano Amarelli, Valentina Mele, Stefania Montagnani
- P3.4** Extracellular matrix composition determines proliferation and survival of cardiac primitive CD117-positive cells
86 Franca Di Meglio, Rita Miraglia, Daria Nurzynska, Clotilde Castaldo, Veronica Romano, Ciro Bancone, Gianpaolo Romano, Stefania Montagnani
- P3.5** Expression of stem cell markers in human cutaneous melanoma
86 Franca Piras, Daniela Murtas, Luigi Minerba, Carlo Floris, Jorge Ugalde, Cristina Maxia, Paolo Demurtas, Maria Teresa Perra, Paola Sirigu
- P3.6** Notch signaling pathway involvement during *in vitro* differentiation of liver mesenchymal stem cells to osteoblasts
86 Aleksandra Bryzek, Ksymena Urbanek, Halina Koryciak-Komarska, Tomasz M. Czeka, Katarzyna Bogus, Piotr Czeka, Wirginia Likus, Aleksander L. Sieroń
- P3.7** The phenotype of newly generated cells in the brain of adult and aged opossums
87 Marta Grabiec, Krzysztof Turlejski, Ruzanna Djavadian
- P3.8** Co-transplantation of bFGF-expressing amniotic epithelial cells and neural stem cells promotes functional recovery in spinal cord injured rats
87 Dong Chen, Xiao-ting Meng, Zhi-yong Dong, Jia-mei Liu, Hui Xue, Dongguan
- P3.9** Characterization of human epidermal stem cells
87 Michał Pikula, Piotr Trzonkowski, Karolina Kondej, Janusz Jaśkiewicz, Andrzej Myśliwski
- P3.10** What about dairy cow stem cells? Semi-quantity and quantity evaluation of Sca1 expression in bovine mammary gland
87 Joanna Bierła, Tomasz Motyl
- P3.11** Transduction of stem cells from deer antler by means of pMINV-EGFP retroviral vector to confirm their contribution to auricle cartilage regeneration in rabbit
88 Marek Cegielski, Ilona Izykowska, Marzena Podhorska-Okolow, Maciej Zabel, Piotr Dziegiel, Dariusz Izycki
- P3.12** Notch regulation of the spinal progenitor fate in the adult rodent
88 Masaaki Kitada, Mari Dezawa
- P3.13** Type I collagen inhibits differentiation of human SVF cells to cardiomyocytes
88 A. Balwier, B. Zapala, A. Polus, A. Sliwa, B. Kiec-Wilk, L. Partyka, A. Dembinska-Kiec
- P3.14** Lectin-binding pattern reveals involvement of glycoconjugates and carbohydrate antigens in neural stem cell niche
88 Masaaki Kitada, Mari Dezawa.

Endothelial cell function and angiogenesis

- P3.15** Adventitial lymphatics of large arteries and atherosclerosis
89 Katarzyna Drożdż, Andrzej Szuba, Piotr Dziegiel, Marzena Podhorska-Okolow, Dariusz Janczak, Dariusz Patrzałek
- P3.16** Regulation of mTOR signaling by free fatty acids in human adipose tissue progenitor cells
89 Urszula Czech, Adriana Balwier, Anna Polus, Wojciech Dudek, Aldona Dembinska-Kiec

- P3.17** Angiogenesis and biochemical parameters in the New Zealand Obese mice
89 Lukasz Wator, Urszula Razny, Anna Polus, Jerzy Stachura, Grzegorz Dyduch, Hans Joost, Aldona Dembinska-Kiec
- P3.18** Hepatocyte retinoid X receptor ? deficient mice respond to high fat diet with downregulation of angiogenesis
89 Urszula Razny, Lukasz Wator, Anna Polus, Yu-Jui Yvonne Wan, Grzegorz Dyduch, Jerzy Stachura, Aldona Dembinska-Kiec
- P3.19** Immunohistochemistry and DNA profiling of VEGFs and VEGFRs during angiogenesis *in vitro*
90 Masumi Akita, Keiko Fujita, Eiko Murata, Hiroaki Shindo, Katsuji Kaneko
- P3.20** Distribution of notch protein members in normal and pathological human endometrium
90 Maria De Falco, Luigi Cobellis, Gabriele Coppola, Lucrezia Manente, Angela Lucariello, Ivan Cavallotti, Nicola Colacurci, Antonio De Luca
- P3.21** Expression of intermediate filament nestin in vascular endothelium
90 Jaroslav Mokry, Dana Cizkova, Jiri Ehrmann, Stanislav Filip, Zdenek Kolář
- P3.22** Role of endothelium in atherogenesis
91 Francesca Bonomini, Luigi F. Rodella, Tony Hayek, Michael Aviram, Shlomo Keidar, Raymond Coleman, Rita Rezzani
- P3.23** Presence of ANP in internal carotid artery (ICA) with and without atherosclerotic lesions
91 G. La Marca, G. La Barbera, F. Valentino, B. Valentino, E. Farina Lipari, D. Lipari, F. Cappello, A. Gerbino, M. Buscemi, G. Peri
- P3.24** AAV-mediated VEGF165 and FGF-4 gene transfer for the treatment of hind limb ischemia
91 Agnieszka Jazwa, Hevidar Taha, Aleksandra Sierpniowska, Anna Zagorska, Paulina Kucharzewska, Justyna Leja, Marco Meloni, Costanza Emanuela, Elisa Vahakangas, Seppo Yla-Herttuala, Alicja Jozkowicz, Jozef Dulak
- P3.25** AAV-mediated VEGF165 and FGF-4 gene transfer for the stimulation of reparative processes in the wounded skin of genetically diabetic mice
91 Agnieszka Jazwa, Aleksandra Sierpniowska, Magdalena Kozakowska, Anna Zagorska, Paulina Kucharzewska, Justyna Leja, Elisa Vahakangas, Seppo Yla-Herttuala, Alicja Jozkowicz, Jozef Dulak
- P3.26** Cellular localization and correlation between NADPH-diaphorase activity and endothelial and inducible nitric oxide synthases immunoreactivity in periovarian vascular complex after testosterone infusion into porcine ovarian artery in the luteal phase
92 Barbara Wąsowska, Ewelina Bajko
- P3.27** Relationships between NADPH-diaphorase staining and endothelial (eNOS) and inducible (iNOS) nitric oxide synthases in porcine uterus vessels after testosterone infusion into ovarian artery during estrous cycle
92 Barbara Wąsowska, Ewelina Bajko
- P3.28** The expression profiles of VEGF, its receptors and angiopoietin receptors Tie-1 and Tie-2 in the early human placenta
92 Ramazan Demir, Yasemin Seval, Leyla Sati
- P3.29** Ultrastructural and immunohistochemical study of the inner vascular retina of the lizard *Gallotia galloti* (Lacertidae)
93 Mc. Alfayate, C. Yanes, E. Santos, M. Romero-Aleman, N. Casanas, R. Vinoly, M. Monzon-Mayor
- P3.30** Human adipose tissue stromal vascular fraction cells differentiate depending on distinct types of media
93 Barbara Zapala
- P3.31** Impaired endothelial function in subjects with multiple cardiovascular risk factors
93 Teresa Domagala, Marek Kaszuba, Katarzyna Kotula-Horowitz, Bartosz Krzyzanowski, Andrzej Szczeklik

Cytoskeleton

- P3.32** Molecular mechanisms of inner ear invagination and otocyst formation
94 Xiao Rei Sai
- P3.33** Redistribution of CacyBP/SIP from neuronal processes to somata during normal aging in rats
94 Gabriela Schneider, Anna Filipek, Anna Mietelska, Izabela Figiel, Grazyna Niewiadomska
- P3.34** Organization of selected cytoskeletal proteins in induced senescence in non-small cell lung carcinoma A549 cell line
94 Anna Litwiniec, Alina Grzanka, Dariusz Grzanka, Dariusz Smoliński
- P3.35** Application of confocal microscopy and scanning cytometry in the cell cytoskeleton imaging
94 Agnieszka Turowska, Michał M. Godlewski, Joanna Cymerys, Anna Chmielewska, Anna Tucholska, Marcin W. Bańbura
- P3.36** Effect of arsenic trioxide on actin reorganization in K562 erythro-leukemia cells
95 Magdalena Izdebska, Alina Grzanka, Dariusz Grzanka, Lidia Gackowska, Maciej Ostrowski
- P3.37** Effects of Taxol treatment on morphology and cell death in CHO AA8 cells
95 Aleksandra Stępień, Alina Grzanka, Dariusz Grzanka, Mariusz Szczepański, Anna Helmin-Basa, Lidia Gackowska
- P3.38** Immunolocalization of dynamin-2 around discoid vesicles in umbrella cells of mouse urinary bladder
95 Nobuo Terada, Nobuhiko Ohno, Sei Saitoh, Chao Dong, Yasuhisa Fujii, Shinichi Ohno
- P3.39** The changes of actin cytoskeleton in CHO AA8 cell line after apoptosis and mitotic catastrophe induction by hyperthermia
96 Dariusz Grzanka, Alina Grzanka, Magdalena Izdebska, Anna Helmin-Basa, Lidia Gackowska, Andrzej Marszałek
- P3.40** Gelsolin in human melanoma A375 cells grown on lumican
96 Agata Radwańska, Monika Mach, Dagmara Baczyńska, Yanusz Wegrowski, Maria Malicka-Błaszkiwicz

ECM and connective tissue biology

- P3.41** Expression of decorin in esophageal cancer in relation to expression of three isoforms of transforming growth factor-beta (TGF- β 1, - β 2 and - β 3) and matrix metalloproteinase-2 activity
97 Katarzyna Augoff, Krzysztof Grabowski, Jerzy Rabczynski, Adam Kolondra, Renata Tabola, Aleksander F. Sikorski
- P3.42** Immunohistochemical studies of decorin expression in polyps and carcinomas of the colon
97 Katarzyna Augoff, Jerzy Rabczynski, Renata Tabola, Leszek Czaplak, Katarzyna Ratajczak, Krzysztof Grabowski
- P3.43** Evaluating the effects of intermittent hypoxia on collagen
97 Mine Gultomruk, Tugba Kotil, Fadime Aktar, Ebru Karabulut, Seyhun Solakoglu, Yurdagül Canberk
- P3.44** Matrix metalloproteinases and their tissue inhibitors in remodeling of bronchoalveolar duct junctions
98 Evguenia Kogan, Vuong Tuong, Sophia Demoura
- P3.45** Stromal reaction at the invasive margin of pT3 colorectal adenocarcinoma
98 Robert Rzepko, Rafał Pęksa, Adam Nałęcz, Wiesław Kruszewski, Kazimierz Jaśkiewicz
- P3.46** Effects of iloprost on calvarial sutures
98 Gökhan Tunçbilek, Petek Korkusuz, Figen Özgür
- P3.47** Expression of MMP9 in guinea pig skeletal muscles after administration of selected neuropeptides
98 Hanna Gendek-Kubiak, Ewa Gendek
- P3.48** Left ventricular fibrillar collagenous network remodeling in ischemic heart disease
99 Jolita Palubinskiene, Dalia Pangonyte, Elena Stalioraityte, Danute Kazlauskaitė, Reda Zhiuraitiene

Electron microscopy

- P3.49** Alterations of testicular morphology and blood-testis barrier in irradiation and hyperthermia exposed rats: evaluation for infertility
100 Gozde Erkanli, Feriha Ercan, Cenk Umay, O Uzel
- P3.50** Ultrastructural and histological alterations of rat testis after bilateral vasectomy
100 Gazi Contuk, Feriha Ercan
- P3.51** Effects of in utero ethanol administration on rat testicular morphology and cell death: evaluation for infertility
100 Yasemin Ersoy, Feriha Ercan.
- P3.52** Adenosine improves burn-induced dermal and gastric injury: a histopathological study
100 Pinar Turan, Yasemin Kocabas, Rumeysa Durak, Berrak Yegen B, Serap Arbak
- P3.53** *In vitro* effect of chitosan on fibroblastic cell activity: immunocytochemical and scanning electron microscopical study
101 Bahar Uslu, Serap Arbak, Ayhan Bilir, Julide Tanbuga, Suna Ozbas, Burcu Biltekin, Seckin Denir
- P3.54** Effects of zinc deficiency on the epithelial and Paneth cells in the rat small intestine
101 Eiko Murata, Masumi Akita, Kumiko Yamada, Yuka Eiki, Makoto Nodera, Kunio Kitamura, Hiroaki Shindo, Katsuji Kaneko
- P3.55** *In vitro* changes of cell proliferation and *in vivo* lung injury after exogenous surfactant treatment
101 Andrzej Marszałek, Lidia Gackowska, Monika Seget, Elżbieta Kaczmarek, Ewa Florek, Wiesława Biczysko
- P3.56** Immunohistochemical and ultrastructural findings on human placental stem villi
102 Leyla Sati, Yasemin Seval, Ramazan Demir
- P3.57** Changes of intercalated disk in case of congenital heart malformations in humans – immunohistochemical, ultrastructural and morphometrical studies
102 Dariusz Tosik, Jacek Moll, Jadwiga Moll, Andrzej Kulig, Hieronim Bartel

New light microscopic techniques

- P3.58** Immunohistochemical study of functional proteins in cells and tissues of living animal organs by the *in vivo* cryotechnique
103 Shinichi Ohno, Nobuhiko Ohno, Nobuo Terada, Sei Saitoh
- P3.59** High resolution microscopy gives insight into DNA repair
103 Grace Chojnowski, Derek J Richard

Live cell imaging in vitro

- P3.60** The influence of bacteria on sperm viability and mitochondrial activity
104 Monika Fraczek, Małgorzata Piasecka, Dariusz Gączarzewicz, Maria Laszczyńska, Maciej Kurpisz
- P3.61** Fluorescence correlation spectroscopy of vesicle movements in the hemocyte cytoplasm
104 Grzegorz Tylko
- P3.62** Quantitative fluorescence recovery after photobleaching (FRAP): The role of photon flux and object translocation
104 Mateusz Kuzak, Dominika Trembecka, Jurek Dobrucki

Sub-cellular molecular tracing in living cells

- P3.63** Detection of the effects of cholesterol level alterations in plasma membrane on the TRH receptor mobility with fluorescence recovery after photobleaching
105 Pavel Ostasov, Zuzana Burdikova, Lucie Kubinova, Petr Svoboda
- P3.64** Solid Lipid Nanoparticles loaded with Doxorubicin and 6-Coumarin (Doxo-CoumSLNs): uptake, distribution and drug release in human U373 astrocytoma cell cultures
105 Calderoni Sara, Strada Alberto, Brioschi Andrea, Colonna Alessandra, Gasco Maria Rosa, Mauro Alessandro

Imaging of nerve cell structure and function

- P4.1** Immunohistochemical changes of tyrosine hydroxylase expression in substantia nigra and VTA rat brain nuclei under dopaminergic hyperfunction
109 Rudolf Khudoerkov, Dmitry Voronkov
- P4.2** Role of TLS in MeCP2-dependent NR1 alternative splicing in neurons
109 Widyawilis Binte Selamat, Loo Chin Wong, Toru Takumi, Ritsuko Fujii
- P4.3** α B-crystallin distribution in the dog central nervous system
109 Siyami Karahan, Tolga Güvenç
- P4.4** Predisposition of distal nodes of Ranvier to injury in a mouse model of Guillain Barre syndrome
110 Rhona McGonigal, Sue Halstead, Hugh Willison
- P4.5** The effects of NOS inhibitor 7-nitroindazole and NO precursor L-arginin on immunoreactive neuronal nitric oxide synthase (nNOS) in rat brains and its relation to memory
110 Süheyla Gonca, Füzuzan Yildiz, Cannur Dağçik, Pelin Coştur Biyiksiz, Hakki Dağçik
- P4.6** Galanin system in the brain of zebrafish (*Danio rerio*)
110 Piotr Podlasz, Ville Salinen, Yu-Chia Chen, Hisaaki Kudo, Natalia Fedorowska, Pertti Panula
- P4.7** Detection of protein gene product (PGP) 9.5 as a marker of epidermal nerve fibre depletion in simultaneous pancreas and kidney transplant patients
110 Alena Lodererova, Ludek Voska, Petr Boucek, Terezia Havrdova, Frantisek Saudek, Milos Adamec, Claudia Sommer
- P4.8** Inflammatory pain induces eNOS and endothelial cell marker alterations in dorsal root ganglia
111 Elisa Borsani, Roberta Albertini, Rita Rezzani, Luigi F. Rodella
- P4.9** Co-localization of IL-6 and BDNF immunostaining in the dorsal root ganglia of rat neuropathic pain model
111 Petr Dubovy, Ilona Klusakova, Ivana Svizenska, Radim Jancalek
- P4.10** The neurosecretion of oxytocin in the hypothalamic magnocellular nuclei of the training rats
111 E. Farina Lipari, A. Lipari, M. Bellafiore, G. Peri, A. Gerbino, M. Buscemi, B. Valentino
- P4.11** Immunolocalization of alpha-synuclein in the rat spinal cord by two novel monoclonal antibodies
112 Giorgio Vivacqua Loredana D'Este
- P4.12** Calcium binding proteins in the hippocampal formation of the guinea pig (*Cavia porcellus*)
112 Małgorzata Kolenkiewicz, Anna Robak
- P4.13** The cocaine- and amphetamine-regulated transcript (CART) immunoreactivity in the amygdala of the guinea pig
112 Maciej Równiak, Anna Robak, Krystyna Bogus-Nowakowska, Barbara Wasilewska, Mariusz Majewski
- P4.14** Mitogenic activity in the ventricular and the subventricular zone of rat brain
112 Faruk Alkan, Sibel Kokturk, Fatma Kaya Dagistanli, Melek Ozturk, Mumin Uzunalan, Bulent Uruluer
- P4.15** Low infra red laser light irradiation protects neural cells from oxidative stress: a confocal microscopy analysis on living cells combined with biochemical and morphological studies
113 Alessandro Giuliani, Luca Lorenzini, Michele Gallamini, Luciana Giardino, Laura Calza
- P4.16** Confocal studies of synaptic connections in the first visual neuropil of *Drosophila melanogaster*
113 Jolanta Górska-Andrzejak, Grzegorz Tylko
- P4.17** Effects of prolonged fasting on serum leptin level and brain leptin receptor expression in aged rats
113 Kotlarz Grazyna
- P4.18** Distribution of CART (cocaine- and amphetamine- regulated transcript) and CaBPs (calcium-binding proteins) in the mamillary region of guinea pigs aged ten days postnatally
114 Anna Robak, Małgorzata Kolenkiewicz, Maciej Równiak, Krystyna Bogus-Nowakowska, Mariusz Majewski

- P4.19** Neurons containing calcium binding proteins in the claustrum and the endopiriform nucleus of the opossum
114 Katarzyna Majak, Beata Domaradzka-Pytel, Jan H. Spodnik, Ruzanna L. Djavadian, Krzysztof Turlejski, Janusz Moryś
- P4.20** GABA-ergic neurons and calcium binding proteins in the rat claustrum and endopiriform nucleus – colocalization study
114 Edyta Spodnik, Jan H. Spodnik, Sławomir Wójcik, Jerzy Dziewiątkowski, Janusz Moryś
- P4.21** Resiniferatoxin (RTX)- and tetrodotoxin (TTX)-induced changes in chemical coding of sympathetic neurons in the inferior mesenteric ganglion (IMG) supplying porcine urinary bladder
115 Agnieszka Bossowska, Joanna Wojtkiewicz, Celina Kowalska, Mariusz Majewski
- P4.22** Calretinin in dopaminergic neurons of the opossum (*Monodelphis domestica*) midbrain nuclei: an immunohistochemical study
115 Ilona Klejbor, Beata Ludkiewicz, Jan Spodnik, Sławomir Wójcik, Krzysztof Turlejski, Janusz Moryś
- P4.23** Forced swim stress changes NGF/c-Fos immunoreactivity in the structures of the limbic system in the aged rats
115 Ewa Badowska-Szalewska, Tomasz Cecot, Ilona Klejbor, Beata Ludkiewicz, Jan Spodnik, Janusz Moryś
- P4.24** Projecting neurons in the rat claustrum and endopiriform nucleus-colocalization study with neurons containing calcium binding proteins
115 Jan H. Spodnik, Edyta Spodnik, Sławomir Wójcik, Jerzy Dziewiątkowski, Janusz Moryś
- P4.25** Comparison of ω -conotoxin (CTX) and botulinum toxin-induced changes in the chemical coding of the inferior mesenteric ganglion (IMG) neurons supplying porcine urinary bladder
116 Joanna Wojtkiewicz, Agnieszka Bossowska, Mariusz Majewski

Varia

- P4.26** Large-scale chromatin remodeling and DNA methylation in immature bovine oocyte during the later phase of growth and differentiation
117 Valentina Lodde, Silvia C. Modina, Federica Franciosi, Irene Tessaro, Alberto M. Luciano
- P4.27** Keratinization of outer root sheath cells is prevented by contact with inner root sheath of rat hair follicles
117 Justyna Niderla-Bielińska, Ewa Jankowska-Steifer, Stanisław Moskalewski
- P4.28** Response to prednisone in relation to NR3C1 intron B polymorphisms in childhood nephrotic syndrome
117 Grzegorz Zalewski, Anna Wasilewska, Lech Chyczewski, Walentyna Zoch-Zwierz
- P4.29** Role of cyclic AMP response element-binding protein in the duct cell differentiation in mouse submandibular gland
117 Shoichi Iseki, Sunisa Keattikunpairroj, Natthiya Sakulsak, Masaki Nakaya, Tomohiko Wakayama
- P4.30** Expression pattern of RET finger protein in developing and young adult mice
118 Hiroyasu Umakoshi, Mayumi Jijiwa, Masahide Takahashi
- P4.31** Distribution and localization of wolframin in adult human tissues
118 Maria De Falco, Angela Lucariello, Luca De Luca, Lucrezia Manente, Rosa Tedesco, Anna Sellitti, Vincenza Laforgia, Antonio De Luca
- P4.32** CYP2B1/2, CYP3A1 and CYP3A2 immunolocalization in normal and regenerating livers of rats of different age
118 Aleksandra Bryzek, Danuta Plewka, Piotr Czekaj, Ryszard Wiaderkiewicz
- P4.33** Pathomorphological evaluation of Alzheimer's disease model induced by sodium azide in rats
118 Mihály Albert
- P4.34** Role of TNF-alpha in pulmonary hypertension induced by monocrotaline in rats
119 Ivan Kocić, Katarzyna Sztornowska, Iga Wapniarska
- P4.35** Level of p53 and p21 protein in pterygium cells
119 Anna Lekawa, Halina Antosz

- P4.36** Collagen-chondroitin sulphate-hydroxyapatite porous composites: a histochemical approach
119 Otilia Zarnescu, Oana Craciunescu, Mirela Balan, Lucia Moldovan
- P4.37** Basal cell marker revealed by RAM11 antibody: delayed expression during epithelial regeneration in rabbits
119 Grzegorz J. Lis, Joanna Zarzecka, Jan A. Litwin, Mariusz Gajda, Tadeusz Cichocki
- P4.38** Seasonal lectin binding pattern in thumb pad of the frog (*Rana ridibunda*)
120 Engin Kaptan, Sehnaz Bolkent
- P4.39** Antigen presenting cell-lymphocytes clusters formation in H-2d mice infected with ectromelia virus
120 Lidia Szulc, Małgorzata Gieryńska, Marek Niemiałtowski
- P4.40** The influence of diet supplementation with selenomethionine and vitamin E on the morphological picture of rabbits' organs in experimental atherosclerosis
120 Barbara Stawiarska-Pięta, Ewa Szaflarska-Stojko, Ewa Birkner, Ewa Grucka-Mamezar, Jolanta Zalejska-Fiolka, Anna Maj, Marta Knapik
- P4.41** Mouse model of the DNA damage response
121 Pavla Lužná, D. Kylarová, S. Takáčová, V. Divoký, E. Kašpárková, E. Nezhybová, L. Piterková, J. Ehrmann
- P4.42** Histochemistry and nuclear magnetic resonance as tools for evaluating meat quality
121 Manuela Malatesta, Federica Perdoni, Barbara Cisterna, Marco Biggiogera, Elena Nicolato, Marco Fontanella, Carlo Zancanaro
- P4.43** The effect of hypometabolizing molecules on transcription as shown by a new two-step pulse-chase method
121 Alessandro Spedito, Manuela Malatesta, Marco Biggiogera
- P4.44** Annual reproductive cycle in two free living populations of three-spined stickleback (*Gasterosteus aculeatus* L.): patterns of ovarian and testicular development
122 Ewa Sokołowska
- P4.45** Bilateral changes of Il-6 protein levels in the rat dorsal root ganglia associated and non-associated with nerve injury
122 Zuzana Veselková, Radim Jančálek, Ilona Klusáková, Ivana Svíženská, Lumír Trenčanský, Petr Dubový
- P4.46** Morphology and function of cells demonstrating expression of beta estrogen receptor in lateral lobe of the rat prostate under hyperprolactinaemia induced with risperidone
122 Maria Laszczyńska, Karolina Chorzępa-Nowicka, Marcin Wylot, Małgorzata Piasecka
- P4.47** CB1 cannabinoid receptors in the central nervous system of *Podarcis sicula*
122 Rossana Favorito, Luigia Cristino, Maria Consiglio Grimaldi, Ida Ferrandino
- P4.48** Biological responses in MG-63 osteoblast-like cell line cultured on sol-gel derived bioglass layers
123 Elżbieta Menaszek, Anna Ścisłowska-Czarnecka, Katarzyna Cholewa-Kowalska, Maria Łączka
- P4.49** Changes of p63 expression pattern during morphogenesis of the mouse submandibular gland
123 Sachiko Matsuura, Akio Kikuta
- P4.50** The effect of the melatonin on survival of ultraviolet-A and ultraviolet-B irradiated human dermal fibroblasts and melanoma cells
123 Ilona Izykowska, Aleksandra Piotrowska, Marek Cegielski, Marzena Podhorska-Okolow, Maciej Zabel, Piotr Dziegiel
- P4.51** Left ventricular cardiomyocyte response to myocardial ischemia
124 Elena Stalioraityte, Dalia Pangonyte, Reda Ziuraitiene, Danute Kazlauskaitė, Jolita Palubinskiene, Lina Utkiene
- P4.52** The lacrimal outflow ducts exfoliative cytology of healthy persons
124 Katarzyna Wesołowska, Andrzej Zieliński
- P4.53** Histamine deficient zebrafish -a step towards understanding the interactions between the histaminergic and other neurotransmitter systems
124 Maria Sundvik, Hisaaki Kudo, Pertti Panula
- P4.54** Ultrastructural study of microfungi under heavy metal contamination
125 Bożena Sosak-Świdowska

Lectures



Poster session 1



Poster session 2



Poster session 3



Poster session 4



Metabolic mapping

Cornelis Van Noorden

Academic Medical Center, University of Amsterdam, Dept. of Cell Biology and Histology, Amsterdam, Netherlands

Contact: c.j.vannoorden@amc.uva.nl

Enzymes play key roles in many pathophysiological processes. To study their exact role, localization and quantification of activity of the specific enzyme that is involved is essential, preferentially under conditions that reflect the *in vivo* situation. It is the activity that represents the function of an enzyme. This is tightly regulated at the posttranslational level by processes such as proteolytic cleavage, glycosylation and binding to endogenous inhibitors. Hence, large amount of inactive and therefore not functional enzymes can be accumulated in a tissue compartment, which can become activated rapidly on demand to keep metabolism under control. Histochemical or cytochemical localization of the activity of an enzyme (also called catalytic histochemistry and cytochemistry, or metabolic mapping) is the approach for functional studies of pathophysiological processes because it links the activity of an enzyme to cell and tissue structure. Localization of the activity of an enzyme is traditionally performed at substrate concentrations that produce maximal amount of colored or fluorescent final reaction product. These concentrations are usually high so that the maximal velocity of the enzyme (V_{max}) is obtained. However, these high substrate concentrations are seldom present *in vivo*. Moreover, the affinity of an enzyme for its substrate(s) (K_m) can also be under posttranslational control, e.g., due to interactions of the enzyme with other macromolecular structures. Variations in V_{max} and K_m greatly affect substrate conversion by an enzyme at physiological concentrations. Because of these considerations, metabolic mapping becomes more and more focused on the visualization of enzyme reactions in living cells and tissues at substrate concentrations that are physiological. In this way, metabolic mapping provides many possibilities to link the actual functioning of an enzyme with pathophysiological alterations so that, for example, specific inhibitors can be tested for therapeutic use. Localization and quantification of the activity of enzymes in living cells and tissues can be performed by the analysis using digital microscopy or flow cytometry of either the production or consumption of fluorescent endogenous molecules, such as NADPH and NADH, or the formation of colored or fluorescent products generated from synthetic chromogenic or fluorogenic substrates. This setup allows quantitative monitoring of enzyme reactions in cells and tissues in time and space while the reaction proceeds. In conclusion, the up-to-date version of enzyme histochemistry or cytochemistry that is called metabolic mapping is an indispensable tool for the understanding of the functional role of enzymes in (patho)physiological processes.

L001

Connectomics in the developing nervous system

Jeff W. Lichtman

Harvard University, Cambridge, Massachusetts, United States

Contact: jeff@mcb.harvard.edu

The ways in which the synaptic circuitry is established remains a central and poorly understood area of developmental biology. My laboratory has focused on this question because in mammals, including humans, the interconnections between nerve cells are dramatically modified during neonatal and adolescent life. These modifications cause many (perhaps the majority) of the axon branches established at earlier stages of development to be pruned. At the same time, surviving connections become stronger. Because all mammals are obligate learners and most learning occurs in early postnatal life, we have wondered whether this rewiring is a physical manifestation of the accumulation of experience. We have studied developmental synapse elimination in several ways, including time-lapse visualization of axonal terminal branches that are pruned and the consequential elaboration of synapses by surviving terminals. In order to image axons *in situ* we use transgenic mice that render neurons fluorescent. By multiple fluorescent colors we can follow the inter-axonal interactions that lead to synapse elimination by visualizing each axon separately. We see evidence of a direct competition between axons that are vying to remain connected to the same target cell. For example, the surviving axon typically occupies much of the postsynaptic territory pre-

viously occupied by the withdrawing inputs. We have also begun to study the circuit implications of axon competition. By computer assisted reconstruction techniques we have traced out the full connectonal maps of all the axons innervating a target tissue. These "connectomes" reveal enormous variability amongst functionally and genetically identical connectivity in mice. Recently we have developed new "Brainbow" transgenic mice that allow tracking many axons as each possesses its own unique combination of a red, green, and blue fluorescent proteins. We are using this tool to examine the "logic" of the developmental reorganization in the neuromuscular system. In addition, we hope this tool will allow study of synaptic reorganization in the central nervous system. Ultimately tools must be developed that can image volumes large enough to encompass whole neural circuits in the CNS while simultaneously providing sufficient resolution to trace the finest neural processes. Moreover unless the procedures are automated it will be unlikely to generate sufficiently large data streams in a reasonable time. To address these general problems, we have constructed a tape-collecting lathe ultramicrotome (ATLUM) which automates both sectioning and collection of ultrathin brain tissue.

L002

Histochemical approaches to visualizing brain feeding pathways

James Blevins¹, Brendan Thatcher¹, Gregory Morton², Diana Williams², Michael Schwartz², Denis Baskin¹

¹VA Puget Sound Health Care System, Seattle, United States

²University of Washington, Seattle, United States

Contact: jblevin@u.washington.edu

Activation of melanocortin receptors (MC3/MC4R) in the paraventricular nucleus (PVN) of the hypothalamus by alpha-melanocyte stimulating hormone results in reduced food intake, but the mechanism is unknown. Using the synthetic MC3R/MC4R agonist, MTII, we tested the hypothesis that CNS melanocortin signaling increases the hindbrain sensitivity to the satiety effects of cholecystokinin (CCK), an intestinal peptide released during meals. We administered MTII directly into the third ventricle (3V) in combination with an I.P. injection of CCK-8 and measured food intake and expression of Fos protein (marker of neuronal activation) in the nucleus tractus solitarius (NTS), a hindbrain area that receives input from the intestinal satiety signals. 3V injection of MTII enhanced the effects of CCK-8 to reduce feeding and induced Fos immunoreactivity in the NTS. To determine if endogenous melanocortin signaling via PVN MC3/MC4R enhances the effects of CCK-8 to reduce food intake, we measured the feeding response following PVN injection of the MC3/MC4R antagonist, SHU9119, in combination with an I.P. injection of CCK-8. SHU9119 attenuated the effects of CCK-8 to reduce food intake, indicating that melanocortin signaling via PVN MC3/MC4R contributes to CCK-elicited satiety. To determine if leptin-stimulated melanocortin signaling through oxytocin (OXY) neurons in the PVN neurons increase the hindbrain sensitivity to CCK, parvocellular PVN (pPVN) neurons projecting to the NTS were identified by injection of the retrograde tracer, fluorescent cholera toxin B (fCTB) in the NTS. A subpopulation of pPVN cells that contained the fCTB tracer from the NTS also showed immunocytochemical staining for both Fos protein and OXY, indicating that leptin and potentially MC3/4R ligands as well, inhibit food intake through activation of descending PVN-NTS OXY neurons. To determine if MC4Rs are expressed by PVN neurons that project to the hindbrain, we used laser capture microdissection to collect pPVN cells containing the fCTB and pooled these cells for real-time quantitative PCR analysis of MC4R mRNA. The pPVN neurons that projected to the NTS were enriched in MC4R mRNA in addition to mRNAs for OXY and corticotropin-releasing hormone (CRH). Taken together, these findings confirm the existence of a neuroanatomical pathway from the pPVN to the NTS that potentially mediates that the anorexic effects of melanocortin ligands in the hypothalamus and support a physiological mechanism in which OXY and CRH neurons in the PVN respond to melanocortin receptor activation by acting on NTS neurons to enhance the satiety response to CCK. Support: Dept. Veterans Affairs, NIH DK-17047, DK-52989, DK-12829, DK-17047-31, NS-32273.

L003

Illuminating the secretory pathway**Rainer Pepperkok**

European Molecular Biology Laboratory, Heidelberg, Germany

Contact: Pepperko@embl.de

Transport of proteins through the secretory pathway involves the sequential action of vesicular coat complexes. In the early secretory pathway COPII concentrates cargo for secretion at ER exit sites, COPI is subsequently recruited to transport carriers and acts in retrieval of recycling proteins back to the ER. Using different live cell imaging techniques and mathematical modeling of the experimental data we show that the presence of transport competent secretory cargo and the interaction of the Sec23/24p COPII sub-complex with the dynactin complex component p150^{Glued} stabilize COPII at ER exit sites. This prevents premature COPII disassembly and provides the time to enable cargo sorting, concentration and subsequent carrier formation. Together, our data suggest a mechanism by which membranes of the early secretory pathway can be linked to motors and microtubules for subsequent organization and movement to the Golgi apparatus. We have also conducted large scale screening projects with the aim to identify further regulators of vesicular Coat proteins. To this end we have developed and applied functional assays to assess the effect of knock-ins by cDNA over-expression and knock-downs by RNAi, on processes such as constitutive protein transport, Golgi integrity and function of vesicular coat complexes. We have applied them to genome-wide siRNA screens to identify and characterize comprehensively the genes involved in constitutive protein secretion.

L004

Imaging of signalling across the plasma membrane**Theodorus W.J. Gadella Jr., Joachim Goedhart, Merel Adjobo-Hermans, Laura van Weeren**

Section of Molecular Cytology & Centre for Advanced Microscopy, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands

Contact: th.w.j.gadella@uva.nl

We use fluorescent biosensors that are designed to specifically visualize signalling dynamics across the membrane from G-protein coupled receptors through G-proteins, phospholipase C and protein kinase C in mammalian cells. Within this signalling module, the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) into diacylglycerol (DAG) and inositoltriphosphate (IP₃) (triggering intracellular calcium oscillations) is the key step. Our aim is to understand in detail the spatiotemporal aspects of this signalling cascade. Our approach is to systematically label all key molecules and visualize their distribution and activity in living cells with multimode quantitative fluorescence microscopy. To visualize the key signalling enzymes we use visible fluorescent protein (VFP)-fusions, some of which were enhanced for brightness in a mutagenesis approach. We monitor the activity of phospholipase C by the visualization of signalling lipids employing highly specific lipid-binding protein domains fused to VFPs. We were able to visualize the activation of the heterotrimeric G protein Gq for the first time using a k²-based FRET ratio imaging technique. In conjunction with FRET-FLIM we could demonstrate that upon activation the G-protein does not dissociate but undergoes a conformational change near the membrane. The activity of protein kinase C is monitored by using genetic encoded ratiometric FRET sensors reporting on phosphorylation kinetics. The calcium release is imaged using translocating Ca²⁺ sensitive lipid binding domains. Using multimodal imaging techniques (including total internal reflection (TIRF) microscopy) we are able to perform multiparameter imaging of the activation of this signalling module in live cells. Our results demonstrate complex spatiotemporal behaviour including transient recruitment and activation of effectors at hotspots adjacent to the plasma membrane.

L005

Imaging and perturbation of receptor endocytosis**Carsten Schultz**

European Molecular Biology Laboratory, Heidelberg, Germany

Contact: schultz@embl.de

Our group is interested in better understanding intracellular signaling pathways. We therefore develop tools to visualize and manipulate signaling

events in living cells. In this respect, lipids are one of the least investigated groups of biomolecules. Bypassing signaling at the receptor level with membrane-permeant derivatives of various phosphoinositides and the use of realtime imaging, we were able to discover a novel form of receptor tyrosine kinase internalization. In collaboration with Rainer Pepperkok's group we find that by artificially elevating the levels of PIP₃ the non-ligand bound EGF receptor is readily endocytosed via clathrin-coated pits. However, in contrast to ligand-induced endocytosis the receptor is not digested in the lysosome but reappears at the plasma membrane some 90 minutes after endocytosis. We therefore identified a novel pathway, which is complementary to classical receptor endocytosis and may contribute to the regulation of receptor tyrosine kinase (RTK) levels at the plasma membrane.

L006

APPL1 and the FSH-induced IP3 pathway for calcium mobilization in human granulosa cells**Joseph E. Mazurkiewicz¹, Richard M. Thomas², Cheryl A. Nechamen², Alfredo Ulloa-Aguirre³, Kerri Kluetzman⁴, James A. Dias⁴**¹Center for Neuropharmacology and Neuroscience, Albany Medical College and Department of Biomedical Sciences, State University of New York at Albany, Albany, United States²Wadsworth Center, David Axelrod Institute for Public Health, New York State Department of Public Health, Albany, United States³Research Unit in Reproductive Medicine IMSS, Mexico City, Mexico⁴Department of Biomedical Sciences, State University of New York at Albany, Albany, United StatesContact: mazurki@mail.amc.edu

Human Follicle stimulating hormone receptor (hFSHR), a class A GPCR found on ovarian granulosa and testicular Sertoli cells, supports normal folliculogenesis and spermatogenesis through its binding of circulatory FSH and the subsequent generation of an intracellular signal in these target cells. The signaling cascades activated after ligand binding are extremely complex and have been shown to include the canonical adenylyl cyclase/cAMP/protein kinase-A signaling pathway as well as mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt, src family kinases and calcium signaling. The adapter protein APPL1, which had been linked to an assortment of other receptors and cytoplasmic signaling proteins, was previously identified as an interacting protein with hFSHR in the first intracellular loop (iL1). Alanine substitution mutations were generated in iL1 of hFSHR and the mutant, FSHRK376A (K376A) was shown to abrogate APPL1 association with hFSHR. When screened for an effect in APPL1 interaction in HEK293 cells, the K376A mutant receptor like the wt hFSHR induced cAMP. However, unlike wt receptor, the K376A mutant did not induce the production of inositol 3-phosphate (IP₃). Cells from the human granulosa cell line, KGN, were then virally transduced to express either wt hFSHR or K376A mutant receptor. Biosynthesis and trafficking to the cell surface, cell surface binding of [¹²⁵I]-hFSH, and induction of progesterone and estradiol production by hFSH were the same for the wt and mutant receptor in KGN cells. To determine if the defect in K376A that was observed in HEK293 cells was manifested in a cAMP independent pathway in KGN cells, calcium mobilization was studied in single cells using confocal microscopy. Pneumophoretic application of hFSH to granulosa cells elicited an increase in [Ca²⁺]_i in KGN cells expressing wt FSHR or the mutant receptor K376A, however, the rise in intracellular calcium was notably less in granulosa cells transduced with K376A than wt-receptor. In cells expressing wt-hFSHR, calcium was mobilized from both extracellular and intracellular sources as determined following removal of extracellular calcium with EGTA and incubation with Thapsigargin to deplete intracellular stores, respectively. In contrast, following EGTA chelation of extracellular calcium, K376A expressing cells did not display a rise in calcium supporting a deficit in IP₃ production. These results suggest that APPL1 interaction with hFSHR may facilitate various G-protein effector molecules such as Gαq/11, Gαh or G12/13 to activate a PLC pathway, that ultimately leads to mobilization of intracellular Ca²⁺. Finally, this study adds to the growing field of APPL1-mediated signal transduction research a role for APPL1 in the FSH-induced PLC/IP₃ pathway and calcium signaling. Supported by NIH HD18407.

L007

Immunofunctional multiple signal detection by quantum dot and spectrum analysis

Johbu Itoh, Yoshiko Itoh, Robert Y. Osamura

Tokai University School of Medicine, Isehara, Japan

Contact: itohji@is.icc.u-tokai.ac.jp

Quantum dots (Qds) are new nanocrystal semiconductor fluorophores consisting of a cadmium selenide core and zinc sulfide or cadmium sulfide shell. They have many advantages over conventional fluorophores including prolonged signal due to photostability, and reinforcement of weak positive reactions. In this study we have used Qds to intensify fluorescent signals in multiple staining for immunohistochemistry. In addition, we introduce a new confocal laser scanning microscopy (CLSM; LSM 510 and /or LSM710) analysis method in which a dye spectrum "META analysis system (Carl Zeiss, Germany)" is utilized. This system ensures optimum specimen illumination and efficient collection of reflected or emitted light and uses an innovative way of separating fluorescent emissions. Evaluation of subcellular structures and its functions by light or fluorescent microscopy is important in many fields including bio-imaging. In addition immunocyto and /or histochemistry, and enzyme histochemistry are key methods used to localize particular substances in subcellular organelles. The immunofluorescent antibody method is the common technique for the detection of antigens and proteins in cells and tissues. However, conventional fluorophores fade and production of a permanent specimen is difficult. In addition, fluorescence-dependent imaging is limited in spatial resolution by the wavelength of light, and is generally not suitable for electron microscopy. We have demonstrated that Qd probes have several advantages over conventional fluorophores, and in addition have shown the usefulness of combining Qds probes with CLSM-META. We have generated single fluorescent emission wavelengths from Qd probes using multiple excitation lasers, and detected multiple Qd signals using a signal excitation source. In addition, weak signals produced by conventional immunofluorescence and/or enzyme-labeled antibody methods have been significantly enhanced using Qd labeling, and comparable signals from Qds in the same specimen area have been detected in both transmittance and META system. The wide application of the fluorescent antibody method is now expanded to include clear detection of conventionally weak signals. We have also succeeded in electron microscopic (EM) observation of an immunofluorescence antibody reaction product which is very difficult with standard techniques. In summary, we believe that Qds represent a breakthrough for the fluorescent antibody method, expanding its already very wide application, and have demonstrated the potential of Qds for the observation of fluorescence microscopy, CLSM, and EM. Our results suggest that very weak immunoreactions seen by traditional immunohistochemical techniques can be greatly intensified, a useful feature for histochemistry and cytochemistry.

L008

Sensitive and stable immunohistochemical detection using combined tyramide signal amplification and quantum dots

Kevin Roth

University of Alabama at Birmingham, Birmingham, AL, United States

Contact: karoth@uab.edu

Quantum dots are relatively recently commercially available photostable fluorophores that offer several advantages over typical organic fluorescent dyes. Quantum dots are remarkably photostable, have a narrow emission spectra, and an apparent large Stokes' shift. The emission spectra of quantum dots vary depending on the size of their semiconductor cores, which consist of cadmium selenide coated with a shell of zinc sulfide. An outer polymer layer increases water solubility and enables conjugation of quantum dots to streptavidin or immunoglobulin molecules used for immunohistochemical detection. Despite these potential advantages, we have found quantum dot conjugated secondary antibodies to be much less sensitive than typical organic fluorophore conjugated antibodies in immunohistochemical applications. Thus, to take advantage of the unique properties of quantum dots while overcoming their relative insensitivity, we developed a tyramide signal amplification (TSA) detection procedure utilizing biotin tyramide deposition and quantum dot conjugated streptavidin for sensitive and stable immunohistochemical detection. TSA is an enzyme amplification technique based on the horseradish peroxidase (HRP)-catalyzed depo-

sition of labeled tyramide molecules at sites of HRP-conjugated antibody binding. Tyramide is converted by HRP into a highly reactive oxidized intermediate that binds covalently to cell-associated protein tyrosine molecules at or near the HRP-conjugated antibody. Organic fluorophore-labeled tyramide can be used for "direct" visualization of the deposited tyramide or biotin-labeled tyramide can be deposited followed by fluorophore-conjugated streptavidin for "indirect" visualization of the deposited tyramide. TSA and in particular TSA Plus, an optimized formulation of TSA buffer and enhancing reagents, dramatically increases the sensitivity of fluorescence based immunohistochemical detection. To take advantage of the amplification power of TSA and the improved photostability and unique excitation/emission properties of quantum dots, we developed a combined TSA and quantum dot immunohistochemical procedure. This procedure uses biotin tyramide and quantum dot conjugated streptavidin to achieve optimal immunohistochemical detection. By carefully adjusting reagent concentrations, non-specific signal can be minimized and immunohistochemical detection limits significantly lowered. Multi-label detection can readily be achieved by combining TSA:quantum dot detection with conventional fluorophore conjugated antibodies and/or additional quantum dot conjugated antibodies. In total, the combined use of TSA and quantum dots provides a simple and sensitive alternative to conventional immunofluorescence techniques.

L009

Imaging the cytoskeleton in cell-matrix dynamicsHinke Multhaupt¹, Atsuko Yoneda¹, Eok-Soo Oh², John Couchman¹¹Copenhagen University, Copenhagen, Denmark²Ewha Womens University, Seoul, Korea (South)Contact: hinke.multhaupt@bric.dk

Cell adhesion to the extracellular matrix is a fundamental property of virtually all cells, and the major class of receptors is the integrins. Cell-matrix adhesion is required in development, homeostasis, immune surveillance and tissue repair. Clustering of integrins into adhesive junctions, known as focal adhesions or focal contacts is readily visualised *in vitro* on planar surfaces, but also occurs *in vivo*. Signalling molecules are recruited to these junctional organelles, including some involved with cell survival, a process underlying anchorage dependence. Also present in focal adhesions is one member of the syndecan family of cell surface proteoglycans, which we have described to signal through protein kinase C α to members of the Rho family of G proteins. Biochemical and imaging data, including FRET microscopy has revealed a role for Rho-GDI α in this cascade. In turn RhoA-GTP activates Rho kinases, notably ROCK I to facilitate myosin II function, which regulates actin cytoskeleton activity. Our data, in part from TIRF microscopy, suggest that the two ROCKs are not redundant, but have distinct properties in controlling cell adhesion, migration, macropinocytosis and extracellular matrix assembly. We are now beginning to understand the basis of the distinct activities of the ROCKs, in part due to their distinct inositol phospholipid interactions.

L010

Cryptic translocations demystified: BAC-FISH assays resolve complex karyotypes in failed human reproduction and cancer

Heinz-Ulli Weier

University of California, E.O. Lawrence Berkeley National Laboratory, Berkeley, United States

Contact: ullweier@gmail.com

Bacterial artificial chromosomes (BAC's) are recombinant DNA molecules capable to maintain and propagate large pieces of genomic DNA. Compared to their predecessors, the yeast artificial chromosomes (YAC's), BAC clones show a much lower rate of chimerisms and insert rearrangements. To facilitate the assembly of physical maps of the genomes of interest and to provide DNA sequencing templates, BAC libraries representing multi-fold coverage of a human genome or that of model organisms have been prepared in labs in the USA and Europe. The low rate of chimeric clones suggests the use of BAC's as DNA probes in genetic analyses using fluorescence *in situ* hybridization (FISH). Furthermore, the average sizes of the genomic DNA inserts in many of today's BAC libraries range anywhere from 100-to 250 kb, which is a very reasonable size for a fluorescence *in situ* hybridization (FISH) target. My laboratory has used BAC clones with human DNA inserts as probes in multicolor FISH assays for a variety of appli-

cations ranging from the detection of numerical chromosome aberrations in prenatal and preimplantation genetic analysis to the delineation of structural chromosome abnormalities in tumors of the thyroid gland, breast and prostate or patients enrolled in *in vitro* fertilization (IVF) programs. A rapid delineation of translocation breakpoints is greatly facilitated by the public online access to physical maps based on the assembly of BAC insert DNA (end-)sequences. Novel assays targeting the use of BAC libraries in *in situ* screening assays for a priori unknown translocations will be presented. This work was supported in part by NIH grants CA88258, CA132815 and HD44313 (to HUGW), and a grant from the Director, Office of Energy Research, Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC-03-76SF00098.

L011

Updated non-radioactive *in situ* hybridization in cell biology and physiology

Takehiko Koji

Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Contact: tkoji@nagasaki-u.ac.jp

For a better understanding of the regulatory mechanism of cell-type specific gene expression, it is essential to localize specific gene transcripts at an individual cell level by *in situ* hybridization (ISH). Our attempts over these 20 years, including the introduction of rRNA as a reference for hybridizable RNA, significantly improved the protocol of non-radioactive ISH. In fact, the improvement allowed us to study the cytoplasmic distribution of mRNAs in detail. However, our understanding of nuclear events underlying the regulation of gene expression was only limited. As a model system to analyze the nuclear events, we attempted to localize 18S rRNA, 28S rRNA and "intron" parts of pre-rRNA in the nucleoli of mouse testicular cells. The nucleolus of mouse Sertoli cell has a very unique structure with central and satellite domains, and we can analyze the nucleoli of germ cells undergoing meiotic prophase. As probes, we synthesized oligodeoxynucleotides (oligo-DNA) complementary to a specific segment of pre-rRNA. After haptenization with thymine-thymine dimer or digoxigenin, the oligo-DNA probes were hybridized *in situ* and the signals were visualized immunohistochemically. When we localized 18S and 28S rRNAs simultaneously in paraffin sections of mouse testis at light-microscopic (LM) level, unexpected heterogenous distribution of those rRNAs in Sertoli cell nucleolus was found. Moreover, the predominant expression of 28S rRNA was detected in the nucleoli of meiotic prophase. However, the resolution at LM level was not high enough to discriminate the distribution of each rRNA precisely in the nucleolus. Thus, we conducted post-embedding electron- microscopic ISH (EM-ISH) to clarify the distribution of various parts of pre-rRNA at EM level. In our post-embedding method, the tissue was fixed in paraformaldehyde and embedded in LR-white and after hybridization with oligo-DNA probes the signals were detected with different-sized colloidal gold. Here it should be noted that the use of nucleotide mixture in place of formamide to reduce T_m is very effective to keep morphological details. As a result, we found a big difference in the distribution of 18S and 28S rRNAs between both domains of Sertoli cell nucleolus, and confirmed the predominant expression of 28S rRNA in the nucleoli of meiotic spermatocytes. Interestingly, the "intron" part of pre-rRNA was located more randomly than that of rRNA parts in nucleolar and extranucleolar area. These results indicate that the distribution of each part of pre-rRNA seems to be sequence-dependent, and that 28S rRNA part may play an important role in the assembly-disassembly process of nucleolus during cell division. Finally, these recent advances in ISH at EM level should contribute to accelerate our understanding of the regulation of specific gene expression in individual cells.

L012

Cytochemical studies of signaling pathways linking nutritional status to fertility

Gwen Childs, Noor Akhter, Zachary Brodrick, Anessa Haney

Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, United States

Contact: childsgwen@uams.edu

Leptin is a hormone produced by fat that plays a key role in regulating energy intake and expenditure. Leptin levels rise to a peak at mid gestation in the human and during postnatal development in the rodent. This rise pre-

cedes the proliferation of somatotropes. This study tested the hypothesis that the postnatal rise in leptin in mice promotes somatotrope development. To test this hypothesis, we crossed mice bearing Cre-recombinase driven by the rGH promoter with mice bearing floxed alleles of either exon 17 or exon 1 of leptin receptor to truncate or ablate Ob-R in pituitary somatotropes and prevent them from receiving leptin signals. Organ genotyping showed that there was no extra pituitary expression of Cre-recombinase. Cytochemical studies of freshly dispersed pituitary cells from deletion mutants showed a significant decrease in % of cells immunolabeled for Ob-R from $38 \pm 1\%$ to $20 \pm 2\%$ (one deleted allele) or $10 \pm 1\%$ (two deleted alleles). Dual immunolabeling for cells with Ob-Rb and growth hormone (GH) showed a dramatic decrease from $16 \pm 2\%$ to $1 \pm 0.3\%$ of the pituitary population in the deletion mutants. There was a significant 40-60% decline in somatotropes from $26 \pm 1\%$ of the population to 10-14%. Studies of growth and pubertal development showed that mice with one or two deleted alleles of Ob-R exon 17 were smaller than normal at 21 days, but grew normally after that. However, some of the mice bearing just one deleted allele of Ob-R exon 1 are 20-40% smaller than their littermates, which correlates with the severity of the exon 1 deletion. With respect to fertility, mice bearing the exon 17 ablation in somatotropes went through puberty normally, however adult ovaries showed significantly lower numbers of corpora lutea/ovary from 8-9 in littermate controls to 2-3 in the deletion mutants. This suggests that F2 generation deletion mutants may be infertile, or have reduced litter numbers. Most female mice in which one allele of exon 1 is deleted are infertile; the males appear to be fertile. However, the litter numbers are abnormally small and no pups are born that bear two deleted alleles of Ob-R exon 1, suggesting that this mutation may be lethal. These studies demonstrate the importance of leptin to the early development of somatotropes, which are critical for normal growth and pubertal development. Humans lacking leptin receptors are infertile and have delayed growth. Thus, these studies suggest that, early in development, leptin may stimulate expansion of somatotropes to levels needed to support growth and maturation. Truncation of the leptin receptor (deleting Ob-R exon 17) on somatotropes, reduces its ability to signal, but is compatible with life. However, deletion of exon 1, a true deletion mutant removes a critical link between leptin and somatotropes needed for survival.

L013

Laser capture microdissection: a contemporary tool for molecular pathology

James L. Wittliff, Sarah A. Andres, D. Alan Kerr II

Department of Biochemistry & Molecular Biology, University of Louisville, Louisville, United States

Contact: jim.wittliff@louisville.edu

A goal of molecular pathology is to assess the molecular basis of clinical behavior and therapeutic response of human disease. To circumvent cellular heterogeneity and evaluate genomic and proteomic differences of normal and diseased cells, Laser Capture Microdissection (LCM) was employed to isolate distinct cell types non-disruptively, allowing independent extraction of DNA, RNA or protein for analyses under stringent conditions (e.g. RNase-free & protease-free). Using breast carcinoma tissue specimens from an IRB-approved BioRepository, RNA from 1000-5000 LCM-procured cells and intact sections of frozen biopsies was extracted, purified and quantified by qPCR using β -actin for relative quantification. RNA was amplified, labeled and hybridized for microarray analyses (Agilent & Affymetrix). Spectrophotometric and Bio-AnalyzerTM analyses evaluated aRNA yield, purity and transcript length. Primer sets were created for qPCR (β -actin 100 base, 200 base, 300 base & 400 base amplicons) to determine gene expression profiles of formalin-fixed, paraffin-embedded (FFPE) biopsies. FFPE-extracted transcripts entailed amplification (ParadiseTM kit, Arcturus) and use of X3P chips (Affymetrix) that required ~200 base intact sequences on the 3' end of aRNA. Unsupervised and supervised methods selected 7000 genes with significant variation. Expression profiles of pure cancer cells were dominated by genes associated with estrogen receptor- α (ER) status; over 3000 genes were identified as differentially expressed between ER+ and ER- cancer types. Other prominent expression patterns of 100 genes divided ER+ carcinomas into two subgroups, which were associated with significantly different clinical outcomes ($p < 0.01$). Using supervised learning methods, we also identified a 141 gene expression signature strongly associated with breast cancer recurrence. From reiterative analyses of various microarray studies, 14 genes appearing in LCM-procured breast carcinoma cells and 18 genes predicted in adjacent stromal cells were identified, whose expression appears related to clinical outcome. An additional 11 genes were identified in two ER+ groups of breast carcinomas exhibiting different survival probabilities. In summary, several cell-specific gene expression

signatures were highly associated with clinical outcome suggesting promise for development of novel diagnostic and prognostic tests for cancer management. LCM of specific cancer cell types also aids in the identification of new molecular targets for drug design and development. Supported in part by grants from Phi Beta Psi Charity Trust, University of Louisville's Office of Technology Transfer and from Arcturus Applied Genomics. SAA & DAK II are recipients of IPIBS Fellowships.

L014

Application of laser assisted microdissection and liquid-based cytology for molecular genetic analysis on surgical specimens

Norimichi Nemoto¹, Yokho Nakanishi², Toshimi Seki³, Fumi Fuchino¹, Eriko Murakami¹, Noriko Watanabe¹, Toshiyuki Ishige¹, Kentarou Kikuchi¹, Keishin Sunagawa⁴, Sumie Ohni¹

¹Department of Pathology, Nihon University School of Medicine and Pathology Division, Nihon University Itabashi Hospital, Tokyo, Japan

²Department of Pathology, Nihon University School of Medicine, Tokyo, Japan

³Pathology Division, Nihon University, Itabashi Hospital, Tokyo, Japan

⁴Department of Pathology, Nihon University School of Medicine, Tokyo, Japan

Contact: nemo@med.nihon-u.ac.jp

In surgical practice, molecular genetic analysis often provides more precise diagnostic evidence in some tumor groups. Despite the usefulness of molecular analysis, reliable sampling of surgical specimens, including both tissue and cell samples is mandatory. For this purpose, laser-assisted microdissection (LMD) is a useful and reliable technique for preparing pure target cell samples from heterogeneous cell populations. Because of its easy handling and sophisticated soft wear, this technique has been widely accepted in pathology laboratories. In addition, liquid-based cytology (LBC), a method of cell preparation originally developed for cervical smears, has been extended into non-gynecologic cytology fields. In this study, we show genetic molecular analysis of non-gynecologic LBC preparations using the LMD technique. Material and method: Urine samples and breast tumor scrapings were processed for LBC. The cell samples were immersed in ThinPrep preservative solution for 1 day to up to 3 months at room temperature. Then thin cell smears were made using ThinPrep 2000 autocytopreparation system (Olympus Co.), routinely fixed in 95% ethanol and stained with toluidine blue or Giemsa stain. Target pure cell samples were obtained by LMD (PALM-EasyBeam, Carl Zeiss). The microdissected cell samples were processed for molecular genetic analysis of cytokeratins (CK) 7 and 20, MMP-2, MMP-9, MT1-MMP and HER2. mRNAs in the samples were quantitatively measured by real time PCR. Results and discussion: In molecular genetic analyses, all the mRNAs of CKs, MMPs and HER2 were satisfactorily detected in cell samples (approximately 50 to 100 cells). In urine samples from the patients with urothelial carcinoma, higher expression of CK 20 mRNA was detected in high grade urothelial carcinomas than in low grade tumors. In breast carcinomas, the expression of HER2 mRNA was well correlated with the HER2 status demonstrated by immunohistochemistry. Although the expression of MMP mRNAs was also detected in some breast and urothelial carcinomas, the clinical significance between the expression and biological behavior was not clearly demonstrated in the specimens examined. We also determined influence of long-term storage in the ThinPrep preservative solution on cell morphology and results of molecular analysis. We obtained a good enough expression of mRNAs of CKs, MMPs and HER2 in cell samples which were stored in the preservatives for up to 3 months, though the morphology showed cell shrinkage and nuclear pyknosis. Conclusion: The combination of LBC and LMD techniques would be a powerful and useful tool for both the cytological evaluation and molecular genetic analysis of non-gynecologic cytology specimens.

L015

Ancillary techniques in the diagnosis and prognosis of bladder cancer

Donna Hansel

The Cleveland Clinic, Cleveland, United States

Contact: hanseld@ccf.org

The diagnosis of bladder cancer encompasses a broad spectrum of cancer subtypes, including urothelial carcinoma, squamous cell carcinoma, adenocarcinoma and small cell carcinoma. Although most of these entities may be readily distinguished on the basis of light microscopy, the use of immu-

nohistochemistry is critical in the evaluation of a subset of pre-invasive and invasive lesions and in the distinction between primary and metastatic carcinoma involving the bladder. Urothelial carcinoma, the most common form of invasive bladder cancer, commonly demonstrates immunoreactivity for high-molecular weight cytokeratin, CK7 and p63, which is especially useful in the distinction from invasive prostatic adenocarcinoma. p53 and p21, both well-characterized tumor-suppressor genes, have also been studied in this tumor type as predictors of disease recurrence and survival. The diagnosis of pre-invasive high-grade urothelial lesions, such as flat urothelial carcinoma *in situ*, is often challenging and examination of patterns of p53, CK20 and Ki67 expression have proven useful in the diagnosis of these entities. Bladder adenocarcinoma, which must be distinguished from metastatic spread of an adenocarcinoma from another location, requires analysis of CK7, CK20, CDX-2 and villin-1, among other markers. Small cell carcinoma of the bladder shares molecular features similar to small cell carcinoma at other anatomic sites, including immunoreactivity for synaptophysin, chromogranin and CD56. Squamous cell carcinoma shows immunoreactivity for CK5/6 and p63, and ancillary techniques are useful in instances of uncommon morphologic variants of this tumor type. Recent work has also identified a potential new marker, smoothelin, to aid in the distinction between muscularis mucosa and muscularis propria of the bladder wall and thereby allow an accurate assessment of depth of tumor invasion in challenging cases. Gene amplification studies for HER2, EGFR, MDM2 and TP53 have been recently reported and may be beneficial in determining prognosis in a subset of bladder cancers. Despite this progress, future studies to identify additional diagnostic and prognostic markers are needed.

L016

Development of peritoneal dissemination of ovarian clear cell adenocarcinoma can vary with sialidase expression

Hiroyuki Nomura¹, Fumio Kataoka¹, Hiroshi Tsuda¹, Yutaka Tamada², Atsushi Suzuki¹, Nobuyuki Susumu¹, Daisuke Aoki¹

¹Department of Obstetrics and Gynecology, School of Medicine, Keio University, Tokyo, Japan

²Kyosai Tachikawa Hospital, Tokyo, Japan

Contact: hnomura@se.ite.keio.ac.jp

Objective: Expression of sialic acid, a carbohydrate antigen, changes as cells become cancerous, contributing to the invasive and metastatic potential of cancer cells. Sialidase, an enzyme that releases sialic acid from carbohydrates, can be involved in this process. Four different types of sialidases (NEU1, NEU2, NEU3, and NEU4) have been identified in humans, however, it is unclear whether they play a role in ovarian cancer pathology. In the present study, the role of sialidase expression in the clinicopathological features of ovarian clear cell adenocarcinoma and its effect on development of peritoneal dissemination were investigated. Materials and methods: (1) To investigate expression of endogenous sialidases derived from ovarian cancer cells, mRNA from 18 different cell lines derived from human ovarian cancer tissues was analyzed using RT-PCR. (2) Expression of sialidases was assessed using monoclonal antibody-based immunohistochemical staining of surgical specimens obtained from 71 patients with ovarian clear cell adenocarcinoma that underwent initial surgery at this hospital. Relationship between expression of sialidases and clinicopathological features in these specimens was also analyzed. (3) Cell lines were constructed that overexpressed sialylated MUC 1 by transfecting ES-2 cells derived from ovarian clear cell adenocarcinoma with cDNA encoding MUC1 containing 42 tandem repeats. These cells were named ES-2/T-42. Control ES-2/mock cells were transfected with only expression vector. These cells were then transplanted into the peritoneal cavity of nude mice to construct mouse models of peritoneal dissemination. Mice were assigned to receive either concomitant sialidase (derived from *Clostridium perfringens*) or 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (DANA, a sialidase inhibitor) at the time of cell transplantation. Differences in the development of peritoneal dissemination and survival time were then evaluated. Results: (1) NEU1 was found to be expressed in all cell lines, while NEU2 and NEU4 were not detected in most cell lines examined. NEU3 was demonstrated to be expressed in all cell lines derived from clear cell adenocarcinoma. (2) The positive rate for NEU3 was 77.5 % in clear cell adenocarcinoma tissues obtained from surgical specimens. Amount of NEU3 expression correlated only with T factors in the pTNM classification, with the rate for stage T3 significantly higher in NEU3-positive cases. (3) In mouse models, peritoneal dissemination was more extensive and survival time shorter in mice that received concomitant sialidase than in the control

group. In contrast, in mice that received concomitant DANA peritoneal dissemination was inhibited and survival time extended ($p < 0.05$). Discussion: Expression of endogenous sialidases was confirmed in ovarian clear cell adenocarcinoma, suggesting that endogenous sialidases may promote development of peritoneal dissemination.

L017

Molecular analysis of lung carcinogenesis with new technical tools

Luis M. Montuenga¹, Isabel Zudaire², Maria J. Pajares³, David Blanco³, Alberto Orta³, Ignacio Fernandez-Garcia⁴, Thomas Pengo⁴, Arrate Munoz⁴, Carlos Ortiz de Solorzano⁴, Ruben Pio⁵

¹Laboratory of Biomarkers. Oncology Division. Center of Applied Medical Research (CIMA) and Department of Histology and Pathology. University of Navarra, Pamplona, Spain

²Laboratory of Biomarkers. Oncology Division. Center of Applied Medical Research (CIMA) and Department of Genetics. University of Navarra, Pamplona, Spain

³Laboratory of Biomarkers. Oncology Division. Center of Applied Medical Research (CIMA). University of Navarra, Pamplona, Spain

⁴Laboratory of Cancer Imaging. Oncology Division. Center of Applied Medical Research (CIMA). University of Navarra, Pamplona, Spain

⁵Laboratory of Biomarkers. Oncology Division. Center of Applied Medical Research (CIMA) and Department of Biochemistry. University of Navarra, Pamplona, Spain

Contact: lmontuenga@unav.es

A decrease in lung cancer mortality will only be possible if tobacco consumption habits are reduced and if early detection techniques and new molecular-targeted therapies are developed. We will review recent progress in the field of biomarkers for early detection of lung cancer and will summarize our work on the analysis of the molecular profile of lung carcinogenesis using a novel technology. Molecular studies show that lung cancer develops in a stepwise process through multiple genetic and epigenetic abnormalities. In recent years, the molecular cytogenetic analysis is becoming an important tool to identify chromosomal alterations as well as oncogenes and tumor suppressor genes involved in cancer pathogenesis. The search for new chromosomal regions associated with lung carcinogenesis is still open, as higher resolution platforms to search for better defined regions are available. 500k SNP arrays are now used to find recurrent chromosomal aberrations, namely copy number alterations or LOH regions in lung cancer cells. Once cancer-associated regions are defined, sensitive and specific detection techniques need to be developed and adapted to the biological fluids and small biopsies obtained from high risk individuals or suspicious patients. We have shown the usefulness of the Multicolor-FICTION technique (*Fluorescence Immunophenotyping and Interphase Cytogenetics as a tool for the Investigation of Neoplasms*) in the analysis of fine needle aspiration, sputum or bronchioalveolar lavage (BAL) specimens from lung cancer patients. M-FICTION technique allows the simultaneous detection of immunophenotypic markers and genetic aberrations. Our group is developing tools for the automation of this analysis. We have also used FICTION to study the biology of telomere elongation in lung cancer cells. To date, significant methodological problems have impeded precise *in situ* examination of telomere maintenance in both normal and cancer cells. We have developed a quantitative image-based *in situ* analysis method that provides simultaneous information about telomere length and nuclear proteins' expression in intact cells. Finally, we are exploring the relevance of the alteration of the alternative splicing process in lung carcinogenesis. We will describe a new tool for the high-throughput analysis of alternative splicing. We have designed and developed a whole-genome splicing-specific microarray to search for differentially expressed splicing isoforms in lung cancer.

L018

Genetic background of undifferentiated thyroid carcinomas

Ryohei Katoh, Tetsuo Kondo, Tadao Nakazawa, Nakamura Nobuki, Tomonori Kawasaki, Tetsu Yamane, Kunio Mochizuki

Department of Pathology, University of Yamanashi, Yamanashi, Japan

Contact: rkatoh@yamanashi.ac.jp

Undifferentiated carcinoma (UC) of the thyroid is a highly malignant tumor that occurs in the elderly, and leads to a mean survival of 6 months after diagnosis. Evidence indicates that an UC arises from the transformation (de-differentiation) of a pre-existing more differentiated carcinoma. We analyzed *RET* rearrangements (*RET/PTC1* and *RET/PTC3*) and *BRAF* mutation in 7 undifferentiated carcinomas having PC components (compo-

site carcinomas) in conjunction with 42 carcinomas showing a single histology (28 PCs and 14 UCs). *RET/PTC* may not be in association with anaplastic transformation from PC. The higher positive rate of *BRAF* mutation in UC components in composite carcinomas may support the hypothesis that *BRAF* mutation plays an important role in anaplastic transformation from PC. Chromosome territories (CTs) are intranuclear subregions occupied by individual chromosomes in an interphase cell. We investigated intranuclear CT positionings of chromosomes 10 (CS10), 18 (CS18), and 19 (CS19) in undifferentiated carcinomas (UCs) using the multicolor fluorescence *in situ* hybridization method. UCs with cells having DNA amplification demonstrated the locational abnormalities of the CS10, CS18, and CS19 radial positions. These findings indicate that alterations of CT positioning could be related to DNA amplification and, morphologically, may explain the nuclear atypia that accompanies the abnormal chromatin feature.

L019

Phospho-beta-catenin and microtubule reorganization in polarized cell migration

Michinari Hamaguchi

Nagoya University School of Medicine, Nagoya, Japan

Contact: mhamagu@med.nagoya-u.ac.jp

β -catenin is a biologically important molecule which plays critical roles in cell adhesion and transcriptional regulation. In addition to these important roles, we found recently that β -catenin played a critical role in microtubule (MT) anchorage and regrowth at centrosome (Huang et al., *Oncogene* 26: 4357-4371, 2007). Given these findings, we examined the role of β -catenin in polarized cell migration. We found that β -catenin was accumulated at the leading edge of the first row migrating cells in scratch-induced cell migration assay. Depletion of β -catenin repressed polarized reorganization of MT, leading to the dysfunction of cell polarized migration, which indicate that β -catenin is required for cell polarity. Furthermore, phospho- β -catenin distributed at the leading edge of the migrating cells and co-localized with reorganized MTs array. A nonphosphorylatable mutant β -catenin, S33A β -catenin blocked the reorganization of MTs and inhibited polarized migration. In contrast, a phospho-mimicking mutant β -catenin, S33/37T41E β -catenin, was accumulated at the leading edge and does not perturb, but rather enhance the polarized reorganization of MT. By microinjection of anti-phospho- β -catenin antibody, but not control antibody, leading-edge cells also lost their polarized reorganization of MTs. Collectively, these data suggest that phosphorylation of β -catenin is involved in cell polarization induced by scratching. Moreover, we found that β -catenin was required not only for the polarized localization of APC and EB1 but also for the reorientation of Golgi and centrosome during scratch-induced cell migration. Taken together, our results demonstrate a pivotal role of β -catenin in cell polarity mediated by MT reorganization.

L020

Evaluation of mitochondrial disorders

Giovanni Meola

University of Milan, Milan, Italy

Contact: giovanni.meola@unimi.it

Mitochondrial diseases are clinically heterogeneous disorders caused by defects of the respiratory chain enzymes, that are the products of two genomes and utilize electrons to generate adenosine triphosphosphate (ATP). In this lecture, I will review several aspects of mitochondrial diseases including: clinical features, pathogenic mechanisms and conundrums, and diagnostic testing. Mitochondrial diseases are often difficult to diagnose because they are phenotypically complex and diverse. Although mitochondrial disorders are typically multisystemic, most often, they affect brain and skeletal muscle and are therefore called "mitochondrial encephalomyopathies". The selective vulnerability of these tissues is likely to be due to their high-energy requirements. Endocrinopathies and cardiopathies are also common in mitochondrial diseases. Within the last decade, we have become aware that mitochondrial patients often have gastrointestinal, hematologic, renal and psychiatric manifestations. Mitochondria are unique mammalian organelles because they possess their own genetic material, mitochondrial DNA (mtDNA) which is a small (16,569 base-pair) circular molecule. Each mtDNA encodes 22 transfer RNAs (tRNA), 13 polypeptides, and two ribosomal RNAs (rRNA). Thus, mitochondria are the products

of two genomes and defects in either genome can cause mitochondrial dysfunction. To date, most of the respiratory chain defects that have been characterized at the molecular genetic level are due to mtDNA mutations, however, the number of identified nuclear DNA mutations is growing rapidly. Among the many syndromes caused by mtDNA mutations, six multi-system disorders that occur frequently are: Kearns-Sayre syndrome (KSS); mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS); myoclonus epilepsy ragged-red fibers (MERRF); neuropathy, ataxia, retinitis pigmentosa (NARP)/maternally inherited Leigh syndrome (MILS), and Leber hereditary optic neuropathy (LHON). While the identification of mtDNA mutations has simplified diagnosis in most cases of mitochondrial encephalomyopathies, it has created new dilemmas. Genetic counseling of patients and their maternal relatives is difficult because heteroplasmy and variability in the tissue distribution of mtDNA point mutations make clinical outcome predictions tenuous. However, in the case of single mtDNA deletions, one can reassure female patients that the risk of transmitting the mutation is low (about 4%). Prenatal diagnosis is also perilous; however, prenatal analyses of chorionic villi and amniocytes for the T8993G mutation have been used successfully to estimate heteroplasmy in fetuses. The molecular genetic information must be handled carefully as it can adversely affect medical insurability, employment opportunities, and the emotional status of patients.

L021

Histochemical and immunohistological approach to neuromuscular disorders

Orlando Paciello

Department of Pathology and Animal Health, University of Naples Federico II, Naples, Italy

Contact: paciello@unina.it

The broad category of neuromuscular disorders covers conditions that involve weakness or wasting of the body muscles. The diagnosis of most neuromuscular disorders rest on clinical evaluation, electromyography, muscle biopsy and in some instances molecular genetic studies. Muscle biopsy, associated to histochemical and immunohistological techniques, plays a key role in diagnosis of many neuromuscular disorders. A number of morphological abnormalities of muscle can be recognized on histological stains such as haematoxylin and eosin and Engel trichrome. This last stain achieved by W King Engel (1963) is important in the identification of red staining structures such as rods, membranous myelin-like whorls of rimmed vacuoles and abnormal proliferation of mitochondria (called "Ragged Red Fibers"). Histochemical techniques are essential for the study of muscle biopsies for four main reasons. First, they demonstrate the non-uniform nature of the muscle highlighting the different biochemical properties of specific fibre type and their selective involvement in certain disease processes. Second, they may show an absence of a particular enzyme. Third, an excess of a particular substrate can be demonstrated. Fourth, they may show structural changes in the muscle which would not be apparent with routine histological stains, such as the enzyme-deficient cores in central core disease "mouth-eaten" fibers, and abnormalities in the distribution of mitochondria. During the past two decades many neuromuscular disorders have been identified due to defects of sarcolemmal, sarcomeric, and nuclear proteins, allowing a more specific and firm diagnosis of muscle diseases. Identification of protein defects relies predominantly on immunohistochemical preparations and on Western blot analysis. While immunohistochemistry is very useful in identifying abnormal expression of primary protein abnormalities in recessive conditions, it is less helpful in detecting primary defects in dominantly inherited disorders. Abnormal immunohistochemical expression patterns can be confirmed by Western blot analysis which may also be informative in dominant disorders, although its role has yet to be established. Besides identification of specific protein defects, immunohistochemistry is also helpful in the differentiation of inflammatory myopathies by subtyping cellular infiltrates and demonstrating up-regulation of subtle immunological parameters. Initial studies of histochemical and immunohistological techniques were established in muscle of human and animals. In recent years there has been a rapid growth of veterinary myology in parallel with human myology. New discoveries of these diseases in animals may have direct application to similar human diseases. While the value of rodent models is well established, spontaneously occurring neuromuscular diseases in animals may provide the much needed bridge between the experimental laboratory and human patients.

L022

Mitochondrial pathogenesis of collagen VI myopathies: from the animal model to the clinical trial

Nadir M. Maraldi¹, Paolo Bernardi², Paolo Bonaldo³, Luciano Merlini⁴

¹Laboratory of Cell Biology, Institute Rizzoli and Department of Human Anatomical Sciences, University of Bologna, Bologna, Italy

²Department of Biomedical Sciences, University of Padova, Padova, Italy

³Department of Histology, Microbiology and Medical Biotechnologies, University of Padova, Padova, Italy

⁴Department of Experimental and Diagnostic Medicine, Section of Medical Genetics, University of Ferrara, Ferrara, Italy

Contact: maraldi@area.bo.cnr.it

Collagen VI is an ECM protein which forms a prominent microfibrillar network in the endomysium of skeletal muscle. Mutations in the genes coding for the three chains of collagen VI cause skeletal muscle diseases: the severe wasting Ullrich congenital muscular dystrophy (UCMD) and the milder Bethlem myopathy (BM). Our group, utilizing *Col6a1* deficient mice, discovered a latent mitochondrial dysfunction that caused increased apoptosis in muscle cells. These effects could be reversed by incubating *Col6a1* null muscle fibers with cyclosporin A (CsA), an inhibitor of the mitochondrial permeability pore; more interestingly, the treatment of *Col6a1* null mice with CoA rescued the muscle phenotype *in vivo* (1). These findings demonstrated an unexpected collagen VI/mitochondrial connection at the basis of the UCMD and BM pathogenesis and suggested a strategy for a possible pharmacological treatment of the diseases. This was assessed by demonstrating that muscle biopsies from patients with UCMD showed abnormal mitochondrial depolarization and that treatment with CsA normalized the mitochondrial phenotype (2). In this study we report the results of an open pilot trial of four UCMD and one BM patients, representing a range of collagen VI deficiencies and having mutations in three of the collagen VI genes. As determined in muscle biopsies prior to treatment, all the patients displayed mitochondrial dysfunction and muscle fibres showed an increased frequency of apoptosis. When patients were treated for one month with a low daily dose of CsA, primary muscle cell cultures of biopsies obtained at the end of the treatment showed decreased apoptosis and increased immunohistochemical signs of muscle fibre regeneration (3). These results confirm that the pathogenic mechanism found in *Col6a1* deficient mice also plays a crucial role in hereditary muscle diseases in humans, and suggest that targeted treatment of these mitochondrial defects in patients with UCMD and BM may be effective in preventing and/or reversing muscle alterations. Because a CsA derivative that has no immunosuppressive activity results to be as effective as the parent molecule, long-term treatment should be used in young patients without exposing them to infective risks. References: 1. Irwin WA, et al. (2003) Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. *Nat Genet* 35:267-71. 2. Angelin A, et al. (2007) Mitochondrial dysfunction in the pathogenesis of Ullrich congenital muscular dystrophy and prospective therapy with cyclosporins. *Proc Natl Acad Sci USA* 104:991-6. 3. Merlini L, et al. (2008) Cyclosporin A corrects mitochondrial dysfunction and muscle apoptosis in patients with collagen VI myopathies. *Proc Natl Acad Sci USA* 105:5225-9.

L023

Evaluation of sarcoglycans in muscular diseases of ureter: an immunohistochemical study

F. Trimarchi¹, G. Santoro¹, A. Consolo¹, A. Duca¹, A. Micali¹, A. Pisani¹, S. Arena², D. Puzzolo¹

¹Dipartimento di Biomorfologia e Biotecnologie, Università di Messina, Messina, Italy

²Dipartimento di Chirurgia Pediatrica, Università di Catania, Catania, Italy

Contact: fatrim@unime.it

Interaction of the dystroglycan-complex (DGC) with components of the extracellular matrix may have an important role in force transmission and sarcolemma protection and the sarcoglycan (SG) complex is an essential component of the muscle membrane-located DGC. Therefore we analyzed the expression of sarcoglycans in two pathological ureters with structural lesions; in particular we studied an ureter affected by alteration of the active valve of the ureterovesical junction (UVJ), causing vesicoureteral reflux (VUR) and an ureter affected by stenosis of ureterovesical junction, respectively. In the first disease, we analyzed a specimen of ureter with low grade of VUR and a specimen of ureter with high grade of VUR. The immuno-

fluorescence analysis of these ureters showed a significant positive correlation between α -SG immunofluorescence intensity and grade of VUR while a negative correlation between ϵ -SG immunofluorescence intensity and grade of VUR was recorded. Other sarcoglycan showed a normal immunostaining patterns. This observation raise the possibility that the structural deficiency of the trigonal ureterovesical junction could provoke a passive stretching of refluxing urine on the ureter deranging the multi-modular tensegrity architecture of sarcoglycan subcomplex or that the SGs could play a key role in physiopathology of VUR. The analysis of ureter affected by stenosis of ureterovesical junction, showed almost complete absence of all tested sarcoglycans in smooth muscle fibers. Interestingly, it is possible to denote a massive presence of α -SG around the muscle fibers. In our opinion, this condition showed a possible compensative role of sarcoglycans in order to rearrange the pathological condition of muscle fibers and to guarantee a signaling of the fibers with other fibers and with extracellular matrix.

L024

Muscle-specific integrins in masseter muscle fibers of chimpanzees: an immunohistochemical study

A. Favalaro¹, G. Speranza¹, S. Rezza², V. Gatta³, G. Vaccarino¹, L. Stuppia³, F. Festa², G. Anastasi¹

¹Dipartimento di Biomorfologia e Biotecnologie, Università di Messina, Messina, Italy

²Dipartimento di Scienze Odontostomatologiche, Università di Chieti, Chieti, Italy

³Dipartimento di Scienze Biomediche, Università di Chieti, Chieti, Italy

Contact: anapuc@unime.it

Integrins are a family of transmembrane receptors, which participate in vital biological processes such as maintenance of tissue integrity, embryonic development, cell differentiation, and cell-extracellular matrix interactions. Integrins are heterodimers composed of two subunits, α and β ; in particular, the β subunits are responsible for interaction with the cytoskeleton, whereas α subunits participate in the determination of the specificity of the ligand binding and signaling. $\alpha 7B$ and $\beta 1D$ are muscle-specific isoforms. In our recent report, we demonstrated that in human muscle, during muscular inactivity conditions, the $\alpha 7A$ could replace $\alpha 7B$ -integrin (1). Considering that the clustering of $\beta 1D$ stimulated tyrosine phosphorylation of pp125^{FAK}, preventing the apoptosis and accelerating the G1/S phase transition, it would be interesting to study these proteins, comprising isoforms $\alpha 7A$ and $\beta 1A$, not in humans but in subjects that represent different behaviours comparing with humans, as chimpanzees. Chimpanzee aggressive behaviour is quite different within and between groups. As within humans, intergroup aggression knows few inhibitions. Our results, interestingly, showed that in: (a) in masseter muscle fibers of female chimpanzee, as in human muscle, $\alpha 7B$ -, and $\beta 1D$ -integrin staining was normal, whereas $\alpha 7A$ -, and $\beta 1A$ -integrin staining was slightly reduced; (b) in masseter muscle fiber of alpha-male $\alpha 7B$ -, and $\beta 1D$ -integrin staining appeared reduced, whereas $\alpha 7A$ -, and $\beta 1A$ -integrin staining was normal or almost increased. It is possible to hypothesize that $\alpha 7A$ and $\beta 1A$, could be the isoforms that characterized the aggressivity of animal. In fact, these isoforms were detected in differentiating myotube and in myogenic precursor cells, then in cells with a wider differentiative potential; these cells could be differentiated in order to reinforce the masticatory muscles and, consequently, to defend the group. (1) Anastasi G et al. *Eur J Histochem* 50: 327-336; 2006.

L025

Input of some feeding-regulating neurons revealed by a genetically encoded tracer

Haruaki Kageyama¹, Fumiko Takenoya², Seiji Shioda¹

¹Showa University School of Medicine, Tokyo, Japan

²Hoshi University School of Pharmacy and Pharmaceutical Science, Tokyo, Japan

Contact: haruaki@med.showa-u.ac.jp

It is clarified that several neuronal nuclei and many kinds of neurons that regulate energy metabolism exist in the hypothalamus. Recently, several neuropeptides such as orexin, galanin-like peptide (GALP), neuropeptide W/B and ghrelin have been discovered from the hypothalamus or stomach. Accumulated evidence shows that hypothalamic neuropeptides are involved in regulating appetite and balancing of homeostatic regulation. We have observed neuronal interactions between feeding-regulating peptide-containing neurons by means of dual fluorescence immunohistochemistry

and dual immunoelectron microscopy. Our and others data have been clarifying neuronal circuit involving feeding regulation. Recently, the development of genetic engineering has made it possible to express a reporter protein such as beta-galactosidase or green fluorescent protein (GFP) under the control of a cell-specific gene promoter and thus label a specific cell type. I have generated a transgenic mouse which express enhanced green fluorescence protein (EGFP) under the control of promoter of GALP gene. We have observed neuronal relationship between GALP and other peptides containing neurons involved in feeding behavior in the transgenic animals using immunofluorescence techniques. I will present new strategies for analyzing neural circuits involving these feeding-regulating neuropeptides in the brain, with research in this field aided by the use of transgenic mouse models. In this study, we generated transgenic mouse lines expressing a fusion protein between the nontoxic C-terminal fragment of tetanus toxin (TTC) and GFP (TTC::GFP) exclusively in orexin neurons. This fusion protein was selectively retrogradely transferred to interconnected neurons. This approach allowed us to visualize, at single-cell resolution, neurons that send synaptic projections to orexin neurons, by visualizing GFP in interconnected neural circuits. We identified GFP-positive cells in multiple specific brain regions, including the basal forebrain cholinergic neurons, GABAergic neurons in the VLPO, and serotonergic neurons in the median raphe (MnR) and paramedian raphe (PMnR) nucleus. From these results, the transgenic animals could be a powerful tool for morphological analysis of a feeding-regulating peptide-containing neuron.

L026

Hypothalamic control of energy homeostasis: the importance of a balanced autonomic nervous system

Andries Kalsbeek¹, Felix Kreier², Eric Fliers³, Ruud Buijs⁴

¹Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience and Department of Endocrinology and Metabolism, Amsterdam Medical Center, Amsterdam, Netherlands

²Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience, Amsterdam, Netherlands

³Department of Endocrinology and Metabolism, Amsterdam Medical Center, Amsterdam, Netherlands

⁴Instituto de Investigaciones Biomedicas UNAM, Ciudad Universitaria, Mexico City, Mexico

Contact: a.kalsbeek@nin.knaw.nl

The biological clock, located in the hypothalamic suprachiasmatic nuclei (SCN), uses its projections to the neuroendocrine and pre-autonomic hypothalamic neurons to control the daily rhythms in hormone release. We showed that a similar control mechanism also exists for the daily rhythm in plasma glucose concentrations. In this case it is the activity of pre-autonomic PVN neurons dedicated to the control of hepatic glucose production that is restrained during the light period by a GABA-ergic inhibition derived from the SCN. The final daily activity pattern of the pre-autonomic neurons connected to the sympathetic innervation of the liver is determined by a balance of GABA-ergic and glutamatergic SCN outputs. On the other hand, the daily activity pattern of the pre-autonomic neurons connected to the parasympathetic innervation of the pancreas seems to be determined by a daily rhythm in GABA-ergic output from the SCN in conjunction with glutamatergic projections from non-SCN areas. Our most recent results show that the SCN control of hepatic glucose production also involves a GABA-ergic projection to the orexin-containing neurons in the lateral hypothalamus. The daily rhythm in adipose leptin production strongly suggests a direct control of adipose tissue activity by the biological clock as well. Indeed, after injection of a viral tracer in the white adipose tissue (WAT), third order neurons were found in the biological clock, among other places. More recently we were able to show that, contrary to general belief, WAT is innervated not only by the sympathetic, but also by the parasympathetic branch of the autonomic nervous system. Additional tracing studies showed that, within the central nervous system, separate sympathetic and parasympathetic motoneurons are in control of subcutaneous and abdominal WAT compartments. This clear somatotopy in the autonomic control of WAT has resulted in hypotheses about the involvement of the CNS in the largely unexplained effects of sex steroids and glucocorticoids on fat distribution, the pathogenesis of fat redistribution syndromes such as AIDS lipodystrophy, and the aetiology of the metabolic syndrome. In a recent series of euglycemic, hyperinsulinemic clamp experiments combined with the ICV administration of neuropeptide Y (NPY), and either sympathetic-, parasympathetic-, or sham-denerations of the autonomic input to the liver, we investigated the possible hypothalamic pathways involved

in the inhibitory effect of increased plasma insulin levels on hepatic glucose production. Together, these results show a highly differentiated hypothalamic control of hormonal rhythms and energy metabolism through the autonomic nervous system, and provide a neuro-anatomical blueprint for a balanced control of energy homeostasis.

L027

Regulation of hypophysiotropic thyrotropin-releasing hormone and corticotropin-releasing hormone-synthesizing neurons by feeding related signals

Csaba Fekete¹, Ronald M. Lechan²

¹Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

²Division of Endocrinology, Tufts-Medical Center, Boston, United States

Contact: feketecs@koki.hu

Hypophysiotropic TRH and CRH neurons reside in the hypothalamic paraventricular nucleus (PVN) and play a critical role in the adaptation to metabolic challenges. These two, neighboring, neuronal populations are embedded in a network of axons containing feeding-related peptides and regulated by fasting and circulating levels of leptin, as well as a number of other physiological signals. To better understand the mechanisms by which these hypophysiotropic, neuronal populations are regulated by energy availability, the origin of the axonal input to these cells from neurons producing α -MSH, CART, NPY and AGRP was studied. All four feeding-related peptides are contained in axons innervating TRH and CRH neurons, with AGRP- and α -MSH-containing axons originating exclusively from the arcuate nucleus. The NPY innervation to TRH neurons derives primarily from the arcuate nucleus, although ~25% of the axons arise from C1-C3 adrenergic neurons in the brainstem. Noradrenergic brainstem neurons also contribute to the innervation of hypophysiotropic TRH neurons, but do not co-contain NPY. In contrast, only ~40% of the NPY innervation to CRH neurons derive from the arcuate nucleus, with the majority arising from the brainstem, including ~40% from adrenergic neurons and ~20% from noradrenergic neurons. The majority of the CART innervation to TRH and CRH neurons similarly derives from the brainstem, primarily medullary adrenergic neurons, comprising ~44% of the CART innervation of TRH and ~60% of the CART innervation of CRH neurons. The arcuate nucleus is only a minor source of the CART innervation of these cells. These data suggest that feeding-related peptides of arcuate nucleus origin have a key role in the regulation of hypophysiotropic TRH and CRH neurons, but the brainstem catecholaminergic neurons that co-synthesize NPY and CART may also be of importance. Since fasting has no effect on NPY and CART gene expression in medullary adrenergic neurons, it is likely that feeding-related peptides of arcuate nucleus and brainstem origin regulate the TRH and CRH neurons under different physiological conditions.

L028

Neuron network of feeding-regulating neurons in the hypothalamus

Fumiko Takenoya¹, Haruaki Kageyama², Seiji Shioda²

¹Department of Physical Education, Hoshi University School of Pharmacy and Pharmaceutical Science, Tokyo, Japan

²Department of Anatomy, Showa University School of Medicine, Tokyo, Japan

Contact: kuki@hoshi.ac.jp

Feeding regulation forms the hierarchical control mechanism that includes hypothalamus and lower brainstem connected with cerebral cortex, amygdala and other brain centers. It is thought that several hormones, cytokines, and neuropeptides participate in the regulation of feeding. The hypothalamus is the center of the autonomic nervous system in which many neurons are connected with neurons within or outside of the hypothalamus and regulate feeding and energy metabolism. Furthermore, hypothalamus receives chemical signals related to metabolism, such as blood glucose, insulin or leptin, directly, and indirectly, neurogenic peripheral information transmitted to the lower brainstem through the vagus nerve. Moreover, many neurons of the ingestion control system exist in the ventromedial hypothalamic nuclei, lateral hypothalamus and arcuate nucleus, and they form close and complicated neural networks. The feeding promotion or the control signal produced in the hypothalamus are transmitted to the medulla oblongata. The feeding regulatory centers in hypothalamus contain various neuropeptides including NPY and POMC in the arcuate nucleus, and MCH,

orexin, ghrelin, GALP, NPW in other nuclei that are reciprocally connected by dense neuronal networks. Moreover, leptin and ghrelin, which are derived from adipocyte and stomach respectively, pass through the blood-brain barriers and act on the feeding-regulating neurons in the hypothalamus through their specific receptors. Therefore, the appetite-regulating neurons in the hypothalamus receive information of neurogenic and humoral origin. In this symposium, we will show the present status of neuron networks involved in feeding regulation in the hypothalamus based on the morphological and physiological data. Research in this field will serve the important role of clarifying causes for appetite dysfunction, and may help in establishing neurologically-based new therapies for patients who suffer from appetite and feeding disorders.

L029

Histology leads the way; what the *in situ* distribution of the mannose receptor and its ligands tells us about the function of this receptor in homeostasis and immunity

Luisa Martinez-Pomares

University of Nottingham, Nottingham, United Kingdom

Contact: Luisa.Martinez-Pomares@nottingham.ac.uk

Being intrigued not only by what happens but also by where it happens, we have exploited the potential of histological analysis to investigate the role of the Mannose Receptor (MR) in homeostasis and immunity. In this presentation I will provide an overview of how by combining the use of chimaeric proteins containing selected regions of MR and anti-MR monoclonal antibodies, we have uncovered novel aspects of macrophage heterogeneity *in vivo* and generated and tested novel hypotheses regarding the biological function of MR.

L030

Synthetic glycoconjugates as probes for flow cytometry and beyond

Nicolai Bovin

Shemyakin&Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia

Contact: professorbovin@yandex.ru

Cell lectins, especially galectins play important role in cell cross-talk. Their architecture on cell surface is sensitive to changes of internal condition to pathologies. Synthetic fluorescent glycoconjugates allow studying the main function of lectins, recognition of carbohydrate ligands. Glycoprobes allow not only the detection of lectins on cells but also studies of lectins' functional activities on cell surface, including interactions with *cis*- and *trans*-ligands. Probes of the type Glyc-PAA-label are convenient for ELISA, flow cytometry, and microscopy. At the same time, the accumulated experience of cell studies with the help of probes, which had been initially designed for the work with individual proteins, has resulted in valuable experience and general strategies for the use of these tools as cell probes with special focus on less 'noisy' labels, i.e. more hydrophilic ones that produce higher signal than fluorescein. Here we present the experimental techniques and describe probing of T-lymphocytes, monocytes, macrophages and tumor cells with emphasis on cell galectins.

L031

Quality control of glycoprotein folding: a glyco-cytochemical perspective

Jürgen Roth

Division of Cell and Molecular Pathology, University of Zurich, Zürich, Switzerland

Contact: juergen.roth@usz.ch

Quality control of glycoprotein folding is a basic cellular phenomenon. Non-native glycoproteins will be recognized and retained to become eventually dislocated and degraded by a process termed ERAD, for ER-associated degradation. This review will focus on (i) machinery proteins such as glucosylase II and glucosyltransferase involved in recognition and retention of non-native glycoproteins through which the subcellular distribution of quality control check points could be established, and (ii) the ERAD component EDEM1 through which a novel quality control vesicular transport pathway out of the endoplasmic reticulum was detected.

L032

An intrinsic feedback mechanism that modulates peripheral pain in the skin

Joseph E. Mazurkiewicz

Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, United States

Contact: mazurkj@mail.amc.edu

The skin is the interface between the external environment and the internal milieu and is thus continuously exposed to a fluctuating environment with potentially noxious stimuli. As such it plays a critical role in maintaining internal homeostasis. The skin is actually a complex sensory structure that can be altered under various pathological conditions, including chronic pain and drug treatments. The outer most layer of the skin, the epidermis, is comprised mostly of keratinocytes that surround sensory nerve endings. Keratinocytes express many of the same receptors as the central nervous system and release many classic neurotransmitter molecules. Although epidermal sensory nerve endings also express receptors for these neurotransmitters, research has traditionally focused on autocrine signaling between keratinocytes and its effects on epidermal homeostasis and wound healing. Recently, it has been shown that keratinocytes are involved in an endogenous analgesic mechanism through the release of β -endorphin and that the release of endogenous stores of β -endorphin from these cells contributes to the peripheral analgesic effects of endothelin-B (ETB) receptor agonists (Nat Med 9:1055, 2003). Endothelin-1 (ET-1) is a pain mediator that is involved in the pathogenesis of many pain states including trauma. ET-1 is synthesized by keratinocytes in normal skin and is locally released after cutaneous injury. ET-1 can trigger pain through its actions on endothelin-A (ETA) receptors present on local nociceptors and, at the same time, it can produce analgesia through endothelin-B (ETB) receptors present on keratinocytes. An endogenous analgesic circuit in the skin is described in which ETB receptor activation induces the release of β -endorphin from keratinocytes and the activation of G-protein-coupled inwardly rectifying potassium channels (GIRKs, also named Kir-3) linked to opioid receptors on nociceptors. These results indicate the existence of an intrinsic feedback mechanism to control peripheral pain in skin, and establish keratinocytes as an ETB receptor-operated opioid pool. Preclinical and clinical studies have shown the usefulness of ETA receptor antagonists for relieving ET-1-induced pain. A complementary strategy could take advantage of existing intrinsic mechanisms of pain inhibition such as this endogenous analgesic pathway present in the skin and ETB receptors and GIRKs might then become new and useful targets for the local treatment of acute peripheral pain.

L033

Multi-laminar epidermal substrates for sensory transduction and integration: a potential contributor to neuropathic pain

Frank Rice

Albany Medical College, Albany, New York, United States

Contact: ricef@mail.amc.edu

Although mostly regarded as a protective barrier, the epidermis is innervated by numerous sensory "free nerve endings" (FNE) that involved in tactile sensation. We have made extensive use of multiple antibody combinations to characterize the chemistry of the FNEs and keratinocytes in rodents, monkeys and humans to show that FNEs and keratinocytes likely function as a stratified structure for transducing and integrating tactile stimuli. These FNE are supplied by unmyelinated C-fibers and lightly myelinated A δ fibers which may lack or contain neuropeptides. Nonpeptidergic FNE (nFNE) express purinergic receptors (P2X3) and capsaicin/thermal receptors (TrpV1). nFNE extend through stratum basalis (SB), stratum spinosum (SS) and stratum granulosum (SG). Peptidergic FNE (pFNE) coexpress the mu-opioid and extend only through SB and partway into SS. The stratified innervation is paralleled by a stratified distribution of numerous neurochemical markers among the keratinocytes. This includes endothelin-1 receptor B (ETB), cannabinoid receptor 2 (CB2) and β -endorphin in SG keratinocytes that mediate an analgesic mechanism. Voltage-gated Na⁺ channels Na_v1.6 and Na_v1.7 are also normally present in SG and mediate a keratinocyte release of ATP, an algescic. Other receptors implicated in thermal transduction and algescia are also preferentially distributed among the layers of keratinocytes. Taken together, the normal stratified

distribution of epidermal FNEs and of keratinocyte chemistry indicates that different types, intensities and durations of stimuli that impact the epidermis will result in a release of different proportions of excitatory or inhibitory ligands. This will in turn generate different patterns of activity among the epidermal endings resulting a wide variety of cutaneous perceptions up to and including pain. In humans and monkeys with chronic painful neuropathic conditions, our research and that of others have shown that the distribution, morphology and immunochemistry of epidermal FNEs can be profoundly altered. These conditions include complex regional pain syndrome 1 (CRPS1), postherpetic neuralgia (PHN) and diabetic neuropathy. These pathologies can include a loss of epidermal endings coupled with increased branching of remaining endings and increased coexpression of CGRP and TrpV1. These conditions also have an increase in the types, distributions and intensities of Nav expression which may contribute to chronic pain by causing an excess release of ATP in response to cutaneous stimuli that would normally not be painful. Thus, pathological changes in the epidermal chemistry as well as in the sensory endings are consistent with potential mechanisms that may contribute to chronic neuropathic pain.

L034

Neuro-immune interactions in skin and their relevance for cutaneous stress-responses and inflammatory disease in skin

Eva Peters

Charité Center 12, Internal Medicine and Dermatology, Psycho-Neuro-Immunology, University Medicine Charité, Berlin, Germany

Contact: eva.peters@charite.de

The constant remodeling of skin innervation during adult life is an instructive example of ongoing neuronal plasticity in the peripheral nervous system. It occurs during hair cycling, after injury or surprisingly after stress-exposure and affects skin trophism and immunology alike. In order to recognize even subtle changes or abnormalities of cutaneous innervation under different experimental conditions and analyze their functional impact, it is critically important to use a quantitative approach. We employ a fast and reproducible quantitative method based on immunofluorescence histochemistry for the exact quantification of peripheral nerve fibers and their interaction with target cells. With this method, it is possible to screen disease models for involvement of neuron-immune interaction in the course of disease development and aggravation (e.g. allergic dermatitis, hair growth termination, alopecia areata, wound healing). Moreover, the effect of pharmaceuticals becomes readily assessable for therapeutic evaluation and this method can be easily transferred to other densely innervated peripheral organs.

L035

Sensing the environment: Regulation of local and global homeostasis by the skin neuroendocrine system

Andrzej Slominski

Department of Pathology and Laboratory Medicine; University of Tennessee Health Center, Memphis, United States

Contact: aslominski@utmem.edu

Described as the body's largest organ, the skin is strategically located at the interface with the external environment where it has evolved to detect, integrate and respond to a diverse range of stressors/signals including solar radiation. A flurry of recent findings has established the skin as an important peripheral neuro-endocrine organ that is tightly networked to central stress axes. Specifically, the epidermal cells produce and respond to classical stress neurotransmitters, neuropeptides and hormones, and their production is stimulated by UVR, biological factors and other agents (Endocrine Rev 21, 457-487; Physiol Rev 80, 979-1020; Physiol Rev 84, 1155-1228; FASEB J 15, 1678-1693; FASEB J 19, 176-194; Mol Cell Endocrinol 265-266, 143-149; J Clin Invest 117, 3166-3169; TEM 19, 17-24). Examples of potent products include biogenic amines (catecholamines, serotonin, N-acetyl-serotonin and also melatonin), proopiomelanocortin-derived ACTH, β -endorphin or MSH peptides, corticotropin-releasing factor and related urocortins, thyroid-related hormones and corticosteroids as well as secosteroids. Crucially, the production of these molecules is hierarchical, following the algorithms of classical neuroendocrine axes (e.g. hypothalamic-pituitary-adrenal axis, hypothalamic-thyroid axis, serotonergic/melatonergic, catecholaminergic and cholinergic systems). More-

over, dysregulation of these axes may lead to skin diseases. They represent an exquisite regulatory layer addressed at restricting maximally the effect of noxious agents in the skin to preserve local homeostasis. Furthermore, the skin-derived factors can activate cutaneous sensory nerve endings to alert the brain to changes in the epidermal environment, or to activate other coordinating centers by direct spinal cord neurotransmission without brain involvement. Thus, the skin cells can coordinate not only cutaneous but also global homeostasis. Furthermore, the common ectodermal origin of the brain and epidermis raises the question on whether the peripheral neuro-endocrine signaling system is an evolutionary duplication of its central homologue or, whether the intracranial and endocrine organs themselves adopted pre-existing in the integument peripheral response system during the evolution (cf. *J Clin Invest* 117, 3166-3169).

L036

Advances in automated cell identification and classification

J. Paul Robinson

Purdue University, West Lafayette, United States

Contact: jpr@flowcyt.cyto.purdue.edu

The field of cytomics broadly defined as the systematic study of biological organization and behavior at the cellular level has begun to mature and establish itself as an integral component in cell biology. The necessary tools for integration of cytomics into the fundamental nature of cell systems analysis are maturing but new tools are demanded to achieve our goals. While there is a long way to go before we have tools that can perform true cytomics analysis, cytometry is a subset of tools that is tremendously powerful and from which we can extract a significant subset of information about many biological systems. It is important therefore to realize that for major advances to be realized new technologies must be developed for cytomics to become a reality. For example there will be a need for essential development of new sensor technologies that provide both sensitivity and selection in the visible and near IR spectrum. Secondly, a better integration between different measurement and detection tools will be needed. We simply cannot make independent measurements and hope to integrate these tools easily. Thirdly, in order to analyze the complex data sets resulting from new technology integration a major advance is needed to accommodate analysis of these data sets. Fourthly, chemistries must advance to permit greater selectivity of tracking tools. These will most likely expand beyond fluorescence to accommodate enhanced scatter analysis as well as chemical composition. This enhances the opportunities for evaluation of the size, shape and texture of cells, features that enrich automated analysis significantly. Together, these advances place the cytomic opportunity into a new dimension for understanding metabolic responses in single cells and ultimately defining new functional populations of cells. The result will be new research tools as well as a toolset for clinical and diagnostic utility.

L037

Contribution of histochemistry in pituitary development and pathology

Robert Y. Osamura

Department of Pathology, Tokai University School of Medicine, Kanagawa, Japan

Contact: osamura@is.icc.u-tokai.ac.jp

Histochemistry has become more practical in clinical and basic research since immunoperoxidase technology became available at light and electron microscopic levels. Recently, GFP and laser microdissection (LCM) technologies have become popular to detect molecular changes in individual cells. In conjunction with immunohistochemistry (IHC), RT-PCR and real time RT-PCR have been the methods of choice to detect trivial amounts of mRNA in a cell-based manner. Analysis of transgenic (Tg) or knockout (KO) animals also relies on IHC methods to clarify the function of particular genes and proteins. The human pituitary adenomas give rise to various types according to the hormones they produce: GHomas, PRLomas, TSHomas, ACTHomas and FSHomas, defined by IHC. Unlike rodents, human pituitary GH cells also co-express α subunit (SU) and occasionally FSH β subunit. By applying IHC methods it was possible to disclose that the majority of clinical "non-functioning adenomas" are immune-positive for gonadotropin (FSH/LH) subunits. The human anterior pituitary cells develop into GH, PRL, TSH, ACTH, FSH/LH by a combination of transcription

factors (TFs) and co-factors similar as in rodents. The corresponding TFs have been identified by real-time RT-PCR and, in cell-based detection, by IHC. By combining IHC-based LCM and real-time RT-PCR, it was found that occasionally, the human adenomas showed "trans-lineage" differentiation of hormones from the same tumors or even from the same tumor cells. The examples included GHomas with ACTH expression or ACTHomas with GH expression. Our analysis using IHC and RT-PCR combined with LCM disclosed "aberrant" expression of transcription factors, i.e. NeuroD1 and Pit-1. Tg and KO animals serve as good models to study oncogenic mechanisms of pituitary cells. The GH-RH Tg mice and rats develop GHomas which are similar to human adenomas. P27/Rb double KO and D2RKO mice are well known to produce POMC(ACTH) in intermediate lobe-derived tumors and PRL, respectively. We showed that Prop-1 Tg mice (1) produce various types of tumors which express GH and PRL or TSH in the Pit-1 lineage. Pituitary tumor transforming gene (PTTG) is a unique multifunctional factor to promote angiogenesis and proliferation. PTTG Tg mice developed tumors with gonadotropin production. The key investigative issue in this multifaceted factor here is the question whether PTTG should be expressed in the pituitary at a particular time during development from the fetal to adult to promote oncogenesis (2). Summary. (Immunohisto)chemistry has contributed a great deal in the field of pituitary studies, especially its tumors. The role of IHC is now expected to be extended to therapeutic approaches in pituitary lesions. In collaboration with: (1) Dr. Sally Camper, (2) Dr. Shlomo Melmed; (3) Dr. Ricardo Lloyd.

L038

LacZ as an *in vivo* gene reporter for real-time magnetic resonance imaging

Niclas E. Bengtsson, Gary Brown, Glenn A. Walter, Edward W. Scott

Program in Stem Cell Biology and Regenerative Medicine, University of Florida, Gainesville, United States

Contact: escott@ufl.edu

Molecular Resonance (MR) imaging is currently hampered by the lack of genetic reporters for *in vivo* imaging. Iron-based contrast agents for cellular and molecular MR imaging are commonly used due to their high sensitivity. We determined, if the commercially available substrate, 3,4-cyclohexene-oesculetin-B-D-galactopyranoside sodium salt (S-GalTM), could be used to detect genetically engineered β -galactosidase expressing cells by MRI. The effect and specificity of the reaction between β -galactosidase and S-GalTM mixed with ferric ammonium citrate (FAC) on MRI contrast enhancement was determined using purified enzyme, tissue phantoms and *in vivo*. β -galactosidase activity in the presence of S-GalTM/FAC, resulted in dark iron-based staining, indicative of successful cleavage of the substrate, that exhibited reduced T_2^* when compared to controls. Furthermore this effect on T_2^* was amplified with increasing external magnetic field strengths (between 4.7-17.6T). When mice bone marrow cells, expressing β -galactosidase, were incubated with a mixture of S-GalTM/FAC there was no significant negative effect on cellular viability and the cells turned dark, stained positive with Prussian blue, and exhibited decreased T_2^* compared to control cells at 4.7T. The decrease in T_2^* compared to control cells was further enhanced by increased magnetic fields, thereby indicating an even larger advantage of using this method at higher external magnetic field strengths. Upon *in vivo* implantation of S-GalTM/FAC labeled cells into C57Bl6J mice or directly delivering S-GalTM/FAC into B6ROSA26 mice, labeled LacZ expressing cells could be detected as hypointense areas on T_2^* weighted gradient-echo images. Similar to phantom studies the detection capability of labeled cells *in vivo* was enhanced at high magnetic fields. Using this methodology we can now detect genetically driven LacZ reporter expression *in vivo* using high field strength MRI. Key words: Cellular MRI, cell tracking, S-Gal, relaxometry, iron, contrast agent, gene reporter.

L039

Malignant and normal stem cells and detection of clonal hematopoiesis in hematologic disorders

Jaroslav Maciejewski

Taussig Cancer Center/Hematologic Oncology and Blood Disorders, Cleveland Clinic, Cleveland, United States

Contact: brutonm@ccf.org

Clonal hematopoiesis, observed in bone marrow failure syndromes, including some cases of aplastic anemia, may be due to stem cell depletion. Such

oligoclonality may result in recruitment of a preexisting defective hematopoietic clone as, for example, in paroxysmal nocturnal hemoglobinuria (PNH) or possibly in low risk variants of myelodysplastic syndrome (MDS). In contrast, in advanced MDS, acute myelogenous leukemia (AML) or myeloproliferative syndromes (MPD), clonality is a result of expansion of a malignant clone and displacement of normal stem cells. In PNH, exogenous permissive factors may be required for dominance of the PIG-A mutated clone, while in MDS stem cells undergo multiple transformation steps leading to growth advantage and clonal dominance. Stem or multipotent progenitor cell involvement in PNH is evidenced by long-term persistence of a clonal defect and its presence in all blood cells. The PIG-A mutation itself or the resulting GPI-deficiency can be used to follow the dynamics of the PNH clone. In MPD, MPD and AML, progenitor or stem cells may be the initial target of transformation. The initial clonal chromosomal aberration may have a "founder effect" and additional defects are secondary. In addition to mutations, such chromosomal defects constitute quantitative/qualitative markers of clonality. Routine metaphase karyotyping measures the proportion of clonal cells within dividing progenitor and stem cells; due to its low resolution, lesions can be found in e.g., only ~50% of patients. This shortcoming may be overcome by the application of whole genome scanning technologies such as single nucleotide polymorphism arrays (SNP-A) allowing for detection of gene copy number changes and loss of heterozygosity in non-dividing cells. This powerful method enhances the ability to detect small cytogenetic defects and allows for a more sensitive assessment of clonality. SNP-A facilitates identification of cryptic lesions in bone marrow failure patients with normal or non-informative cytogenetics and allows for detection of copy-neutral loss of heterozygosity as a result of uniparental disomy, a lesion frequently found in myeloid malignancies but undetectable by metaphase cytogenetics. Recent results demonstrate the utility of this technology in cytogenetic diagnostics of hematopoietic stem cell disorders. The concept of clonality allows for insights into the kinetics of the stem cell compartment in hematologic diseases; clonal derivation of peripheral blood cells indicates profound depletion of the stem cell compartment or expansion of a singular permissive, malignant hematopoietic clone.

L040

Myogenic stem cells

Thomas Braun

Max-Planck-Institute of Heart and Lung Research, Bad Nauheim, Germany

Contact: office.braun@mpi-bn.mpg.de

Satellite cells are the stem cells of skeletal muscles. The mechanisms, which lead to a decline of the number and the fitness of muscle stem cells during life time are not understood. A stimulation of muscle stem cell renewal and differentiation holds an enormous potential to combat muscle-wasting disorders and to improve the clinical conditions of patients suffering from muscle dystrophies. We have approached several pathways that most likely control self-renewal, asymmetric cell division, and lineage selection of muscle stem cells. We also initiated studies to explore the heterogeneity of satellite cells, which are composed of distinct subpopulation of cells owing distinct capacities to regenerate skeletal muscle. One focus was on the transcription factors Pax7, Myf5 and MyoD, which seem responsible for the maintenance and/or renewal of muscle stem cells and for the acquisition of the fate of muscle stem cells. We have used a combination of genetic, cell biology and molecular biology techniques to access the epistatic regulation of the aforementioned regulators and devised new strategies for the manipulation of muscle stem cells. Another focus has been on the development of distinct myogenic lineages. Previous work had demonstrated that the development of myogenic cells is mainly determined by expression of two myogenic factors, Myf-5 and MyoD, which genetically compensate for each other during embryogenesis. More recently we have demonstrated by conditional cell ablation in mice that Myf-5 determines a distinct myogenic cell population *in vivo* that also gives rise to MyoD positive cells. Ablation of this lineage uncovered the presence of a second autonomous myogenic lineage, which superseded Myf5-dependent myogenic cells. In contrast, ablation of Myogenin expressing cells erased virtually all differentiated muscle cells indicating that some aspects of the myogenic program were shared by most skeletal muscle cells. We concluded that Myf5 and MyoD define different cell lineages with distinct contributions to muscle precursor cells and differentiated myotubes. Individual myogenic cell lineages seem to substitute for each other within the developing embryo.

L041

Neurogenesis in the adult brain: genetic dissection

Robert K. Filipkowski, Leszek Kaczmarek

Laboratory for Molecular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland

Contact: l.kaczmarek@nencki.gov.pl

In the adult mammalian brain, there is limited proliferation and differentiation of neuronal precursors (neurogenesis) within the brain. Despite proposed role of adult neurogenesis in learning and action of antidepressants, its functional significance for the brain function remains poorly elucidated. We have previously established that mice with no cyclin D2 (D2 KO) do not generate new neurons in the brains of adult animals (Kowalczyk et al., *J Cell Biol*, 167, 209-13; 2004). Recently, we have tested D2 KO mice in several behavioral paradigms including those in which the role of adult neurogenesis has been postulated. Mutant mice showed no impairment in sensorimotor tests with only sensory impairment in an olfaction-dependent task. However, D2 KO mice showed proper procedural learning as well as learning in context and trace fear-conditioning, Morris water maze, novel object recognition test, and in a multifunctional behavioral system – Intellicages. D2 KO mice also demonstrated correct reversal learning. Our results suggest that adult brain neurogenesis is not obligatory for learning, including the kinds of learning where the role of adult neurogenesis has previously been strongly suggested. In conclusion, the role of the adult brain neurogenesis still remains enigmatic.

L042

Identification of very small embryonic like (VSEL) stem cells in adult tissues

Mariusz Z. Ratajczak

Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, Louisville, United States

Contact: mzrta01@louisville.edu

Bone marrow (BM) was for many years primarily envisioned as the "home organ" of hematopoietic stem cells (HSC). Augmenting evidence demonstrates, however that BM in addition to HSC also contains a heterogeneous population of non-hematopoietic stem cells (non-HSC). Recently our group identified in BM and other adult tissues including brain, kidney, pancreas, skeletal muscles, liver, spleen and lungs a population of very small embryonic like stem cells (VSELS) which express several markers characteristic for pluripotent stem cells (PSC) that are characteristic for epiblast/germ line-derived stem cells (*Leukemia* 2006; 20:857-869 & *Leukemia* 2007; 21:297-303). We hypothesize that VSELS are a population of epiblast-derived cells that are deposited during early gastrulation in developing tissues/organs and play an important role in turnover of tissue specific/committed stem cells. In this context VSELS deposited in BM may give rise to long term repopulating HSC. VSELS could be also mobilized into peripheral blood (PB) and their number of these cells circulating in PB increases during stress and tissue/organ injuries. Finally, we envision that in pathological situations VSELS are involved in development of some malignancies (e.g., teratomas, germinal tumors, pediatric "small round blue cell" sarcomas).

L043

Biology, transplantation and tissue regeneration potential in human umbilical cord blood

Arnon Nagler

Head of Hematology Division BMT & CBB, Tel Hashomer, Israel

Contact: a.nagler@sheba.health.gov.il

Human umbilical cord blood (HUCB) has established itself as a legitimate source for hematopoietic stem cell transplantation. We have developed some innovative approaches to try to increase the number of harvested stem/progenitor cells and to overcome the 20-30% of cells lost while thawing the cryopreserved HUCB grafts. For doing so, we have developed a closed, sterile disposable system that enables the "semi"-controlled collection of HUCB and lyophilized HUCB MNC, using a directional freezing technology that enables the precise control of ice

crystal morphology during the freezing process, thus making the use of intracellular cryoprotectant agents (CPAs) such as DMSO not necessary. Additional approach for increasing HUCB derived stem/progenitor cells is *ex vivo* expansion. For this purpose we took advantage of the multidrug transporter MDR1 (ABCB1) gene product (Pgp) reported as being over-expressed in various stem cells relative to their differentiated progeny. We compared the expression level and activity of Pgp in HUCB-derived CD133⁺ HSCs relatively to CD133⁻ cells. Analyses of freshly isolated CD133⁺ HSCs from various donors (n=6) indicated that the majority (>92%) of these HSCs express functional Pgp on the cell surface. At optimal colchicin (novel MDR inhibitor) dose (2.5 ng/ml), we were able to achieve 2.9±0.5 fold expansion of HUCB CD133⁺ HSCs. The last approach that we have tried in an attempt to improve and facilitate engraftment post HUCB transplantation is to co-administer the HUCB cells with mesenchymal stem cells (MSCs). In addition, we have assessed the potential of HUCB derived cells in tissue regeneration for neurologic and cardiac applications. Results of both studies will be presented and the tissue regeneration potential of HUCB will be discussed.

L044

"Decoding The Dot": The ImageStream system as a novel supportive tool for flow cytometric analysis

Ewa Zuba-Surma, Magdalena Kucia, Janina Ratajczak, Wan Wu, Mariusz Ratajczak

University of Louisville, Stem Cell Biology Institute, Louisville, KY, United States
Contact: ekzuba01@louisville.edu

ImageStream system (ISS) is a new technology combining the capabilities of a flow cytometer with the high resolution imaging of a fluorescent microscope in one instrument. These capabilities make ISS a major achievement in the field of cellular imaging and analysis. Similar to flow cytometer, the ISS analyzes a large number of cells in suspension while simultaneously acquiring their images. Multiple morphological and fluorescence-related features of the acquired cells can be quantitatively measured based on the acquired images including size, shape, texture, and location of probes within, on or between cells (Folia Histochem Cytobiol 2007;45:279). The ISS is the first technology which is capable of "decoding the dots" by analyzing the images of the acquired objects which were visualized until now only as "dots" (events) on the flow cytometric dot-plots. Recently, our group identified a rare population of very small embryonic like stem cells (VSELs) in adult bone marrow (BM) and human cord blood (CB) that i) are CXCR4⁺Oct-4⁺SSEA-1⁺Scal-1⁺CD45⁻lin⁻ in mice (Leukemia 2006;20:857) and CXCR4⁺Oct-4⁺SSEA-4⁺CD34⁺AC133⁺CD45⁻lin⁻ in humans (Leukemia 2006;21:297); ii) exhibit features of pluripotent stem cells *in vitro*; iii) contain large nuclei with primitive euchromatin and iv) are very small in size (<5µm) explaining why VSELs were missed during classical isolation procedures applied in cell sorting (J Cell Mol Med 2008;12:292). Employing classical flow cytometry complemented by ISS, we were able to investigate the content of VSELs in various murine and human tissues. The combination of these two methods is a useful approach to distinguish these small nucleated cells from cellular debris and falsely "positive" artifacts especially in enzymatically digested tissues (murine organs) or samples treated with erythrocytes' lysing solutions (BM and CB samples). Moreover, we used ISS to characterize the morphological features of murine and human VSELs that have been indicated as indicators of the primitive nature of cells, including their size and nuclear to cytoplasm ratio. By using ISS, we confirmed that VSELs are smaller than erythrocytes but larger than platelets. We also employed ISS to confirm the expression of the above mentioned VSELs surface markers and markers related to pluripotency such as Oct-4, SSEA-1 and SSEA-4. Further, we confirmed the pluripotent phenotype of sorted VSELs using confocal microscopy. By employing multi-dimensional methodological approach, including ImageStream technology, flow cytometry, classical epifluorescence, confocal microscopy and electron microscopy; we demonstrate the presence of very small stem cells, smaller than erythrocytes, in various murine organs and human tissues. This is the first multi-step systematic and comprehensive approach employing ImageStream system in the stem cell research field as a supportive tool for classical flow cytometry.

L045

A glioblastoma-specific kinase expression profile

Philip De Witt Hamer¹, Shahryar Mir², Cornelis Van Noorden³, Tom Wurdinger²

¹Free University Medical Center, Amsterdam, Netherlands

²Free University Medical Center and Cancer Center Amsterdam, Amsterdam, Netherlands

³Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands

Contact: c.j.vannoorden@amc.uva.nl

Specific kinase inhibition induces clinically relevant responses in kinase driven human cancers. The best kinases to target by small molecular inhibitors are often unknown. We address the issue of the best treatment targets for glioblastoma in relation to other human malignancies, such as lung, breast, colon, renal and prostate cancer, based on differential gene expression compared to normal tissues. For this purpose, we retrieved array expression data from 34 publicly available datasets on the web of a wide variety of tumor types, including two glioma datasets, and extracted the differential gene expression data for the kinase gene family consisting of 518 protein kinases and 33 lipid kinases. Kinases were ordered based on levels and frequencies of overexpression in the patient population. The upper 5% of percentile fold changes was considered to be substantial overexpression and expression in more than 20% of the patient population was considered as frequent overexpression. In both glioma datasets, 9 kinases appeared to be substantially and frequently overexpressed, MAPK7, DDR2, EGFR, WEE1, TTK, AURKA, CDC2, MELK and BUB1. Two of these 9 selected kinases, EGFR and AURKA, have been described previously as overexpressed in human glioblastoma samples, confirming the plausibility of the glioblastoma-specific kinase profile. Moreover, selective inhibition of EGFR has shown response in a subgroup of glioma patients. We also confirmed overexpression of the 9 kinase genes with quantitative RT-PCR in samples of glioblastoma and glioma cell lines compared to normal brain from our institution's Brain Tumor Bank. Four of the 9 kinases were exclusively overexpressed in glioblastoma, MAPK7, DDR2, EGFR and WEE1, whereas MELK, TTK, AURKA, CDC2 and BUB1 were frequently overexpressed in various cancer types. The four glioblastoma specific overexpressed kinases and especially WEE1 are putative treatment targets. We selected WEE1 to further investigate its role in gliomagenesis and the effects of its inhibition by either a selective small-molecular inhibitor or siRNA in both *in vitro* and *in vivo* experimental models. Histochemical and cytochemical analysis revealed a crucial role of WEE1 in the relatively strong radiotherapeutic resistance of glioma and its sensitization by application of the WEE1 inhibitor. This study indicates the usefulness of screening of relevant datasets available on the web as a starting point for a research project.

L046

Systematic analysis of the healthy and diseased brain using human antibody based proteomics

Jan Mulder

Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom

Contact: j.mulder@abdn.ac.uk

A need exists for mapping the protein profiles in the human brain both during normal and disease conditions. Using antibody-based proteomics, it is possible to systematically generate affinity reagents to a large portion of the proteome and subsequently use these for a wide range of functional assays. In 2005 an antibody-based human protein atlas covering 48 human tissues including four areas of the brain has been released (www.proteinatlas.org). Because of its size, availability, optimized tissue processing and wide use in medical sciences, we are currently producing detailed protein expression maps of the rodent brain. So far over 800 antibodies generated against human gene products with orthologs in rodent have been tested on rat brain sections, and have been validated for specificity using Western blot analysis. Approximately 12% of the antibodies revealed a distinct staining pattern in the rat brain and passed specificity validation. Initially we are investigating protein expression and sub-cellular distribution in 25 brain areas involved in i.a. higher cognitive functions, sensation, emotion, maintenance of internal homeostasis, sleep and motor and sexual behaviors. This growing collection of over 100 specific antibodies against brain expressed proteins can be utilized for the identification of novel diagnostic and therapeutic biomarkers in neuropsychiatric and neurodegenerative disorders using animal models and/or human post-mortem tissue. In an initial study, we have applied 105 antibodies to the spinal cord and lumbar dorsal root ganglions (DRG's) of rats after a unilateral transection of the

sciatic nerve, and have identified 14 proteins that are up-regulated, and 2 proteins that are down-regulated. Interestingly, most of these proteins have not been investigated, or associated with nerve injury before. Within the Human Protein Atlas program, antibody generation for 16,000 genes has been initiated, and it has been estimated that a first draft of the human proteome can be achieved by 2015. Currently we are expanding our collection of protein expression maps in the rat and mouse brain, which will become available for the public in the future. Furthermore we recently started analyzing expression, cellular distribution and post translational modification of a large set of proteins in human brain tissue affected by Alzheimer's disease and brains from Alzheimer transgenic mice.

L047

Analysis of proteolysis in a three-dimensional tumor-microenvironment architecture

Kamiar Moin, Mansoureh Sameni, Quanwen Li, Julie Dosesco, Hitchintan Kaur, Raymond Mattingly, Bonnie Sloane

Wayne State University School of Medicine, Detroit, United States

Contact: kmoin@med.wayne.edu

The increase in mammographic screening has led to greater numbers of women being diagnosed with mammary ductal carcinoma *in situ* (DCIS). A major problem is that some DCIS lesions rapidly progress to invasive ductal carcinomas (IDC) whereas other DCIS lesions remain indolent, and we are not presently able to predict which is which. Key proteases are increased in expression in human breast biopsies at the point of transition from pre-invasive DCIS to invasive carcinomas. Proteolytic pathways also are regulated through other mechanisms such as secretion and activation. We have shown that aberrant signal transduction that is driven by dysregulated activation of Ras small GTPases and kinases can lead to increased pericellular proteolysis. Clearly, the constituents of the proteolytic pathways that contribute to malignant progression need to be defined and validated before considering their potential as therapeutic targets. We are testing the hypothesis that the transition from pre-invasive DCIS to invasive carcinomas are mediated through alterations in proteolytic pathways, both in the epithelial cells and DCIS-associated cells, and that dysregulated activation of Ras small GTPases and kinases contributes to the induction of these aberrant proteolytic pathways. We modeled progression of normal mammary tissue through premalignant lesions to carcinoma using 3D reconstituted basement membrane (rBM) overlay cultures of a progression series of cell lines derived from MCF10A breast epithelial cells grown alone and in triple layer cocultures with breast fibroblasts and breast myoepithelial cells. To analyze effects on proteolysis, we used live cell confocal imaging. Our 3D triple layer coculture model is designed to recapitulate mammary tissue *in vitro*: MAME (mammary architecture and microenvironment engineering). Cell lines of the MCF10A lineage [10A; 10.DCIS; and CA1d (carcinoma)] were grown alone and in triple layer cocultures with WS-12T₁ (tumor-associated human breast fibroblasts). The MAME cocultures also exhibit the progression observed in the 3D rBM overlay cultures, with the addition of fibroblasts increasing the amount of proteolysis observed and enhancing the invasiveness. Addition of breast myoepithelial cells [a kind gift of K. Polyak (Dana Farber)] reduces the proteolysis and reverses the invasive phenotypes of the 10.DCIS and CA1d cocultures. The MAME coculture model recapitulates the architecture of human breast tissue, thus serving as an *in vitro* tool for screening of small molecule inhibitors. Validating proteases and proteolytic pathways, key to progression of pre-invasive DCIS to IDC, and the pathways that regulate them should discover potential targets for therapeutic intervention as well as candidate biomarkers to identify those DCIS lesions that will rapidly progress to IDC.

L048

Expression and clinical relevance of zinc-finger proteins in human cancer cells

James L. Wittliff, Traci L. Krueger

Department of Biochemistry & Molecular Biology, University of Louisville, Louisville, KY, United States

Contact: jim.wittliff@louisville.edu

The superfamily of nuclear hormone receptors (NHR) consists of transcription factors regulating proliferation, differentiation, metabolism and

apoptosis, which bind a variety of small, often lipophilic, molecules. NHR subfamilies share structural and functional properties e.g., a ligand-binding domain (LBD) and a conserved DNA-binding domain (DBD) with two zinc finger motifs. The DBD recognizes response elements in the 5'-flanking regions of hormone-responsive genes. The LBD coordinates ligand-dependent activation functions (AF-2). The amino-terminal domain (A/B region) plays a role in ligand-independent activation of transcription (AF-1). Thus far, 48 genes encoding NHRs appear in the human genome using protein sequence homologies. Activation of transcription requires ligand association and recognition of various protein partners termed coactivators and corepressors. In contrast to NHR, ligands for a structurally-related group of proteins termed orphan receptors are unknown. Of the NHR, estrogen receptor-alpha (hER) and progesterin receptor are the most frequently utilized in breast cancer, serving as prognostic factors for clinical management and as predictive indicators of therapeutic response. While investigating estrogen response element (ERE) binding properties of hER in human breast carcinomas, lower molecular weight ERE-binding proteins (ERE-BP) were observed. Recognition properties of ERE-BP were evaluated by electrophoretic mobility shift (EMSA) with ERE sequences present in the 5'-flanking region of estrogen responsive genes, e.g., pS2, Cath D, VitA2. Cytosols, prepared from breast cancer tissues, were incubated with [³²P]ERE sequences and separated by EMSA. When ERE-BP levels were correlated with tumor marker levels, grade, stage, race, age, no correlation was observed between ERE-binding activities and patient age, race, nodal status, cancer grade or stage. However, when overall survival of these groups of patients was analyzed as a function of either above or below median ERE-binding protein expression, a surprising result was detected from Kaplan-Meier plots. Patients in the above mean group show a lower overall survival than patients in the below mean group. Addition of ERE-binding protein expression within a single hER status (i.e., either positive or negative) identified subsets of patients exhibiting decreased overall survival. Strikingly, breast cancer patients with hER negative status and high ERE-binding protein expression exhibited the poorest overall survival of any of the groups. Although these data are preliminary, they clearly support the hypothesis that the presence of these novel ERE-binding proteins in a breast carcinoma is an indicator of poor prognosis for breast cancer patients. Supported in part by grants from the NIH/NCI 1 R43 CA106059-01A1 & Phi Beta Psi Sorority Charity Trust. TLK is a recipient of an IPBS graduate fellowship.

L049

Seeing more with light microscopy

Tetsuro Takamatsu

Kyoto Prefectural University of Medicine, Kyoto, Japan

Contact: ttakam@koto.kpu-m.ac.jp

Recent developments in the application of fluorescent dyes and laser techniques have enabled us to visualize functional molecules not only in cultured cells but also in living tissue, or at higher order in a living body. In spite of the limitations, such as poor light penetration into the deeper area, laser toxicity, and undesirable staining, the *in vivo* imaging technique appears to be an extremely valuable tool for integrated study of physiology and pathology. The aim of my talk is to introduce recent advances made in the optical microscopic technologies for *in vivo* imaging. They include second harmonic generation (SHG) microscopy, fluorescence lifetime imaging microscopy (FLIM), Raman scattering microscopy and photoacoustic (PA) microscopy. The former three microscopic technologies give us a solution to the problems of undesirable staining. The latter is one means of overcoming poor tissue penetration of light. These technologies can potentially further our understanding of the direct relation between the subcellular dynamics of functioning molecules and the integrated organ function.

L050

Vibrational (FTIR and Raman) microspectroscopies and spectral imaging in cells and tissues: biomedical applications

Michel Manfait, Olivier Piot, Elodie Ly, Ganesh Sockalingum, Ali Tfayli, Florence Draux, Abdel Beljebbar

Université de Reims Champagne-Ardenne, Reims, France

Contact: michel.manfait@univ-reims.fr

Vibrational microspectroscopies (FTIR and Raman) are highly potential tools to investigate biological samples at various scales from cells' analysis to *in*

in vivo development. This presentation will consider biomedical applications of spectral micro-imaging (hyperspectral and multimodal), from frozen and paraffin embedded (without chemical dewaxing) tissue sections. Examples will focus on characterisation of i/ cancer lesions of skin, peritoneum and colon, and ii/ aortic pathologies. Perspectives of an *in vivo* Raman analysis with an up to date dedicated instrumentation will be shown.

L051

Fast dynamics of the cytoskeleton in 3D

Ulrike Engel¹, Margaret Clarke², Günther Gerisch³

¹Nikon Imaging Center of the University of Heidelberg, Heidelberg, Germany

²Oklahoma Medical Research Foundation, Oklahoma City, United States

³Max Plank Institute for Biochemistry, Martinsried, Germany

Contact: engel_nic@uni-hd.de

The study of cytoskeletal dynamics and intracellular transport requires imaging at high temporal and spatial resolution. While instrumentation for real time acquisition is readily available (fast cameras, fast scanning devices), phototoxicity and bleaching in living cells often make analysis of cytoskeletal dynamics an extremely difficult task. This is especially true if the specimen needs to be reconstructed in 3D over time. We use microlens-enhanced spinning disk confocal microscopy to analyze microtubule dynamics in primary neurons, which are especially sensitive to phototoxicity. Using GFP-tagged microtubule binding proteins, we can follow single pioneer microtubules in the growth cone of outgrowing axonal protrusions. In *Dictyostelium* amoebae, we achieved continuous 3D reconstruction of actin dynamics for up to 30 minutes, documenting spontaneous repolarization and actin-dependent protrusion. We have also used spinning disk microscopy to study the fast transport of endocytic vesicles and investigate the spatial recruitment of actin and myosin-1B during phagocytosis.

L052

Novel techniques for biomedical imaging in microscopy

Bernhard Zimmermann

Carl Zeiss MicroImaging GmbH, Jena, Germany

Contact: b.zimmermann@zeiss.de

Modern methods and instruments for optical microscopic imaging are important tools for direct visualization in biomedical research. Conversely, the demand to understand bio-molecular networks on various levels (from the subcellular to the organism) creates challenges to be met by novel developments in microscopy. In the center of interest of today's basic biomedical research are investigations of complex dynamic processes in cells, tissues and model organisms. These processes take place at different time scales and within different spatial ranges. In particular, these research activities encompass questions such as the ontogenesis of organs and organisms, investigations of signal transmission pathways mediated by ions within cells and through membranes, as well as vesicle and protein transport processes within cells. In today's post-genomic world all the above-mentioned questions are tightly related to the more general question of how identified proteins are networked in cells and cell tissues. Common to all these investigations is the study of function arising from the dynamic interplay of molecules and structures at different levels. In this context optical imaging technologies continue to play an important role as tools of direct visualization and of quantitative analysis. However, it is only by utilizing novel techniques that the requirements of the modern biomedical research can be met. The approaches can be classified according to two strategies: 1. expanding the basis of microscopy by establishing instruments with novel functionalities; 2. expanding the horizon of current instruments by developing new methods often utilizing special biomolecular or photochemical processes. In this lecture I will give an overview over some of the more recent developments, address their advantages and applications, and discuss limitations.

L053

Simultaneous imaging of intracellular calcium dynamics and membrane potentials in the heart revealed by dual-view rapid scanning confocal microscopy

Hideo Tanaka, Katsuji Fujiwara, Tetsuro Takamatsu

Kyoto Prefectural University of Medicine, Kyoto, Japan

Contact: hideotan@koto.kpu-m.ac.jp

With the advent of fluorescent-labeling technologies, laser-scanning confocal microscopy has enabled us to visualize not only precise intracellular structures within the tissue but also dynamic behaviors of functional molecules within the cells *in vivo*. Here we show an advanced approach for living-cell image to elucidate cellular functions in the heart with high spatiotemporal resolution. We developed novel *in situ* dual-view, rapid scanning confocal microscopy, a new method for detecting cellular functions of the working heart *in vivo*. With this confocal system, we succeeded in detecting fast fluorescence images (~500 frames/s) of subcellular behaviors of the electrical activity (transmembrane potentials) and intracellular calcium dynamics in the heart under combined loading with the voltage-sensitive dye RH237 and calcium indicator fluo4/AM. We revealed an intimate relationship between intracellular calcium and membrane potentials that determines not only the excitation/contraction process under physiological conditions but also a mechanistic link between the cellular events of abnormal calcium handlings in the heart. This novel approach will provide important insight into understanding the genesis of abnormal excitation and conduction of the heart.

L054

Revisiting the insulin factory: What has cellular tomography added to our understanding of the pancreatic beta cell

Brad Marsh

Institute for Molecular Bioscience, ARC Centre of Excellence in Bioinformatics, Centre for Microscopy & Microanalysis and School of Molecular & Microbial Sciences, The University of Queensland, Brisbane, Australia

Contact: b.marsh@imb.uq.edu.au

We study structure-function relationships in the pancreatic beta cell function to better understand changes in basic beta cell/islet function leading to dysfunction. We combine fast-freezing/freeze-substitution with electron microscope tomography (ET) to conduct comparative structure-function studies of pancreatic islets isolated from mice and humans. Over 50 dual-axis tomograms encompassing over 300 cubic microns of islet beta cell volume at ~5nm resolution have now been computed/analyzed for different beta cells imaged by ET cultured under steady-state or stimulated conditions (5.6mM versus 11mM glucose, respectively). Such 3D snapshots of parts of beta cells have provided powerful new insights into the spatial complexity of inter-organelle relationships in insulin-secreting cells. However, even such relatively 'large area' (e.g. ~4 microns x 4 microns) tomograms still represent just a narrow cross-sectional slice through a cell, typically about ≤1% of an entire islet beta cell's volume. Clearly, a multi-scale/multi-resolution approach that utilizes efficient methods for spatially marking up and quantifying subcellular compartments for large numbers of 3D cellular reconstructions is required to understand how the distribution/size of organelles changes with respect to time and physiology. To complement insights from these high-resolution tomograms of parts of cells, we have undertaken two different approaches to reconstructing mammalian (beta) cells in toto in 3D at the EM level. We have developed new expedited approaches for ET that yield whole cell data with ≤15nm resolution in ~3-4 weeks; tomograms of four pancreatic beta cells (two glucose-stimulated, two steady-state unstimulated) reconstructed in toto in 3D in this manner represent the largest serial section mammalian cell tomograms to date and have already afforded unique insights into cell structure-function relationships. In parallel, we have developed tools/methods that demonstrate proof-of-concept for reconstructing entire mammalian cells in 3D at ≤5nm resolution. Such complete sets of 3D spatio-temporal coordinates for cells at multiple resolutions will uniquely inform advanced in silico studies of 3D cellular/molecular organization, and will underpin the world's first navigable 'Visible Cell™ atlas'.

L055

High-resolution 3D imaging of larval zebrafish: from brain structure to function

Pertti Panula, Maria Sundvik, Yu-Chia Chen, Madhusmita Priyadarshini, Juha Kolehmainen, Ville Sallinen

Neuroscience Center and Institute of Biomedicine, Univ Helsinki, Helsinki, Finland
Contact: pertti.panula@helsinki.fi

Zebrafish is a small teleost fish suitable for detailed analysis of structure-function relationships due to its rapid development, small size of the brain, and similarity of neurotransmitter systems with those of mammals. Genetic manipulation is also easy, and hundreds of mutants are available. We developed methodology to stain larval zebrafish brains to visualize essentially complete neuronal networks in fixed brains. In parallel with morphological methods, we applied quantitative methods for locomotor analysis of up to 96 fish simultaneously. A total of 17 catecholaminergic groups were identified using tyrosine hydroxylase (TH) immunohistochemistry. Neuron numbers in these groups were larger than those previously reported. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a toxin that produces experimental Parkinson's disease, produced a transient decline in TH-ir neurons in many subgroups, most prominently in the pretectal and one diencephalic cell group. This alteration was accompanied by a decline in swimming speed. In one TH-ir neuron group, translation inhibition of PINK1 gene, mutations of which cause hereditary PD, also decreased neuron number in two nuclei, and this decline was enhanced by a dose of MPTP that alone was subeffective. Similar structural analyses were done for the 5-HT system of the larval zebrafish brain. Nine distinct neuron groups were identified in 5-day-old fish. Inhibition of monoamine oxidase altered the pattern of 5-HT immunoreactivity: a dramatic decline was found in the number of immunoreactive neurons in some identified nuclei, whereas 5-HT immunoreactivity appeared in ectopic diencephalic neurons which do not express tryptophan hydroxylase. These changes were accompanied by reduced locomotor activity. Cellular and behavioral abnormalities were normalized by a serotonin uptake inhibitor and synthesis inhibitor, respectively. This suggests that the behavioral and cellular effects are causally related: inhibition of MAO induces accumulation of 5-HT and activates uptake in ectopic neurons, which lead to a characteristic hyperserotonergic behavioral pattern. The results suggest that the larval zebrafish brain is suitable for detailed structure-function studies. The results may be useful for understanding basic mechanisms in human brain diseases like PD. Supported by Technology Development Fund of Finland (TEKES), Academy of Finland, Finnish Parkinson Foundation.

L056

3D architecture of membrane undercoat and spatial specificity of actin binding proteins revealed by immuno-freeze-etching and cryo-electron microscopy

Jiro Usukura¹, Takashi Watanabe², Nobuhiro Morone³, Koza Kaibuchi⁴

¹EcoTopia Science Institute, Nagoya University, Nagoya, Japan
²Institute of Advanced Research, Nagoya University, Nagoya, Japan
³Department of Ultrastructural Research, National Center of Neurology and Psychiatry, Tokyo, Japan
⁴Department of Neuropharmacology, Graduate School of Medicine, Nagoya University, Nagoya, Japan
Contact: usukuraj@esi.nagoya-u.ac.jp

Three-dimensional architecture of membrane undercoat (membrane cytoskeleton) was investigated by freeze deep etched replica and cryo-electron microscopy combined with immuno-labeling. The membrane undercoat consisted of abundant actin filaments, intermediate filaments and microtubules together with their associated proteins independently from cytoplasmic cytoskeleton. Cryo-electron microscopy using native and hydrated specimen without any treatment revealed microtubules as well as actin filaments extending with wavy curvature. Such filamentous images, in particular microtubules, were quite different from the fixed specimen showing linearity. Careful observation of freeze-deep-etching replica let us classify actin filaments into three types according to spatial distribution. The first type are the actin filaments adhered firmly with the membrane surface in all extending areas. The second type are the ones extending parallel to the membrane surface while contacting salutatory with the membrane at several points. The third type are the so called stress fibers with no contact with the membrane except at their proximal ends. We also examined the localization of IQGAP1, an actin-binding protein that is

involved in the formation of lamella-podia and one of the effectors of Cdc 42. Interestingly, IQGAP1 was found predominantly on the actin filaments contacting salutatory with the membrane in cortical areas (type2 actin filaments), but not on stress fiber. Thus, localization of IQGAP1 showed spatial specificity. On the other hand, cryo-electron microscopy using unroofed native samples revealed characteristic distribution of smooth ER that is located just beneath the membrane. Although rough ERs were detected easily and distributed in central cytoplasm of cell in thin section, it is not obvious where the smooth ERs are in cells. It is first morphological finding that smooth ER network surrounds the entire cytoplasm along the cell membrane.

L057

Regulation of caveolin-1 trafficking and formation of caveolae

Carlos Enrich¹, Laia Cubells¹, Thomas Grewal²

¹Department of Cell Biology. Fa. Medicine. University of Barcelona., Barcelona, Spain
²Faculty of Pharmacy. University of Sydney., Sydney, Australia
Contact: enrich@ub.edu

The molecular mechanisms regulating the exit of caveolin from the Golgi complex are not fully understood. We recently demonstrated that high expression levels of annexin A6 (AnxA6) is associated with a sequestration of cholesterol in late endosomes, lower levels of cholesterol in the Golgi and the plasma membrane, both likely contributing to a retention of caveolin-1 in the Golgi apparatus and a reduced number of caveolae at the cell surface. Consequently, a significant diminution in the number of caveolae at the cell surface and a strong reduction of cholesterol efflux was observed. However, cholesterol availability also affects Golgi vesiculation events and most probably is responsible for the inhibition of the cytosolic phospholipase A2 activity (cPLA₂), in cells overexpressing Anx6. AnxA6-mediated downregulation of cPLA₂ activity was overcome upon addition of exogenous cholesterol or transfection with siRNA targeting AnxA6. These findings indicate that AnxA6 interferes with caveolin transport through the inhibition of cPLA₂. Further strengthening these findings, knock-down of AnxA6 and the ectopic expression of the Niemann-Pick C1 (NPC1) protein in AnxA6 overexpressing cells restores the cellular distribution of caveolin-1 and cholesterol, respectively. In summary, in this study we demonstrate that elevated expression levels of AnxA6 perturb the intracellular distribution of cholesterol, which indirectly inhibits the exit of caveolin from the Golgi complex.

L058

Neutral lipid storage: dynamics and regulation

Christoph Thiele

MPI-CBG Dresden, Dresden, Germany
Contact: thiele@mpi-cbg.de

Lipid droplets are the intracellular storage sites for neutral lipids. In contrast to other organelles, they do not have a bilayer membrane but consist of a neutral lipid core covered by a phospholipid monolayer. Using a family of novel fluorescent lipids we have studied the dynamics of lipid transport to lipid droplets and have found that the final storage lipid, triglyceride, is not transported from the ER to the droplets but rather formed on the droplet itself. Also, the droplet monolayer membrane can be synthesized locally, making the droplet independent from vesicular exchange with other cellular organelles. The implications of these findings for droplet biogenesis will be discussed. References: 1. Kuerschner, L., C. Moessinger, and C. Thiele. 2008. Imaging of Lipid Biosynthesis: How a Neutral Lipid Enters Lipid Droplets. *Traffic*. 9:338-352. 2. Thiele, C., and J. Spandl. 2008. Cell Biology of lipid droplets. *Current Opinion in Cell Biology*: in press.

L059

Analysis of lipid supramolecular structures by electron microscopy

Toyoshi Fujimoto

Nagoya University Graduate School of Medicine, Nagoya, Japan
Contact: fujimot@med.nagoya-u.ac.jp

The lipid droplet (LD) and the membrane microdomain are two examples of the lipid supramolecular structure, in which lipids play major roles in its for-

mation. The LD had been often regarded as an inert lipid accumulation, but recent studies have revealed that it is not only important for the intracellular lipid trafficking, but also plays various unexpected roles. In cultured hepatoma cells, apolipoprotein B-100 (ApoB) shows a peculiar LD-associated localization, which we termed "ApoB-crescent". The ApoB-crescent increased drastically upon inhibition of proteasomes or autophagy, and was thought to harbor ApoB destined for degradation. Interestingly, ApoB molecules making the ApoB-crescent were lipidated as well as ubiquitinated. Electron microscopy revealed the ApoB-crescent is an ER cistern fused to a LD and that ApoB is in the cisternal lumen. The LD making the ApoB-crescent was topologically equivalent to a lipid ester globule intercalated between the two leaflets of the ER membrane. The results indicated two things: 1) the LD is the platform for degradation of lipidated ApoB, and 2) the lipid ester can accumulate in the membrane according to the prevalent LD biogenesis hypothesis. Concerning the second point, similar intramembrane lipid ester deposits are not observed in the normal cell, suggesting that the lipid ester may leave the ER membrane continuously, and that the nascent LD may be too small to be identified by conventional techniques. The mechanism of how those small LDs grow to become larger visible ones remains to be elucidated.

L60

Cellular cholesterol distribution and trafficking

Elina Ikonen

Institute of Biomedicine, Anatomy, University of Helsinki, Helsinki, Finland
Contact: elina.ikonen@helsinki.fi

In mammalian cells, cholesterol is enriched in the plasma membrane and endosomal compartments. Cholesterol moves rapidly between compartments and its distribution is in part regulated by sphingolipids, towards which it has high affinity (Ikonen, Nat. Rev. Mol. Cell Biol. 2008, 9:125). For several intracellular cholesterol transport processes, the actual transfer mechanisms are not well understood. We employ live cell sterol imaging and sterol biochemical assays (e.g. compartment-specific enzymatic processing) in combination with RNAi/human disease mutations of sterol-binding proteins to address these issues. From these approaches we have recently discovered that one of the key regulators of endosomal cholesterol removal and egress to physiological extracellular acceptors is the small GTPase Rab8 (Linder et al., Mol. Biol. Cell 2007, 18:47). In further studies we are addressing the mechanism of how Rab8 mediates this effect. It is evident that a minor change in the structure of cholesterol can modulate specific membrane protein-sterol affinity and protein function (Jansen et al., J. Biol. Chem. 2008, 283:14610). This is of importance for cholesterol trafficking because cholesterol processing may serve as a mechanism to mobilize the sterol (Ikonen and Jansen, Curr. Opin. Cell Biol. 2008, epub May23). Structural specificity is also important to take into account when cholesterol analogs are used to monitor sterol trafficking.

L061

Decorin and biglycan signaling in inflammation and fibrosis

Liliana Schaefer

University of Frankfurt/Main, Germany, Frankfurt am Main, Germany
Contact: Schaefer@med.uni-frankfurt.de

Decorin and biglycan, two structurally related small leucine-rich proteoglycans (SLRPs), have been shown to directly signal through different receptors and pathways, thereby giving rise to distinct biological outcomes. Decorin in non-carcinoma cells (endothelial, renal tubular epithelial cells, renal fibroblasts) signals via IGF-IR and the PI3K/Akt pathway, thus protecting cells from programmed cell death. Furthermore, the interaction of decorin with the IGF-I receptor/mTOR/p70 S6 kinase signaling pathway leads to enhanced translation of fibrillin-1 and its deposition in the extracellular environment, both *in vitro* in renal fibroblasts and *in vivo* in unilateral ureteral obstruction and in diabetic nephropathy. Thus, decorin acts as a signaling molecule through the canonical IGF-signaling cascade, and directly regulates cell death and synthesis of other matrix constituents thereby influencing the pathophysiology of several disorders. In inflammatory conditions the ECM turns into a dynamic microenvironment, generating a host of regulatory molecules, which interact with resident and infiltrating cells. Biglycan is an ubiquitous ECM component, however, its biological role has not yet been elucidated in greater detail. Recently, we showed that biglycan acts as an endogenous ligand of the innate immunity Toll-like

receptor-4 (TLR4) and -2 (TLR2) in macrophages, leading to rapid activation of p38, Erk and NF- κ B, thereby stimulating the expression of TNF- α and macrophage inflammatory protein-2 (MIP-2). Importantly, macrophages, normally not expressing biglycan, start to synthesize biglycan when stimulated by proinflammatory factors. Thus, biglycan, upon release from the ECM or from macrophages, is capable of boosting inflammatory reactions by signaling through TLR4 and TLR2, thereby enhancing the synthesis of TNF- α and MIP-2. This novel concept, that under certain conditions matrix components may act as ligands to distinct immunity receptors and thereby regulate inflammatory reactions, might be of considerable significance for the pathogenesis of and the therapeutic approach to inflammatory disorders.

L062

Matrikines, the products of extracellular macromolecules

Yanusz Wegrowski, Stephane Brezillon, Francois-Xavier Maquart

Reims University Medical Faculty, Reims, France

Contact: yanusz.wegrowski@univ-reims.fr

Extracellular matrix is a theatre of numerous physiological and pathological processes including embryogenesis and organogenesis, wound healing, inflammation pathogen penetration or tumor growth and metastasis. Local proteolysis and glycolysis play a crucial role in all this phenomena. The fragmentation of matrix macromolecular components by physical or enzymatic processes liberate smaller molecules which are informative for the cells and sometimes possess new properties either different from whole molecules or enhancing native molecules' activity. Such fragments are called matrikines. The best characterised matrikines are derived from collagen. Type I collagen peptides are potent stimulators of neutrophil activity including oxidative burst. Broadly studied type IV collagen fragments hold anti-tumoral and anti-angiogenic activities. They are all derived from C-terminal non-collagenous portion (NC1 fragment) of the molecule and were named arresten, constatin, or tumstatin from alpha1, 2 and 3 chains respectively. Similar C-terminal fragments were isolated from collagen type XV (restin) or type XVIII (endostatin). Some of them were used in clinical assays as anti-angiogenic agents. Non-collagen glycoproteins and proteoglycans from extracellular matrix also have matrikine features. Particularly, decorin, a small proteoglycan of the SLRP family, apart from its role in the organisation of fibrous collagen network and the neutralisation of different growth factors including TGF-beta, has anti-tumor properties. Our studies are focused on another SLRP molecule, lumican. Lumican is present in the dermis as a glycoprotein, and its expression is negatively correlated with breast cancer prognosis. We have shown that, in melanoma development, lumican is mainly expressed by stromal fibroblasts around the tumoral tissue. Overexpression of lumican inhibits melanoma growth in mouse model but also inhibits lung metastasis. Recombinant lumican enhanced cellular adhesion and decreased cell migration through beta1 type integrin receptor. The inhibition of migration was also done by lumican fragments. This phenomenon was accompanied by aberrant focal adhesion formation by beta1 type integrins on the cell/substratum contacts and altered intracellular focal adhesion proteins' distribution in the connections with actin cytoskeleton. All these data indicate that lumican or its fragments have matrikine properties and may be considered as a new anti-tumor agent.

L063

Regulation of matrix metalloproteinases in inflammation: effects on cell adhesion and migration

Jelena Gavrilovic

University of East Anglia, Norwich, United Kingdom

Contact: J.Gavrilovic@uea.ac.uk

Matrix metalloproteinases (MMPs) and ADAMs are involved in several aspects of cell behaviour including cell proliferation and migration through generation of matricryptic sites in extracellular matrix molecules or liberation of membrane bound growth or motogenic factors. Here the roles of MMPs and ADAMs in cell migration of several cell types will be considered. Atherosclerosis is now often considered to be a disorder with inflammatory features and migration of vascular smooth muscle cells (VSMC) can be both beneficial (generation of a stable plaque) or deleterious (neointima formation). We have shown previously that VSMC respond to a degraded collagen matrix in combination with PDGF-BB with highly

increased migration, without any effect on proliferation. Now we show that metalloproteinases (probably ADAMs) are involved additionally through the generation of soluble motogenic ligands. Thus VSMC migration is a complex process likely involving both MMPs and ADAMs. More detailed knowledge of the roles of ADAMs in cell migration comes from recent work with ADAM15 splice variants. Cells expressing alternatively spliced ADAM15 variants show altered migration which is probably due to the signalling capacities determined by different ADAM15 cytoplasmic tails. Finally we have recently undertaken MMP profiling of macrophages treated with LPS as a mimic of bacterial infection. These studies reveal novel MMP regulation and these MMPs could prove to be potential novel targets for the regulation of chronic inflammatory conditions.

L064

Desmosealin and other elements of the epidermal extracellular matrix

Marek Haftek

Université Lyon1, CNRS, Lyon, France

Contact: haftek@univ-lyon1.fr

Extracellular matrix is a molecular scaffold secreted by cells and is particularly prominent in the connective tissues. Various matrix molecules are deposited by cells presenting specific phenotypes and corresponding to particular stages of the cellular activity. The cells recognize matrix molecules using surface receptors and respond to the composition of their environment with a distinct behavior, e.g.: more matrix deposition, enzyme secretion and matrix re-modeling, adherence, migration, division. For a long time, molecules present in the intercellular spaces of solid epithelial tissues were largely neglected by researchers. However, the extracellular compartment in the living part of the epidermis is filled with several proteins and carbohydrates indicating the presence of epidermal extracellular matrix, with all the resulting functional implications. So far, hyaluronic acid and proteoglycans remain best albeit incompletely studied. Recently, we have described a new epidermal chondroitin sulfate proteoglycan, desmosealin, synthesized by keratinocytes and delivered to the interkeratinocyte spaces through the lamellar bodies. There is an increasing gradient of desmosealin expression from the basal to the granular epidermal layer. Once excreted, desmosealin integrates with the extracellular portions of desmosomes, and so in a differentiation-dependent manner. It persists in the lowermost layers of the stratum corneum compactum but at this level it is visualized only in the corneodesmosomes, not in the lipid-rich extracellular spaces. Apparent affinity of desmosealin to the desmosomal components may have important functional consequences that remain to be further studied.

L065

Cell-matrix adhesion, cytoskeleton and the activated fibroblast

John Couchman, James Whiteford, Hinke Multhaupt, Atsuko Yoneda

University of Copenhagen, Copenhagen, Denmark

Contact: john.couchman@bric.dk

Fibroblast activation can occur in many pathological settings, including wound repair, inflammation, fibrotic disease and tumor stroma. It is known that cytokines such as transforming growth factor β can promote this phenotype. A common feature is the incorporation of α smooth muscle actin into substantial microfilament bundles that terminate in large focal adhesions. We have been interested in the assembly and turnover of focal adhesions, which are not only points of strong adhesion between cells and their pericellular matrix, but also signalling organelles that are associated with cell survival. Besides the essential integrins, cell surface proteoglycans of the syndecan family are present in focal adhesions. Syndecan-4 is a type I membrane protein with three heparan sulphate chains externally that interact with extracellular matrix molecules. It can promote focal adhesion assembly through a signaling pathway that involves protein kinase C α and Rho GTPases. Recent data suggest that syndecan-4 may be an essential partner in the conversion of fibroblast to the activated form (i.e. myofibroblasts), since cells derived from null embryos have altered actin cytoskeleton, with reduced stress fibres and focal adhesions. Specific changes in α -smooth muscle actin are seen. Roles of the heparan sulphate chains and protein domains of syndecan-4 core protein in fibroblast activation will be discussed.

L066

Advantages of laser microdissection in histochemistry, molecular biology and pathology

Susumu Takekoshi, Noboru Egashira, Kajiya Hanako, Shinobu Umemura, R. Yoshiyuki Osamura

Tokai University, Isehara, Japan

Contact: takekos@is.icc.u-tokai.ac.jp

Many tissues are complex structures composed of heterogeneous mixtures of morphologically and functionally distinct cell types. Laser microdissection (LMD) is a technique that has recently become available to biologists and also pathologists to isolate defined populations of cells from heterogeneous histological tissue sections or cytologic preparations for molecular analyses under direct visualization. Herein, we focus on the advantages of LMD in histochemistry, molecular biology and pathology. The mutation of the p53 tumor suppressor gene is one of the most frequent genetic alterations observed in human cancers. LMD selected cells were procured from the formalin-fixed paraffin embedded sections of colorectal adenocarcinoma and the cytologic specimens of breast tumor. And these samples were analyzed by single-strand conformation polymorphism (SSCP) and direct sequencing after PCR amplification for the exons 5, 6, 7 and 8 of p53. As a result, in the LCM microdissected samples, the mutation of the p53 gene could be confirmed to tissue-cell specific lesions. For RNA profiling, cytospin preparations of the dissociated and suspended growth hormone (GH) secreting pituitary adenoma cells were immunostained for prolactin (PRL) and subjected to RT-PCR analysis of GH, PRL and estrogen receptors alpha and beta. Additional expression of ER was considered to be a major factor in obtaining additional PRL production from the GH-secreting adenomas cells. On the other hand, the combined use of LMD and DNA microarray techniques is a useful method for monitoring gene expression in pathological lesions. Carbon tetrachloride (CCl₄) induced fatty liver through the generation of carbon trichloride radicals by cytochrome P-450 enzymes. CCl₄-induced fatty changes occur at the centri lobular regions of the liver because cytochrome P450 enzymes localize in that area and fewer content of anti-oxidative enzymes such as glutathione peroxidase. The unfixed frozen cryosections were prepared for LMD. The RNA was extracted from captured tissues and amplified. Amplified RNAs were converted to labeled cDNA to obtain probes that were hybridized onto DNA microarrays. As a result, the expression of 46 genes, including cytochrome P-450 enzymes, was increased in CCl₄-injured lesions. In contrast, the administration of CCl₄ decreased the expression of 24 genes including anti-oxidative enzyme such as glutathione S-transferase. These results suggest that the alterations of gene expression by CCl₄ stimulate radical generation and lipid peroxidation, resulting in exacerbated fatty change in the liver. In conclusion, the LCM technique is useful for mutation analysis and RNA profiling for specific pathological lesions.

L067

A decade of non-invasive visible light imaging: What have we accomplished and what are the current directions?

Peter Lassota

Caliper Life Sciences, Hopkinton, MA, United States

Contact: Peter.Lassota@CaliperLS.com

The idea of using non-invasive visible light imaging to follow biological processes in experimental animals was born more than a decade ago. Since then the relentless pursuit to improve the technology, the tools and the instrumentation led to creation of imaging systems capable of reliably detecting less than five luciferase-labeled cells located under the skin of a mouse, or a single metastatic nodule in the lungs of a rat. Luciferase-based bioluminescence as well as fluorescence can now be registered on one instrument, and mathematical algorithms allow visualizing the sources of light emission in 3-dimensions. At the same time various luciferases utilizing different chemical substrates as well as numerous fluorescent dyes, quantum dots and fluorescent proteins make possible following multiple biological processes in experimental animal models simultaneously. It seems that at least some of the technological advances have already reached the point where further improvements will be limiting by the physical properties of the light and of the matter the light passes through (absorption, dispersion, autofluorescence, etc.). Thus, further improvements in our non-invasive visible light imaging "tool box" may become rather slow, expensive and incremental. What can be improved though is the way we are utilizing these tools, particularly in the areas of medical

diagnostics and drug discovery and development. Although there are already excellent examples of how non-invasive bioluminescence imaging helped bringing new medications to patients (Zometa® and Tasigna® in oncology and Cubicin® in infectious diseases are the most prominent examples), the full potential of it has yet to be explored. This technology can be used to create pharmacodynamic readouts which allow rapid and seamless translation of the *in vitro* drug screening results to *in vivo* animal models virtually eliminating the need for the time consuming pharmacokinetic and efficacy assays. It can also reduce the time needed for efficacy studies (particularly in Oncology) by at least 50%, it can double the throughput of screens looking for novel antibiotics while making possible following responses of resistant biofilms to treatments *in vitro*, and *in vivo*. It also provides a unique opportunity to study cancer stem cells which can be isolated from the residual tumors after treatment. Examples of the above described assays and studies will be presented and discussed.

L068

Quantitative colocalization analysis of confocal immunofluorescence microscopy images

Vadim Zinchuk¹, Olga Grossenbacher-Zinchuk²

¹Kochi University, Nankoku, Japan

²Bern University, Bern, Switzerland

Contact: zinchukvs@yahoo.com

Colocalization is an important observation detected in various cell and molecular biological studies. In histochemical experiments, colocalization is observed when staining of two or more antigens in the same section, labeled by correspondent antibodies with different excitation spectra and therefore visualized in different colors, overlaps. Finding of colocalization not only helps to identify the exact location of the structures of interests, but also provides support for better understanding of their common characteristics. Colocalization of molecules is determined by their compartmentalization and binding. According to these processes, molecules are targeted to their specific areas in cells where they eventually colocalize. Understanding of these processes provides clues for exploring the nature of the molecules themselves. Reliable interpretation of confocal microscopy images with colocalization is impossible without its quantitative estimation. Quantitative colocalization analysis relies on calculation of a number of coefficients, such as Pearson's correlation coefficient, overlap coefficient according to Manders, overlap coefficients k_1 and k_2 , and colocalization coefficients m_1 and m_2 . These coefficients provide important information about colocalization process and often complement each other. In addition, precise quantification of colocalization requires the use of proper digital images, such as the images that were correctly acquired and processed. In the present study, we show the applicability of quantitative colocalization analysis of confocal immunofluorescence microscopy images using normal and pathologically changed tissues of conjunctiva, brain, and liver. We explain the significance of colocalization observations, clarify biological meaning of coefficients, and illustrate how the results of colocalization experiments can possibly assist in understanding the mechanisms of trafficking of regulatory proteins. This knowledge helps to accomplish quantification colocalization analysis on a variety of tissues and cells, ensuring reproducibility of colocalization coefficients calculations and allowing unbiased comparisons of the results between different quantitative colocalization experiments.

L069

Endothelial-neural progenitor cell interactions in the neurovascular niche: implications for recovery from CNS injury & tissue engineering

Joseph, A. Madri¹, Erin Lavik²

¹Yale University School of Medicine, New Haven, United States

²Yale University, New Haven, United States

Contact: joseph.madri@yale.edu

Neural stem cells (NSCs) exist in vascularized niches. Co-cultures of NSCs with brain-derived endothelial cells (BECs) elicited vascular tube formation & maintenance mediated by induction of vascular VEGF & BDNF and activation of vascular VEGFR2 & TrkB by NSC NO. NO scavengers & sequestration of VEGF & BDNF blunted induction of tube formation. Addition of exogenous NO donor, rBDNF & rVEGF rescued the induction of tube formation. rBDNF enhanced NSC eNOS activation & NO generation, suggesting an inducible positive feed-back signaling loop between

NSCs & BECs, providing for homeostasis and responsiveness of the resident NSCs & BECs comprising the neurovascular niche. These findings demonstrate the importance of reciprocal modulation of NSCs & BECs in induction & maintenance of the neurovascular niche and underscore their dynamic interactions. These interactions are crucial in recovery from a variety of CNS injuries. Chronic hypoxia results in cognitive & motor disabilities. Evidence suggests that there is variable recovery over time. One possibility that explains this variability entails variable neurogenic responses in the subventricular zone (SVZ). We characterized responses to chronic hypoxia of two mouse strains that represent a wide range of susceptibility to chronic hypoxia and determined that C57BL/6 pups and their NSCs exhibit a blunted response to hypoxic insult compared to CD-1 pups and NSCs. C57 pups and NSCs exhibited blunted proliferative and increased apoptotic responses to hypoxic insult and exhibited lower baseline levels and hypoxia-induced levels of selected transcription factors, growth factors and receptors that determine, in part, the responsiveness to chronic hypoxic insult compared to CD-1 pups and NSCs, providing insight into the recovery from CNS injuries. A 3-dimensional microvascular network is critical for the survival & function of most tissues. We investigated the potential of NSCs to augment the formation & stabilization of microvascular networks in a 3-dimensional macroporous hydrogel (an open macroporous network that supports the formation of tubular structures by endothelial cells) and the ability of this engineered system to develop a functional microcirculation *in vivo*. After subcutaneous implantation of hydrogel co-cultures in mice, blood flow in new microvessels was noted with perfused networks established on the surface of implants. Compared to endothelial cells cultured alone, co-cultures of BECs and NSCs had a greater density of vascular structures. These findings support the concept that NSCs promote the formation & maintenance of endothelial cell tubes in co-culture and the development of a functional microcirculation *in vivo*. Creating stable microvascular networks to support engineered tissues of desired parenchymal cell origin has widespread potential in the development of vascular scaffolds for tissue engineering.

L070

New fluorescent tools give new insight in cell biology

Carsten Schultz

European Molecular Biology Laboratory, Heidelberg, Germany

Contact: schultz@embl.de

Molecular tool development opens new doors to elucidate complex biological events. Ideally, the probes used are non-invasive. Therefore, in cell biology optical techniques are often preferred over more destructive methods, mainly because they provide much temporal and spatial resolution to help answering functional as well as structural questions. Spatial resolution is particularly important when localized events such as plasma membrane repair are monitored or when enzymes exhibit different activity patterns in particular cellular locations. To address these two subjects, we recently introduced in collaboration with Philippe Bastiaens a novel FRET method for imaging the formation of enzyme-substrate (ES) complexes in living cells. We show that under certain conditions the amount of ES complex in a particular area of the cell reflects the status of enzyme activity in this region. After cells are injured by physical impact, crucial steps of the local cellular repair mechanism can be unraveled using fluorescence microscopy in real time. The same cells can subsequently be used for EM analysis (correlative microscopy) of the impact site and essential cellular structures including the 'scab' formation initially sealing the lesion. With the help of multiparameter imaging, it is then possible to identify the recruitment of repair enzymes to the impact site. Finally, a novel FRET-based reporter series is introduced that allows to locate the activity of macrophage elastase (MMP-12) in lungs.

L071

Cellular actions of angiogenesis inhibitors on the vasculature of tumors

Donald M. McDonald

University of California-San Francisco, San Francisco, United States

Contact: donald.mcdonald@ucsf.edu

Blood vessels in tumors have bizarre defects that involve all components of the vessel wall, including changes in endothelial cells, pericytes (mural cells), and the vascular basement membrane. Endothelial cells of tumor

vessels overexpress growth factor receptors, undergo sprouting and proliferation, and become dependent on VEGF or other growth factors for survival. Structural defects result in impaired endothelial barrier function, vessel leakiness, poor blood flow, and altered entry of immune cells. Despite the dense vascularity of tumors, local blood flow to tumor cells may be inadequate for viability. Loss of blood flow may lead to tumor necrosis in some regions and redirect flow to other regions of tumor cells that grow even faster. Inhibition of VEGF signalling (i) stops sprouting angiogenesis in tumors, (ii) triggers regression of VEGF-dependent vessels, and (iii) normalizes tumor vessels that survive. Endothelial cells of regressing tumor vessels lose endothelial fenestrations, patency, and blood flow within hours and then disappear. Many pericytes and empty sleeves of vascular basement membrane persist after the endothelial cells regress and provide a scaffold for rapid blood vessel regrowth when the inhibition ends. Increasing use of agents that inhibit VEGF signalling in the treatment of cancer is advancing the understanding of the actions of angiogenesis inhibitors in tumors and increasing familiarity with the adverse effects associated with their use. Some adverse effects of VEGF inhibitors are consequences of blocking the actions of VEGF in normal physiology. These effects may be exaggerated by the presence of existing pathological conditions. Although the essential role of VEGF during embryonic development is well established and widely accepted, this dependency was thought not to persist into adult life. Yet actions of VEGF in normal organs of the adult include maintenance of function and survival of certain blood vessels, regulation blood pressure, and renal, neurological, and hepatic function. The efficacy of angiogenesis inhibitors in the clinic provides proof of concept and stimulates the search for agents that have even more robust effects in tumors while preserving the normal vasculature. However, the actions of these inhibitors are still not fully understood. Better understanding of the growth factor dependency of the vasculature in tumors and in normal organs will be accompanied by more powerful strategies for selectively controlling blood vessel growth and regression in tumors with minimal adverse consequences.

L072

Computational time-lapse analysis of vascular patterning, *in vivo*

Charles Little¹, Andras Czirok¹, Brenda Rongish¹, Evan Zamir¹, Cheng Cui¹, Rusty Lansford²

¹Department of Cell Biology and Anatomy, Kansas University Medical Center, Kansas City, United States

²Biological Imaging Center, California Institute of Technology, Pasadena, United States

Contact: clittle@kumc.edu

During embryogenesis new cellular patterns form within constantly expanding tissue scaffolds. This fact raises the problem of relative motion. For example, primordial endothelial cells, in bird and mammalian embryos, assemble early vascular patterns in the context of continuous tissue expansion/deformation. Our novel computational time-lapse software and algorithms are designed to quantify large-scale tissue drift and deformations in intact, warm-blooded, quail embryos. The computer code enables recording of multiple fluorescently colored objects, i.e., cells and ECM fibers. Two methods are available for marking quail vascular progenitor cells with fluorescent tags. One method, electroporation, allows introduction of DNA plasmids encoding fluorescent proteins into exceedingly early stage embryos (HH Stage 2), well prior to specification of the endothelial cell lineage – which permits mapping the position fate of vascular cells. The second method employs Tiel-YFP germline transgenic quail, allows labeling the endothelial cell lineage from approximately HH Stage 7 onward. Computation of tissue displacement fields using the motion of ECM fibrils as *in situ* markers for tissue drift – while simultaneously tracking individual endothelial cells in the same time – allows calculation of active cell-autonomous motility versus passive convective tissue motion. The data demonstrate that passive tissue motion contributes significantly to apparent embryonic vascular cell trajectories. In other words, tissue drift significantly impacts the *de novo* assembly of vascular networks. Previous notions regarding "cell migration" in warm-blooded embryos will have to be revisited in light of these data. The work has major implications for understanding embryonic cell guidance mechanisms, such as cellular chemotaxis in response to proposed gradients of molecules such as VEGF. Supported by AHA fellowships (EZ, CC), NIH/R01 awards (CDL, AC) and the G. Harold and Leila Y. Mathers Charitable Foundation (CDL, BJR, AC).

L073

Novel imaging strategies to assess myocardial angiogenesis

Albert Sinusas

Yale University, New Haven, United States

Contact: albert.sinusas@yale.edu

Many patients suffering from advanced coronary artery disease and chronic myocardial ischemia cannot be adequately managed by a combination of anti-anginal medication, and angioplasty or coronary artery bypass surgery. Therefore, therapeutic stimulation of vascular growth in the management of chronic myocardial ischemia seems to be a useful strategy in treating such patients. Myocardial angiogenesis should result in improved perfusion and tissue oxygenation, resulting in reduced hypoxia, diminished myocardial ischemia, and improved regional left ventricular function. Thus, angiogenesis may be indirectly evaluated non-invasively by analysis of standard physiological parameters like regional myocardial perfusion and function. However, there is a tremendous need for development of non-invasive approaches for direct evaluation of the molecular events associated with angiogenesis in order to more effectively track therapeutic angiogenesis. Targeted single photon emission tomographic (SPECT) imaging of angiogenesis may be a useful approach for monitoring angiogenic therapy in the heart. Application of this type of noninvasive evaluation of angiogenesis in clinical practice may help predict post-infarct remodeling and permit risk stratification of patients following ischemic injury. Potential biological targets for imaging angiogenesis fall into three principal categories; 1) endothelial cell markers of angiogenesis, 2) non-endothelial cells involved with angiogenesis, and 3) markers of the extracellular matrix (ECM). The $\alpha v\beta 3$ integrin is expressed in angiogenic vessels in response to hypoxia, and represents a potential novel target for imaging myocardial angiogenesis. This lecture will discuss the feasibility of non-invasively tracking hypoxia-induced $\alpha v\beta 3$ -integrin activation within the myocardium as a marker of angiogenesis after myocardial infarction. This targeted $\alpha v\beta 3$ -integrin imaging approach has been affectively applied in several pre-clinical models of myocardial injury, and in evaluation of gene therapy targeted at stimulating angiogenesis. This type of targeted imaging will benefit from the availability of novel targeted radiotracers and hybrid SPECT-CT imaging systems which will permit registration of molecular and physiological information with 3-dimensional anatomical structure. Quantitative targeted imaging of a biological marker of this type will be critical for understanding the angiogenic process and tracking novel molecular or genetic therapies directed at stimulating angiogenesis.

L074

Noninvasive *in vivo* molecular imaging of peripheral angiogenesis

Lawrence W. Dobrucki

Yale University School of Medicine, New Haven, CT, United States

Contact: wawrzyniec.dobrucki@yale.edu

Nuclear cardiology has historically played an important role in detection of cardiovascular disease as well as risk stratification. Recent therapeutic interventions which have come with the growth of molecular biology require new diagnostic imaging approaches. Noninvasive targeted radiotracer based imaging strategies for evaluation of peripheral angiogenesis would be valuable in risk stratification of patients with peripheral ischemia and for the assessment of response to therapies directed at stimulation of angiogenesis. Recent progress has been made possible with the advancements in genomics along with many technological advances. Future adaptations of the developing experimental procedures and instrumentation will allow for the smooth translation and application to clinical practice. Ischemia-induced angiogenesis is a complex process involving initial hypoxic stimulus, degradation of extracellular matrix (ECM) by matrix metalloproteinases (MMPs), migration and proliferation of endothelial cells to form tubular-like structures. During this process multiple vascular growth factors (like VEGF) and mediators (NO, $\alpha v\beta 3$ integrin) are activated. We will review studies focused at non-invasive molecular imaging of peripheral angiogenesis using tracers targeted at hypoxia, MMPs and $\alpha v\beta 3$ integrin as markers of angiogenic process. This lecture is intended as a brief overview on the subject of molecular imaging of peripheral angiogenesis. Basic concepts and historical perspective of molecular imaging will be reviewed first, followed by description of current technology, and concluding with current applications. The emphasis will be on the use of a single

photon emission computed tomography (SPECT) radiotracers, although other imaging modalities will be also briefly discussed. The specific approaches presented here will include imaging of natural peripheral angiogenesis in animal models of hindlimb ischemia in diabetic animals and animals lacking endothelial nitric oxide synthase, and imaging of therapeutic angiogenesis stimulated by use of specific AMPK activator (AICAR). In addition novel techniques to analyze images of peripheral angiogenesis will be reviewed.

L075

Vascular oxidative stress: relative importance of pro- and anti-oxidative enzymes

Huige Li, Ulrich Förstermann

Department of Pharmacology, Johannes Gutenberg University, Mainz, Germany

Contact: huigeli@uni-mainz.de

Endothelial cells control vascular homeostasis by generating paracrine factors that regulate vascular tone, inhibit platelet function, prevent adhesion of leukocytes, and limit proliferation of vascular smooth muscle. The dominant factor responsible for many of those effects is endothelium-derived nitric oxide (NO). Endothelial dysfunction characterized by enhanced inactivation or reduced synthesis of NO, alone or in combination, is seen in conjunction with risk factors for cardiovascular disease. Endothelial dysfunction can promote vasospasm, thrombosis, vascular inflammation, and proliferation of the intima. Vascular oxidative stress and increased production of reactive oxygen species contributes significantly to mechanisms of vascular dysfunction. Oxidative stress is mainly caused by an imbalance between the activity of endogenous pro-oxidative enzymes (such as NADPH oxidase, xanthine oxidase or the mitochondrial respiratory chain) and antioxidative enzymes (such as superoxide dismutase, glutathione peroxidase, heme oxygenase, thioredoxin peroxidase/peroxiredoxin, catalase and paraoxonase). Furthermore, small-molecular-weight antioxidants might have a role in the defense against oxidative stress. Increased concentrations of reactive oxygen species reduce bioactive NO through chemical inactivation, forming toxic peroxynitrite, which in turn can uncouple endothelial NO synthase (eNOS) resulting in a dysfunctional superoxide-generating enzyme that contributes further to vascular oxidative stress. The role of oxidative stress in vascular dysfunction and atherogenesis, and strategies for its prevention will be discussed in the lecture.

L076

Multiple roles of caveolin in tumor and post-ischemic angiogenesis

Olivier Feron

Department of Pharmacology and Therapeutics, University of Louvain Medical School, Louvain, Belgium

Contact: olivier.feron@uclouvain.be

Caveolin, the structural protein of caveolae, interacts with the endothelial nitric oxide synthase and inhibits its activity in basal conditions. Since nitric oxide is a key angiogenic mediator, we examined a possible relationship between changes in caveolin abundance and angiogenesis. We first exploited the propensity of cationic lipids to target endothelial cells (EC) lining tumor blood vessels to selectively deliver a caveolin plasmid. The resulting caveolin overexpression was found to inhibit both the vascular reactivity and the neo-angiogenesis in solid tumors. Paradoxically, in a model of adaptive angiogenesis obtained after femoral artery resection, we found that angiogenesis was altered in caveolin-deficient (*Cav^{-/-}*) mice. Isolation of *Cav^{-/-}* EC allowed us to document that upon VEGF stimulation, NO production and endothelial tube formation were dramatically decreased when compared with *Cav^{+/+}* EC. Caveolin transfection in *Cav^{-/-}* EC redirected the VEGFR-2 in caveolar membranes and restored VEGF signaling, emphasizing the critical role of caveolae in ensuring the coupling between VEGFR-2 stimulation and downstream mediators of angiogenesis. In the same model, we also found that intravenous infusion of endothelial progenitor cells (EPC), but not bone marrow transplantation, rescued the defective neovascularization in *Cav^{-/-}* mice, suggesting a defect in vasculogenesis. We documented that the adhesion of *Cav^{-/-}* EPC to bone marrow stromal cells was resistant to the otherwise mobilizing SDF-1 exposure. The lack of caveolin was here associated with a defect in the internalization of the SDF-1 cognate receptor CXCR4. Finally, we recently got interested in

the roles of caveolin in angiogenesis-related phenomena, namely vessel permeability and vascular maturation. In tumor-bearing *Cav^{-/-}* mice, we found that fibrinogen accumulated in early-stage tumors to a larger extent than in *Cav^{+/+}* animals. These results were confirmed by a consecutive elevation of the interstitial fluid pressure and a late deficit in albumin extravasation in *Cav^{-/-}* tumors. Immunostaining analyses of *Cav^{-/-}* tumor sections further revealed a higher density of CD31-positive vascular structures and a dramatic deficit in alpha-smooth muscle actin-stained mural cells. *in vitro* assays led us to document that the decrease in caveolin abundance observed in the tumor vasculature actually prevented the termination of angiogenesis by inhibiting PDGF-mediated mural cell recruitment.

L077

Nitric oxide as a marker of vascular endothelial function

Leszek Kalinowski

Department of Clinical Chemistry and Biochemistry, Medical University of Gdansk, Gdańsk, Poland

Contact: lekal@amg.gda.pl

Over the last two decades, it has become evident that the endothelium is not merely an inert, single-cell lining that covers the internal surface of blood vessels, but in fact plays a crucial role in regulating vascular function. Although it is an extremely active endocrine and paracrine organ that produces a large variety of molecules participating in complex biochemical processes, it seems that its simple product, nitric oxide (NO), generated by nitric oxide synthase (eNOS) is the key molecule for the maintenance of vascular homeostasis. Endothelium function/dysfunction has commonly been assessed as impaired endothelium-dependent vasodilatory response (EDVR) that represents the reduced eNOS-derived NO activity. A strong rationale for this approach has been based on the assumption that an impairment of EDVR represents a surrogate indicator for other important functions of NO. Indeed, NO not only produces vasodilation but also inhibits platelet aggregation, vascular smooth muscle migration and proliferation, lipid peroxidation and expression of adhesion molecules, all of which show antiatherogenic properties. Among various mechanisms responsible for the impaired EDVR, the increased NO breakdown by superoxide (O_2^-) seems to play central role in the reduced NO bioavailability. Among several enzymatic systems that are capable of producing O_2^- , NAD(P)H oxidase and eNOS itself apparently are the main sources of O_2^- in the endothelial cells. In the absence of substrate, L-arginine, or cofactor such as BH_4 , eNOS was revealed to synthesize O_2^- in preference to NO, that is called enzyme "uncoupling". Activation of NAD(P)H oxidase could initiate a vicious cycle of O_2^- formation, loss of L-arginine and BH_4 and altered eNOS function toward uncoupling state, with increased O_2^- and decreased NO production. The reaction between O_2^- and NO not only results in removing the beneficial effects of NO, but also increases the damaging effects of the reaction product, peroxynitrite ($ONOO^-$), that is highly reactive and cytotoxic. eNOS uncoupling seems to take place in numerous common diseases, such as atherosclerosis, hypertension and diabetes or in the presence of typical risk factors predisposing to these diseases. This is important caveat of potential clinical relevance because in such cases eNOS upregulation might even aggravate vascular damage. Also, it is totally unclear whether O_2^- production by dysfunctional eNOS occurs only under a specific pathological conditions or whether it may be present to some extent in normal endothelium that is not exposed to the cardiovascular risk factors. Because of an extremely rapid reaction between NO and O_2^- , there is always some O_2^- reacting with NO within endothelial cells and in extracellular space. The mechanisms keeping a minute balance between NO, O_2^- and $ONOO^-$ at different redox levels within native endothelial cells may explain the existence of individual predisposition to endothelial dysfunction.

L078

The structure and dynamic properties of mammalian chromosomes – linking nuclear structure and function

Dean Jackson, Apolinar Maya-Mendoza, Chi Tang, Dorota Feret

University of Manchester, Manchester, United Kingdom

Contact: dean.jackson@manchester.ac.uk

Nuclei of higher eukaryotes are highly structured, yet the extent to which this structure is a fundamental determinant of function or passive by-product remains a matter of intense debate. A key determinant of structure-

function links reflects the way in which the folding of chromatin partitions the genome in functional domains, the organisation of which has the potential to act as a fundamental regulator of gene function and maintain chromosome stability. It is well-known that the chromosomes of somatic mammalian cells are arranged in nuclei as essentially discrete chromosome territories. The basic structural units of the territories are DNA foci, which contain about 1 Mbp of DNA. DNA foci play a central role in structure-function links: as well as key structural units they are also functional targets during DNA replication. We will present a series of recent experiments that provide a detailed analysis of the structure of DNA foci and how this contributes to the efficiency of the replication process in mammalian cells. Using high-resolution 3D imaging, we have assessed the contribution of the spatial and genetic continuity of adjacent foci within individual chromosome territories. Using a nearest neighbour analysis we show that with extremely high precisions (>90%) newly activated foci within early S phase lie immediately adjacent to foci that recently completed synthesis. Using spread DNA fibres we also show that this high degree of spatial continuity correlates with the genetic continuity of DNA along the chromosome fibre. As the structure of early S phase is stable over many cell division cycles we evaluated how this structure function link might be established. Using the histone deacetylase inhibitor TSA we show that the structure of DNA foci is disrupted when regulation of histone acetylation is perturbed and that this eventually prevents foci from operating as substrates for DNA synthesis. During this analysis, the live cell labelling protocols used for analysis of DNA foci were also used to monitor the dynamic behaviour of foci within individual territories and the spatial interaction of foci on the boundaries of adjacent territories. Together these experiments show that the structure of DNA foci is maintained epigenetically and that preservation of the structure of foci is a fundamental determinant of S phase progression.

L079

The modification and dynamics of histones in human cells as revealed by a panel of specific monoclonal antibodies

Hiroshi Kimura¹, Yoko Hayashi-Takanaka¹, Naohito Nozaki²

¹Osaka University, Osaka, Japan;

²Kanagawa Dental College, Yokosuka, Japan

Contact: hkimura@fbs.osaka-u.ac.jp

In eukaryotic nuclei, DNA is wrapped around a histone octamer containing two copies of each of H2A, H2B, H3, and H4, forming nucleosomes, the fundamental units of chromatin. In living cells, the histone-DNA interaction and chromatin structure are expected to be remodeled and/or disrupted during transcription, DNA replication and repair. On the other hand, epigenetic marks on histones are preserved to maintain the transcription status of specific genomic loci. Thus, the fluidity and stability seem to be well balanced in nucleosomes in living cells, and the regulation of chromatin remodeling and epigenetic modifications is crucial in cell growth, development and differentiation. Indeed, the epigenetic profiles are often altered in diseased cells including cancer. We have found that histone H3 and H4 are more stably associated with DNA in nucleosomes compared to H2A and H2B in living cells by using the GFP-tagged proteins. To elucidate the mechanism and biological significance of H2A-H2B exchange, we developed a permeabilized cell-based assay of histone assembly and exchange in the chromatin context. By purifying factors assisting in the exchange of H2A-H2B, we identified protein phosphatase 2C-gamma in addition to previously known nucleosome assembly proteins. Further analyses suggested that this phosphatase may link the deposition and dephosphorylation of H2A-H2B (and H2AX-H2B) during DNA damage recovery. As the dynamics and modification of histones appear to be closely linked, we have begun to investigate the function of histone modifications. While the modification-specific antibodies are widely available, most commercial ones are rabbit polyclonal and so the specificity can vary from lot to lot. We therefore generated our own mouse monoclonal antibodies, whose specificity should remain constant over time, after extensive screening. By using these antibodies for immunofluorescence, we found that di- and tri-methylation on histone H3 lysine 4 (H3K4me2 and H3K4me3) was diminished during replicative senescence. As H3K4 methylation was reported to be found on most genes regardless of productive transcription in embryonic stem cells, these modification may be related to the plasticity of chromatin. These highly-reliable and fully-characterized monoclonal antibodies may facilitate future epigenomic studies on normal and diseased cells by a variety of approaches including histochemistry and cytochemistry.

L080

Immunocytochemical detection of DNA damage: activation of ATM and Chk2, and phosphorylation of histone H2AX and tumor suppressor protein p53 on Ser15

Zbigniew Darzynkiewicz

New York Medical College, Valhalla, United States

Contact: darzynk@nymc.edu

DNA in live cells is exposed to exogenous and endogenous genotoxins. DNA damage if improperly repaired accumulates with age and this contributes to cell senescence as well as preconditions to neoplasia. The sensitive reporters of DNA damage are activation of ATM (A-ATM) through Ser1981 phosphorylation, phosphorylation of histone H2AX (P-H2AX) on Ser139, activation of Chk2 through Thr68 phosphorylation (A-Chk2) and phosphorylation of tumor suppressor protein p53 on Ser15 (P-p53Ser15). Phospho-specific antibodies to these proteins were used in combination with multiparameter flow and laser scanning cytometry to correlate their respective phosphorylation with activation of caspase-3 (A-C3), the marker of apoptosis and with cellular DNA content, the marker of the cell cycle phase and reporter of cell cycle arrest. The method was applied to measure DNA damage by several exogenous genotoxins and metabolically generated oxidants. Exposure of cells to UV-B induced A-ATM and P-H2AX followed by A-C3 maximally in early-S phase. DNA topo1 inhibitor topotecan triggered A-ATM, P-H2AX, A-Chk2, P-p53Ser15 and A-C3 selectively in S-phase cells, with maximum at mid-S. DNA topo2 inhibitors mitoxantrone and etoposide induced A-ATM, P-H2AX, A-Chk2 and P-p53Ser15 in all phases of the cycle, with maximum in G1. Apoptosis, however (A-C3) was preferential to S-phase cells. Replication stress caused by aphidicolin, hydroxyurea or thymidine led to P-H2AX and A-C3 (in S-phase) but did not trigger A-ATM. Oxidative DNA damage by exogenously applied H₂O₂ affected (A-ATM, P-H2AX, A-Chk2, P-p53Ser15) preferentially DNA replicating cells. Constitutive A-ATM and P-H2AX, were seen in untreated normal or tumor cells and they reflected the ongoing DNA damage caused by metabolically generated oxidants. The level of constitutive A-ATM and P-H2AX was many-fold higher in mitogenically stimulated- compared to G₀ lymphocytes. Extent of constitutive A-ATM and P-H2AX, which was maximal during S and G₂, was reduced by antioxidants and ROS scavengers (vitamin C, N-acetyl-cysteine, COX-2 inhibitors), metabolic inhibitors (2-deoxyglucose, 3-bromopyruvate) and growth at hypoxia or low serum concentration. The multiparameter cytometry to concurrently measure A-ATM, P-H2AX, A-Chk2, P-p53Ser15, A-C3 and DNA content is useful in studies of association between DNA damage, recruitment of DNA repair machinery, activation of cell cycle checkpoints, and induction of apoptosis. Analysis of constitutive A-ATM and P-H2AX provides the sensitive means to measure effectiveness of agents that through lowering aerobic metabolism and/or neutralizing radicals protect DNA from damage.

L081

Heterochromatic protein 1 and repair of DNA damage – a new job for the old fellow?

Jurek Dobrucki

Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Div. of Cell Biophysics, Kraków, Poland

Contact: jurek.dobrucki@gmail.com

Heterochromatin protein 1 (HP1) is a dominant suppressor of position-effect variegation and a major component of heterochromatin. It plays an important role during development as embryos with homozygous deletions die during the third larval instar. HP1 is a member of a group of highly conserved non-histone chromatin proteins found in fungi, plants and animals, with multiple members often being found in one species. They typically contain an amino-terminal chromatin organisation modifier domain – or chromodomain (CD) – which binds non-specifically to DNA and specifically to methylated lysine 9 on histone H3 to induce transcriptional repression. A carboxy-terminal chromoshadow domain (CSD) is highly conserved; it can dimerize to form both homo- and hetero-dimers with a non-polar groove that acts as a docking site for various proteins containing the consensus sequence PxVxL. As a result, members of the HP1 family can interact with transcriptional regulators and chromatin-modifying proteins, DNA replication and repair proteins, chromosome-associated proteins, and structural nuclear proteins. It is now clear that HP1 play diverse roles, including the regulation of higher-order structure and activity of both eu- and

heterochromatin. As a result, it is now believed that HP1 members play a role in establishing and maintaining epigenetic marks in chromatin. We now report a previously unknown role for HP1. After introducing localized oxidative damage into the DNA of mammalian cells, HP1 accumulates transiently in damaged regions [1]. Based on our studies of recruitment we hypothesize that HP1 acts after the repair of damage in DNA to restore chromatin structure with its epigenetic information. Reference: [1]. M. Zarebski, E. Wiernasz, A. Rapp, P. Verschure, R. van Driel, P.R. Cook and J.W. Dobrucki. The recruitment of HP1 to sites of photo-oxidative damage: a role for HP1 in BER or the repair of epigenetic information?

L082

Visualizing the fate of nuclear proteins during apoptosis

Carlo Pellicciari¹, Maria Grazia Bottone¹, Anna Ivana Scovassi², Marco Biggiogera¹

¹Department of Animal Biology, University of Pavia, Pavia, Italy

²Institute of Molecular Genetics, CNR, Pavia, Italy

Contact: pelli@unipv.it

During apoptosis, in parallel with chromatin condensation, there is a severe reorganization of the ribonucleoprotein (RNP)-containing structures which are involved in the synthesis and processing of RNAs: already in early apoptosis, the nucleoplasmic RNPs, i.e. perichromatin fibrils (PF), perichromatin granules (PG), and interchromatin granules (IG), coalesce in the interchromatin space where they associate with segregated nucleolar components, to form heterogeneous ectopic RNP-derived structures (HERDS). Both in spontaneous and induced apoptosis, PF first clusterize and aggregate to a core of IG to whom both chromatin-associated and/or nucleolar components are eventually added. Since nuclear HERDS were also found to form whenever RNA synthesis is arrested, it is tempting to speculate that RNA degradation might be involved in HERDS formation. Actually, different apoptosis-activated RNA-degrading nucleases have been described (e.g., RNase L and endonuclease G) which could effectively cleave RNA at the transcription sites, i.e. at the level of PF. Under normal conditions, a balance exists between the amount of nascent RNAs and the quantity of the associated proteins which are needed for their processing: we hypothesize that, whenever the amount of newly formed hnRNA significantly decreases, RNP proteins become exceedingly predominant and the different nuclear RNP-containing structures accumulate in HERDS, as in a final storage-site. Starting from middle apoptosis, when irreversible degradation of the architecture and functional properties of the nuclear envelope has taken place, HERDS move from the nucleus to the cytoplasm: here, they are still recognized morphologically and can be immunolabeled even in the apoptotic blebs which are released at the cell surface, in late apoptosis. This demonstrates that the protein components of these RNP-containing aggregates may have been only partially (or even not) cleaved. It is worth noting that several RNPs and non-RNP factors involved in pre-mRNA processing (e.g., the Sm antigen and SC-35) are found in nuclear and cytoplasmic HERDS, whereas other proteins (such as RNA polymerase II or the cleavage factors CFI_m and CStF) do not co-locate therein. Immunolabeling experiments demonstrated that several other chromatin- or nucleolus-associated proteins also move to the cytoplasm, during apoptosis, independently from HERDS, and such a transit does not occur simultaneously for all the protein or nucleoprotein complexes. This likely reflects the timing of protein dissociation from chromatin or the interchromatin domain and may also account for the final heterogeneity of apoptotic blebs.

L083

Post-translational modifications, subcellular relocation and release in apoptotic bodies: apoptosis turns nuclear proteins into potential autoantigens

Jürgen Dieker, Marion Decossas, Sylviane Muller

Immunologie et Chimie Thérapeutiques, IBMC, Strasbourg, France, Strasbourg, France

Contact: j.dieker@ibmc.u-strasbg.fr

Autoantibodies against particular nuclear components, such as chromatin and snRNPs, are a characteristic feature of the autoimmune disease systemic lupus erythematosus (SLE). The last decade evidence has suggested that apoptotic cells are the main source of autoantigens in this disease. Therefore it has been proposed that protein modifications occurring during

apoptosis lead to the formation of neo-epitopes which can break the tolerance when apoptotic cells are not properly cleared. Indeed, many lupus autoantigens are prone to apoptosis-associated post-translational modifications and/or cleavage by caspases. In addition, lupus autoantigens are relocated from the nucleus to apoptotic blebs on the cell surface. Therefore, to understand why certain nuclear proteins become autoantigens during apoptosis, it is important to know the apoptotic processing of these proteins. The U1-70K protein is part of the U1 snRNP particle, which involved in splicing of mRNA, and is an important autoantigen in SLE. Early during apoptosis the U1-70K protein undergoes a specific sequence of changes, which include an increase in the phosphorylation of Ser140 in the RNA-binding domain, a dephosphorylation of other residues by protein phosphatase 1 and cleavage by caspase 3. Simultaneously, the U1-70K protein is clustered in heterogeneous ectopic RNP-derived clusters (HERDS), which are formed in the nucleus, relocalize to apoptotic blebs on the cell surface and are finally released in small apoptotic bodies. Interestingly, many known RNA-associated or nucleolar autoantigens are also relocalized to HERDS. On the other side, chromatin is cleaved by endonucleases and histones undergo specific post-translational modifications (such as the acetylation of histone H4) during apoptosis. Chromatin is also partially relocated to apoptotic bodies. Finally, apoptotic bodies, containing condensed chromatin or HERDS are released very early during the apoptotic process (during or even before the phosphatidyl serine exposure) and seem to be very stable structures. Importantly, autoreactive T and B cells target proteins in apoptotic blebs/bodies and especially epitopes which are modified during apoptosis, i.e. the RNA-binding domain of the U1-70K protein phosphorylated at Ser140, specific acetylated residues in the N-terminus of histone H4 and dephosphorylated SR proteins. These data suggest that apoptotically processed nuclear proteins, clustered in apoptotic bodies are the main target of the immune system during SLE. Therefore, further characterization of the contents of apoptotic bodies and their encounter by the immune system in healthy or autoimmune situations should shed more light on the origins of this disease.

L084

Apoptotic nucleases – a role and regulation

Piotr Widlak

Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland

Contact: widlak@io.gliwice.pl

Apoptosis, or programmed cell death, is a fundamental process essential for both development and maintenance of tissue homeostasis, which allows to eliminate excessive, unnecessary or damaged cells from multicellular organisms. Cells undergoing apoptosis show specific biochemical and morphological features. One of the hallmarks of the terminal stages of apoptosis is nuclear breakdown. First, large fragments of about 50 kb and more are excised from chromosomes, which reflects domain structure of the chromatin. This large scale DNA fragmentation is followed by more advanced internucleosomal cleavage termed "DNA laddering". Although several enzymes have been implicated in such DNA cleavage reactions, caspase-activated DNase (CAD), also termed DNA fragmentation factor (DFF), is the major endonuclease responsible for internucleosomal DNA cleavage during apoptosis. In non-apoptotic cells, DFF exists in nucleus as a heterodimer, composed of CAD/DFF40 latent nuclease subunit, and ICAD/DFF45 chaperone and inhibitor subunit. Apoptotic activation of caspases (mostly caspase-3) results in the cleavage of the inhibitor and release of the nuclease, which form active homo-oligomers. The nuclease can be further stimulated or inhibited by other regulatory factors, including abundant chromatin proteins histone H1, HMGB1 or topoisomerase II. Another nuclease involved in apoptotic internucleosomal cleavage is endonuclease G, which in normal cells resides in the mitochondrial intermembrane space, and is released into the nucleus upon apoptotic disruption of mitochondrial membrane. DNA breakdown is usually temporally and functionally correlated with chromatin condensation, characteristic morphological hallmark of cells undergoing apoptosis. Although apoptotic cell death can occur without significant DNA degradation, this phenomenon apparently facilitates the engulfment of resulting apoptotic cell corpses by phagocytes and eliminates the transforming potential of any damaged or mutated DNA. Importantly, defects in apoptotic DNA fragmentation could be associated with a number of disease, including cancer and autoimmunity.

L085

Advantages of microlens-enhanced dual spinning disk confocal microscopy in functional imaging of chemo- and/or mechanoreceptors in the lung

Jean-Pierre Timmermans, Ian De Proost, Isabel Pintelon, Inge Brouns, Dirk Adriaensen

Univ. Antwerp, Dept. Veterinary Sciences, Lab Cell Biology & Histology, Antwerp, Belgium

Contact: jean-pierre.timmermans@ua.ac.be

Combined use of dual spinning disk confocal microscopy and a recently developed model based on vibratome slices of living lungs allows long-term ex vivo experiments to study the role(s) of pulmonary chemo- and/or mechanoreceptors [1,3]. Such an integrated approach of live cell optical recording, immunocytochemistry, biosensors and electrophysiology of these receptors in their original microenvironment offers much greater perspectives to study their function in health and disease compared to *in vitro* experiments on isolated cell lines in culture. Apart from studying the specific features of pulmonary neuroepithelial bodies (NEBs) this ex vivo lung slice model also enables to visualize and analyze paracrine signalling pathways within the NEB environment in control lungs and disease models. In this way we were able to show that ATP is released in a quantal manner from stimulated NEBs and activates through a P2Y₂-receptor mediated pathway the surrounding Clara-like cells. References: 1. De Proost et al. (2007) Selective visualisation of sensory receptors in the smooth muscle layer of ex-vivo airway whole-mounts by styryl pyridinium dyes. *Cell Tissue Res* 329: 421-431. 2. De Proost et al. (2008) Functional live cell imaging of the pulmonary neuroepithelial body microenvironment. *Amer J Respir Cell Molec Biol* (in press, doi:10.1165/rmb.2008-0011oc). 3. De Proost et al. (2008) Evidence for the release of ATP by activated pulmonary neuroepithelial bodies. Abstract XVIIth Meeting of the International Society for Arterial Chemoreception, Valladolid, Spain.

L086

Fluorescence imaging of neuroplastic changes induced by peripheral axotomy

Lars Klimaschewski

Medical University Innsbruck, Innsbruck, Austria

Contact: lars.klimaschewski@i-med.ac.at

Peripheral nerve lesions are common. They cause motor and sensory deficits with often serious clinical consequences such as prolonged paralysis, anaesthesia and neuropathic pain. Therefore, improvement of long-distance axonal regeneration is important for fast elongation of axons into target muscles which atrophy in the absence of reinnervation. Over the recent years the cellular basis for insufficient or incorrect axonal regeneration and consequent lack of functional recovery has been unraveled in various laboratories. Applying neuronal fluorescent tracers, multiple-labeling immunofluorescence and a variety of molecular techniques, we have demonstrated profound alterations of neuronal metabolism, survival, transmitter phenotype and regenerative capacity in lesioned neurons. This 'axotomy-response' is contributed by transcriptional changes triggered by the loss of trophic support from target organs and by signals generated at the injury site. Primary neurons derived from peripheral ganglia are particularly suitable to study regeneration-associated changes in response to either denervation from peripheral targets or from the central nervous system where the preganglionic autonomic neurons are located and the sensory afferents terminate, respectively. Their axons are capable of regeneration after lesion due to the intrinsic regenerative properties of peripheral neurons and because of the permissive environment provided by Schwann cells, extracellular matrix and neurotrophic activities. Various families of neurotrophic factors exist, e.g., the neurotrophins, neurotrophic peptides, neuropoietic cytokines and members of the fibroblast growth factor family that exert direct effects on neurons. Some of the different FGF proteins and their receptors have been shown to play a prominent role during axonal growth not only in developing neurons but during regeneration in the adult nervous system as well. My presentation will focus on fluorescence imaging techniques which we use to study the expression, regulation and possible functions of some of these trophic molecules in lesioned peripheral neurons (www.neuroanatomy.at).

L087

The esophagus as a source of unorthodox ideas on gastrointestinal innervation

Winfried Neuhuber

Institute of Anatomy, University of Erlangen-Nuremberg, Erlangen, Germany

Contact: winfried.neuhuber@anatomie1.med.uni-erlangen.de

Despite its ostensibly simple structure and function as a mere conduit for the passage of food from pharynx to stomach, the innervation of the esophagus turned out to be rather sophisticated. In contrast to the classical concept of vagal motor fibers to striated muscle and vagal preganglionic fibers to myenteric ganglia and farther to smooth muscle for efferent control, and vagal tension-sensitive afferents for afferent control of peristalsis, recent studies indicated an unexpected complexity of both motor innervation of striated muscle and vagal sensors. Striated esophageal muscle of non-mammalian and mammalian species, including human, receives innervation from both cholinergic vagal motoneurons and nitergic/peptidergic myenteric neurons. These findings challenged both the dogma of mononeuronal innervation of adult striated muscle and Langley's classical view of the autonomic nervous system as innervating everything except for striated muscle. As to afferent innervation, the esophagus provided a model for studying the so-called IntraGanglionic Lamellar Endings (IGLEs), the prominent vagal sensors in the gastrointestinal tract. Being low-threshold mechanosensors, IGLEs display several features which point to a local effector function, probably influencing myenteric neurons. These new discoveries rest mainly on histochemical and immunohistochemical studies complemented by functional experiments and, in aggregate, point to complex local reflex circuits particularly in striated esophageal muscle.

L088

Immunohistochemical investigations on the plasticity of enteric neurons in the course of some infectious and non-infectious diseases of the gastrointestinal tract

Jerzy Kaleczyc

Division of Animal Anatomy, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

Contact: jerzy.kaleczyc@uwm.edu.pl

It has been found that neurons can change their chemical phenotype under pathological conditions. Among peripheral autonomic neurons, enteric neurons are thought to be particularly highly plastic in their response to inflammation. These adaptive changes include both up and down regulation of transmitter expression and the induction of new genes in enteric nerve cells. They are developed not only to help enteric neurons to survive under pathological conditions but also to help the inflamed part of the gastrointestinal tract to recover. Most of the data on the plasticity of the enteric nervous system (ENS) have been gained in experiments performed on small laboratory mammals involving chemically induced intestinal inflammation. On the other hand, information on the adaptive changes of the enteric neurons in the course of inflammatory processes associated with infectious, invasive or systemic diseases affecting the gastrointestinal tract is very limited. This paucity of data deals especially with the ENS of the stomach and regards even such fundamental information as that on potential changes in the chemical coding of the enteric neurons during gastropathies. Recent studies performed on pigs have revealed distinct differences in the chemical properties of the enteric neurons supplying the gastrointestinal tract between the normal animals and animals suffering from swine dysentery or porcine proliferative enteritis. In general, they consisted in an increase in numbers of neurons expressing neuropeptides investigated except NPY and NOS, but different substances were up-regulated in particular intramural plexuses of different parts of the gastrointestinal tract. The most remarkable differences included an increased number of GAL- (from approx. 16% in control to 64% in dysenteric pigs) or VACHT- (56% vs. 87%) positive neurons in gastric submucosal plexuses, GAL-positive nerve cells in the inner submucosal plexus of the caecum (12% vs. 49%) or VIP-positive neuronal somata in the myenteric plexus of the ascending colon (8% vs. 23%). The results obtained suggest that acetylcholine, GAL, VIP, SOM, CGRP and SP have a specific role in the functioning of the inflamed porcine gastrointestinal tract in the course of gastroenteropathies. A better understanding of ENS plasticity during inflammation could be instrumental to develop new therapeutic strategies to treat some systemic diseases affecting the gastrointestinal tract.

L089

Neurotoxins as tools for "sculpturing" of the peripheral nervous system

Mariusz Majewski

Department of Human Physiology, Faculty of Medical Sciences,
University of Warmia and Mazury, Olsztyn, PolandContact: mariuszm@moskit.uwm.edu.pl

It is well-known that the correct functioning of the internal organs is under a continuous control of the peripheral nervous system. To achieve this goal, neuronal cells are equipped with a broad range of receptors and cellular ion channels (often fulfilling also receptor-like functions). As may be judged from many recent studies, a remarkable number of "neurogenic" diseases, which may be diagnosed, for example, in the lower urinary tract (LUT), is caused in fact by a pathology of a very specific, often only one, ion channel and/or receptor of the neuronal cell. Thus, it may be of interest to establish such kind of therapeutic agents that would be able to "recover" (or either "switch off") the function(s) of affected neurons by acting on pathologically changed ion channels and/or receptors. This "fine sculpturing" of the functional status of cells involved in the neural control of affected organ(s) may become in the future a component of new therapeutical protocols, supporting the "ordinary" pharmacological therapy. However, in order to achieve this goal, we need information considering mutually the mode of action(s) of such a putative drugs and changes in the chemical coding of affected neurons (their so-called "plasticity") they are able to evoke (both under the physiological and pathological conditions), as well as possible adverse side-effects. One of the most promising "groups" of such biologically active compounds involves neurotoxins, substances known to be able to discriminate between even a subtype of a receptor/ion channel when binding to the neuron. The present lecture is therefore aimed at unravelling the "state-of-the-art" of our knowledge considering the use of selected neurotoxins (like capsaicin, resiniferatoxin, botulinum toxin, ω -conotoxin GIVa, conatokine, tetrodotoxin and guanethidine) in the field of both the human and veterinary medicine, with special regards to their putative therapeutical applications in the neurourology.

L090

Impact of light stimuli on the maturation in neonatal mice and potential effects of the abnormal expression of stromal glycoprotein Lumican

Guo-min Zhou, Li-yun Liu

Fudan University, Shanghai, China

Contact: gmzhou@shmu.edu.cn

Purpose: We established the lighting ahead model in CD1 mouse and corroborated the temporal and spatial profile of the ectopic lumican expression in the light-affected retina with an attention to the neuronal growth in the lumican accumulated region. The results may contribute to the exploration of pathological mechanisms involved in development of retina influenced by early lighting. Methods and results: (1) Light stimuli inhibits retinal cell proliferation: At P9 light stimuli almost diminished the PCNA nuclear immunoreactivity dispersed in the marginal region of ONL due mostly to the susceptibility of the photoreceptor cells, while no significant alterations of general retinal cell proliferation were observed within P6-P9, the cellular growth promoting phase. However, during P10-P12, the cellular growth inhibiting phase, the negative effects of light stimuli on cell growth further extended to the intermediate neurons in INL and few of faint PCNA positive signals could be detectable in INL and the physical PCNA augment was inhibited absolutely within GCL. (2) Light stimuli influence retinal cell apoptosis: TUNEL staining was applied to estimate cell apoptosis. Neural cells within INL and ONL possess quite different apoptosis peak: for ONL apoptosis peaked at P12, the native eyelid separation, while for INL apoptosis reached the summit at P6. Lighting exposure alleviated cell apoptosis of photoreceptors and exhibited more complicated effects on INL cells. (3) Light stimuli activate ectopic lumican expression in retina: Early lighting initiated the abnormal lumican expression at P9, peaked at P12, and reversed to silence till P21. At P9, slight lumican was measurable in the retinal total protein extract and the product accumulated rapid afterwards. A 65kDa band evident at P12, implies that lumican deposited in retina is glycoprotein instead of KSPG, which was distributed predominantly in the inner portion inside the outer plexiform layer, including INL, GCL, outer and inner plexiform layer as well.

(4) Lumican ectopic expression and relative neuronal proliferative inhibition: With the lumican accumulation in INL and GCL, the renewal cells sharply decreased in the relative region of neural retina. Whereas lumican was mostly accumulated at P12, the nuclear PCNA signals dropped moderately, and PCNA boost observed in normal developing retina vanished completely in GCL. (5) Lumican ectopic expression and relative neuronal apoptosis maintenance: Immunofluorescent assay showed that caspase-9 could be detectable in the PCD cells within INL and GCL, with no unexpected label of ONL cells. Western-blot analyses demonstrated that the isoform distributed in these two layers is prominently p39 and no 49kDa band (whole length) was observed, proving it is a perfect marker for PCD cell labeling specially in INL and GCL.

L091

Varying colocalization of extracellular matrix components in the perineuronal nets of cortical parvalbumin-expressing neurons revealed by quantitative analysisOlga Grossenbacher-Zinchuk¹, Marco Celio², Vadim Zinchuk³

1Institute of Anatomy, University of Bern, Bern, Switzerland

2Department of Anatomy, University of Fribourg, Fribourg, Switzerland

3Department of Anatomy and Cell Biology, Kochi University Faculty of Medicine, Kochi, Japan

Contact: olga.grossenbacher@ana.unibe.ch

Perineuronal nets represent a unique form of extracellular matrix surrounding distinct populations of cortical neurons. They consist of several chondroitin sulfate proteoglycans, such as spectrin, restrictin, aggrecan, phosphacan, etc., and hyaluronic acid. The function of the nets, however, remains unclear. With the help of specialized computer software, we examined the pattern of colocalization of parvalbumin with spectrin and restrictin in parvalbumin-expressing neurons located in the layer V of temporal cortex. Colocalization was calculated using confocal images of neurons following background correction procedure using area selection. Both Pearson's correlation and Manders' overlap coefficients showed significant differences in the degree of colocalization of parvalbumin-expressing neurons double-stained with spectrin and restrictin. Quantitative analysis revealed three populations of neurons with high, moderate, and low degree of colocalization, representing 24.4%, 46.3%, and 29.3% for parvalbumin-spectrin stained neurons and 27%, 55.1%, and 17.9% for parvalbumin-restrictin stained neurons, respectively. Furthermore, neurons with nets double-stained for spectrin and restrictin displayed varying degree of colocalization as well. These findings show that temporal cortical parvalbumin-expressing neurons are of heterogeneous nature and that the relationship of their extracellular matrix components is very dynamic and more complex than it was previously thought. Varying colocalization of these components may be indicative of the rapid changes occurring in the perineuronal nets as a result of their stabilization and/or plasticity and should be taken into consideration when interpreting their functional characteristics. These conclusions only became possible after quantification of the images of double stained sections.

L092

Dynamic imaging of cancer cell invasion and plasticity

Peter Friedl

Radboud University Nijmegen, Nijmegen, Netherlands

Contact: peter.fr@mail.uni-wuerzburg.de

Cancer cell dissemination and metastasis *in vivo* result from a diverse set of migration strategies including individual cells and multicellular strands and clusters, referred to as collective invasion. Using 3D collagen lattices and *in vivo* intravital microscopy of cancer cell invasion, we have reconstructed at high resolution the subcellular location of pericellular proteolysis during the migration process, the resulting ECM remodeling, and invasion mechanism. Collective invasion *in vitro* was confirmed using *in vivo* xenografts monitored by intravital multiphoton microscopy. The findings show how cell invasion and proteolytic ECM remodeling form a functional unit to generate collective cell invasion along realigned tissue structures and further reveal novel compensation strategies that rescue single-cell migration after protease inhibitor-based treatment.

L093

Imaging of cytoskeletal functions of disseminating cancer cells

Erik Sahai, Silvia Giampieri, Caroline Hill

Cancer Research UK, London Research Institute, London, Great Britain

Contact: Erik.Sahai@cancer.org.uk

Here we use intravital confocal imaging to demonstrate a reversible transition to a motile, single cell state as cancer cells spread. Imaging primary tumours reveals considerable heterogeneity in cell morphology and cell motility. Two modes of motility are observed: collective and single-cell, which can be used to enter vessels. This motile behaviour is not maintained at secondary sites and cells revert to a more epithelial morphology. By monitoring the localisation of Smad2 and the activity of a TGF β -dependent reporter gene during these events *in vivo* we demonstrate that TGF β signalling is transiently and locally activated in motile single cells. Blockade of TGF β receptor or Smad4 function prevents cells moving singly *in vivo* but does not inhibit cells moving collectively. This work provides direct evidence for localised, transient and reversible changes in TGF β signalling driving changes in cell behaviour during metastatic dissemination.

L094

Visualizing stromal cell dynamics in different tumor microenvironments by spinning disk confocal microscopy

Mikala Egeblad

University of California, San Francisco. Dept. of Anatomy, San Francisco, United States

Contact: mikala.egeblad@ucsf.edu

The tumor microenvironment consists of stromal cells and extracellular factors that evolve in parallel with carcinoma cells. To gain insights into the activities of stromal cell populations, we developed and applied multi-color imaging techniques to analyze the behavior of these cells in different tumor microenvironments in the same live mouse. We found that regulatory T-lymphocytes migrated close to blood vessels. Dendritic-like cells, myeloid cells and carcinoma-associated fibroblasts all exhibited higher motility in the microenvironment at the tumor periphery than within the tumor mass. Since oxygen levels differ between tumor microenvironments, we tested if acute hypoxia could account for the differences in cell migration. Direct visualization revealed that regulatory T-lymphocytes ceased migration under acute systemic hypoxia, whereas myeloid cells continued migrating. We next experimentally subdivided the myeloid cell population in the same mouse and microenvironment: uptake of fluorescent dextran defined a low-motility subpopulation that expressed markers of tumor-promoting, alternatively activated macrophages. In contrast, fluorescent anti-Gr1 antibodies marked myeloid cells that patrolled inside tumor vessels and in the stroma. Our techniques allow real-time dynamic analysis of stromal cell populations based on spatial location, gene expression, cell behavior and cell surface molecules within intact tumors and reveal that stromal cell behavior varies with the microenvironment.

L095

Visualization of chemokine signaling in morphogenetic tissue movements *in vivo*

Darren Gilmour

European Molecular Biology Laboratory Cell Biology and Biophysics, Heidelberg, Germany

Contact: gilmour@embl.de

Thanks to the development of powerful *in vivo* imaging techniques, it is now possible to study the role of putative guidance molecules and their receptors in regulating tumour metastasis within living specimens. One useful complementary method for addressing how guidance molecules control such movements *in vivo* is to study their function during the normal process of embryonic morphogenesis, when complex organs are formed through precisely controlled collective cell migrations. Of all systems currently used, the zebrafish embryo has a number of intrinsic features, such as rapid, external development and unparallelled optic clarity, which make it the ideal specimen for imaging dynamic cell behaviours *in vivo*. Our model tissue is the primordium of the lateral line organ, a migrating epithelium of approximately 150 cells whose function is to deposit a series of mechanosensory organs throughout the skin of the fish. Previous genetic studies have revealed that the primordium migra-

tes along a pre-patterned stripe of the chemokine CXCL12/SDF1, which it detects through its receptor CXCR4, a ligand-receptor pair that is known to determine the homing destination of a number of human tumour types. By combining *in vivo* imaging with a number of functional approaches – such as genetic mosaics, ectopic expression and laser nanosurgery – we have begun to address how changes in the level of chemokine signaling allow a variety of migratory behaviours within a moving tissue.

L096

Cell migration assay of glioblastoma cell using an automated screening methodMarco Rossi¹, Cinzia Giordano¹, Patrizia Tunicci¹, Massimiliano Salerno¹, Alessandra De Robertis¹, Silvia Matteoni¹, Roberto Raggiaschi¹, Elisa Mori¹, Letizia Magnoni¹, Hendrick Bothmann¹, Serena Pellegatta², Annette Bakker¹¹Siena Biotech S.p.A., Siena, Italy²Istituto Nazionale Neurologico Besta, Milan, ItalyContact: mrossi@sienabiotech.it

Glioblastoma multiforme (GBM) is the most common primary malignant tumor of the adult central nervous system and has a median survival time after diagnosis is of 14.6 months [1]. The highly lethal nature of this tumor partly derives from the acquisition of an invasive phenotype, which allows the tumor cells to infiltrate the surrounding brain tissues [2]. The tumor cells leading to the deal of recurrent GBMs highly contribute to the lack of success in eradicating this disease. Identification and characterization of genes that drive the invasive behavior of GBM might serve as reliable diagnostic and prognostic markers, as well as potential key targets for effective therapy. In order to obtain this focus the setup of an assay to study correctly the cell migration is very important and fundamental. The scratch assay is an easy, low-cost and well-developed method to measure cell migration *in vitro*. We have developed a screening assay based on scratch assay with two GBM cell lines stable expressing AcGFP protein to distinguish with an automated fluorescence microscope the cell migration and morphology. Since RAC1 plays a key role in motility [3], siRNA for RAC1 was used to validate the assay. Subsequently, we have analyzed the effect on cell motility of 10 known anti-tumor compounds used for treatment of GBM and other cancers. The results in 2D scratch assay showed that Panzem-(2ME2), HSP90 inhibitor and GSK3b inhibitor compounds are able to inhibit the cell motility of GBM cells at 10 μ M with an inhibition of 70%, 50% and 30% respectively in T98G cells and an inhibition of 50% for Panzem-(2ME2), and 65% for GSK3b and HSP90 inhibitor compounds in DBTRG-05MG cells. Recent evidence suggests that a small population of stem-like cells present in brain tumors could be responsible for the GBM tumour initiation and renewal. These cells share many properties with normal neural stem cells, including their capacity to self renew, to differentiate and to grow as floating 3D neurospheres (NS) in well-defined culture conditions. Microarray and proteomic analysis demonstrated that cellular movement is one of the top processes that drive GBM stem-like cell (grown as NS) behavior. Therefore it was considered essential for us to develop a 3D cell migration assay to measure the cell spreading motility of NS. In conclusion, the developed methods are suitable to evaluate the functional anti-migratory effects of small molecules and/or siRNAs in both 2- as well as in 3-dimensional culture conditions. Bibliography: 1. Stupp R. et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005 10;352:987-96. 2. Giese A, Bjerkvig R, Berens ME, Westphal M. Cost of migration: invasion of malignant gliomas and implications for treatment. *J Clin Oncol.* 2003; 21:1624-1636. 3. Keely PJ, Westwick JK, Whitehead IP, Der CJ, Parise LV. Cdc42 and Rac1 induce integrin-mediated cell motility and invasiveness through PI(3)K. *Nature* 1997;390:632-6.

L097

Force generation during leukocyte locomotion

Michael Sixt

Max Planck Institute of Biochemistry, Martinsried, Germany

Contact: sixt@biochem.mpg.de

The current paradigm of cell migration is a multistep process: initial protrusion is driven by actin-polymerization at the leading edge. This is followed by myosin dependent contraction of the cytoskeleton that imposes rearward pulling forces on transmembrane adhesion receptors to allow forward movement of the cell body. Finally adhesions at the trailing edge are

disassembled and the cell body is retracted. While this model applies to slow migrating cells on two-dimensional surfaces, we asked if adhesion-mediated traction is also the force generating principle for the 100 times faster migration of leukocytes. As integrins are the essential adhesion receptors for metazoan cell migration, we employed mouse genetics to generate primary leukocytes (dendritic cells, granulocytes) that are completely devoid of this receptor family. Using time-lapse videomicroscopy based *in vivo* migration assays (in skin and lymph node) and *in vitro* 3D chemotaxis assays we demonstrate that interstitial leukocyte migration in 3D environments does not require integrin-mediated adhesion while adhesion is essential for movement over 2D surfaces. Functional analysis of the non-adhesive migration mode revealed that leading edge actin protrusion is the sole driving force of forward locomotion. However, such protrusive migration is incapable of overcoming large extracellular counter forces and the cells use auxiliary myosin II dependent trailing edge contraction at sites where they must squeeze their rigid nucleus through narrow pores. Having established that directional actin-treadmilling is the main determinant of leukocyte movement, we analysed the functional consequences of perturbed actin flow on leukocyte locomotion in different extracellular environments. Using *cdc42*-deficient leukocytes, we demonstrate that coordinated polarization of the cytoskeleton is absolutely essential for migration in 3D interstitial environments while movement on 2D surfaces is less affected.

L098

The dynamics of immune cells *in vivo*

Matthias Gunzer

Otto-von-Guericke University, Magdeburg, Germany

Contact: matthias.gunzer@med.ovgu.de

The ability to autonomously migrate is a fundamental property of all immune cells. In addition, different cell types need to interact physically in order to exchange information or function. In contrast, during an ongoing infection, immune cells need to interact with pathogenic particles for phagocytosis and defence. The interaction zone between antigen presenting cells (APC) and helper T cells has been termed "immunological synapse" (IS). In the first part of my talk I will present our findings on the motility of T cells and different antigen presenting cells, how they interact with each other and what consequences this has for the molecular structure of their IS. I will demonstrate what the functional outcome of these synapses is with respect to T cell function and that this might have direct implications for novel therapeutic approaches. In the second part of the talk I will focus on the innate immune system and its function under normal circumstances as well as conditions of infection with a focus on polymorphonuclear neutrophil granulocytes (PMN). PMN are the most efficient migrators identified so far in our systems. They present with potent motility at the most distinct environments. Despite analyzing their motility *in vivo* we have generated a highly resolved analyses of the phagocytosis process of different fungal elements by PMN and other phagocytes. We can demonstrate a massive influence of the environmental dimensionality as well as the type of pathogen encountered by the cells on the phagocytosis process. In addition, different phagocytes use different strategies for defence. In summary, the dynamics and interactions of immune cells in all body areas are extensive and their investigation by molecular microscopy uncovers previously unknown mechanisms of immune protection and its control.

L099

Endoplasmic reticulum stress and cellular toxicity of mutant huntingtin

Yufeng Jiang, Huiping Tan, Yinong Zhang, Weixi Wang, He Li

Division of Histology and Embryology, Department of Anatomy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Contact: heli@mails.tjmu.edu.cn

Huntington's disease (HD) is a dominant hereditary neurodegenerative disorder characterized by severe neuronal loss in the brain, particularly in the striatum and cerebral cortex. At a molecular level, HD is caused by expansion of a polyglutamine repeat in the amino-terminal region of huntingtin (Htt) protein. Polyglutamine expansion causes the mutant Htt to accumulate and aggregate in the nuclei and cytoplasm of neurons. Accumulation of unfolded and misfolded proteins induces endoplasmic reticulum (ER) stress, activating caspase-12 located on the ER, finally causing apoptosis. Little is known about the relationship between ER stress and mutant Htt. Here, we analyzed the role of the ER stress in cellular toxic-

ity of mutant huntingtin. In neuroblastoma (N2a) cells, transfected mutant Htt induced upregulation of glucose-regulated protein 78 (GRP78), the chaperone that increases protein folding in the ER lumen, and activation of caspase-12. In HD transgenic mouse brain and N2a cells expressing mutant Htt, GRP78 was found to be colocalized with the aggregates of mutant Htt. Overexpression of GRP78 could inhibit formation of mutant Htt aggregates, reducing activation of caspase-12 and increasing cell viability, whereas silencing of GRP78 by siRNA promoted formation of mutant Htt aggregates, elevating activation of caspase-12 and enhancing the decrease in cell viability. Moreover, it was found that mutant Htt-induced upregulation of GRP78 and activation of caspase-12 could be significantly diminished by edaravone, a potent scavenger of free radicals, and by X2628, a blocker of IP₃R which mediates Ca²⁺ release from ER. Thus, mutant Htt could stimulate ER stress through inducing peroxidation and Ca²⁺ release from ER. Stimulation of ER stress is involved in the cellular toxicity of mutant Htt. This work was supported by the National Natural Science Foundation of China (30430260).

L100

Exploratory study of co-transplantation of neurotrophin-3 gene modified Schwann cells and neural stem cells in the recovery of spinal cord injury

Yuan-shan Zeng

Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China

Contact: zengysh@mail.sysu.edu.cn

To provide a new strategy of treating spinal cord injury, the present study explores whether (i) co-transplantation of neurotrophin-3 (NT-3) gene modified Schwann cells (SCs) and neural stem cells (NSCs) or/and NSCs induced by retinoic acid (RA), or/and (ii) NSCs modified by neurotrophin-3 receptor (TrkC) gene could promote the recovery of structure and function of rat spinal cord transected completely. SCs were transfected by recombinant adenovirus vector carrying NT-3 gene, (NT-3-SCs). In the meanwhile, NSCs were induced by RA (RA-NSCs) and then NSCs transfected by recombinant adenovirus vector carrying TrkC gene (TrkC-NSCs). Then NT-3-SCs+ NSCs, NT-3-SCs+RA-NSCs and NT-3-SCs+TrkC-NSCs, respectively, were grafted into the transected site of spinal cord. 60 days after the transplantation, the hind limb locomotive functions of the rats were observed and recorded by BBB score. Fluorogold was injected into the spinal cord 3mm caudal to the transected site, which labeled retrogradely the regenerative nerve fibers of corticospinal tract. Biotinylated dextran amine (BDA) was injected into the somatosensory motion area of cerebral cortex, which labeled anterogradely the regenerative nerve fibers of the corticospinal tract. The cortical motor evoked potential (CMEP) and cortical somatosensory evoked potential (CSEP) of the rats were detected. The spinal cords were taken after rats were sacrificed under anesthesia and sectioned for morphological observation. The results displayed that the survival of injured neurons, regeneration of nerve fibers, recovery of CSEP and CMEP, and score of BBB in completely transected spinal cord were increased after the co-transplantation of NT-3-SCs plus NSCs, NT-3-SCs plus RA-NSCs, and NT-3-SCs plus TrkC-NSCs. Meanwhile, the differentiation of grafted NSCs into neuron-like cells, which had a potency of forming synapses, was promoted. The results suggest that co-transplantation of NT-3-SCs plus NSCs, NT-3-SCs plus RA-NSCs, and NT-3-SCs plus TrkC-NSCs may enhance partial recovery of structure and function of injured spinal cord.

L101

Neurodegenerations: from neuropathology to structural biology

Pawel P. Liberski

Department of Molecular Pathology and Neuropathology, Medical University of Lodz, Lodz, Poland

Contact: lucy@csk.umed.lodz.pl

Neurodegenerations are a group of central (and peripheral) nervous system diseases that are caused by pathologically misfolded proteins. It includes prion diseases (an abnormal isoform of the prion protein, PrP^{Sc}), Alzheimer diseases (A β and A β peptide), tauopathies (MAP-t) and synucleinopathies. The transition of a normal, usually α -helical peptide into its mostly β -sheet pathological isoform underlies the pathogenesis. In this review, I will show examples of conformation disorders diagnosed by electron-microscopy, immunoelectronmicroscopy and immunohistochemistry along the data of structural biology.

L102

Progression of Alzheimer disease correlates with the increased neuronal DNA replicationAttila Tarnok¹, Birgit Mosch², M. Morawski², Thomas Arendt², Anja Mittag³¹Department of Paediatric Cardiology, Cardiac Centre, University Leipzig, Leipzig, Germany²Paul Flechsig Institute of Brain Research, Department of Neuroanatomy, University of Leipzig, Leipzig, Germany³Translational Center for Regenerative Medicine (TRM-Leipzig), University of Leipzig, Leipzig, GermanyContact: tarnok@medizin.uni-leipzig.de

Reactivation of the cell cycle, including DNA replication, might play a major role in Alzheimer's disease (AD). A more than diploid DNA content in differentiated neurons might alternatively result from chromosome mis-segregation during mitosis in neuronal progenitor cells. It was our objective to distinguish between these two mechanisms for aneuploidy and to provide evidence for a functional cell cycle in AD. Using slide-based cytometry, chromogenic *in situ* hybridization, and PCR amplification of alu-repeats, we quantified the DNA amount of identified cortical neurons in normal human brain and AD and analyzed the link between a tetraploid DNA content and expression of the early mitotic marker cyclin B1. In the normal brain, the number of neurons with a more than diploid content amounts to approximately 10%. Less than 1% of neurons contains a tetraploid DNA content. These neurons do not express cyclin B1, most likely representing constitutional tetraploidy. This population of cyclin B1-negative tetraploid neurons, at a reduced number, is also present in AD. In addition, a population of cyclin B1-positive tetraploid neurons of approximately 2% of all neurons was observed in AD. Our results indicate that at least two different mechanisms need to be distinguished giving rise to a tetraploid DNA content in the adult brain. Constitutional aneuploidy in differentiated neurons might be more frequent than previously thought. It is, however, not elevated in AD. In addition, in AD some neurons have re-entered the cell cycle and entirely passed through a functional interphase with a complete DNA replication.

L103

Quetiapine facilitates oligodendroglia development and prevents mice from demyelinationLan Xiao¹, Yanbo Zhang², Xinmin Li²¹Department of Histology and Embryology, Third Military Medical University, Chongqing, China²Neuropsychiatry Research Unit, Department of Psychiatry, University of Saskatchewan, Saskatoon, CanadaContact: xiaolan35@hotmail.com

Antipsychotic drugs are the main medications for schizophrenia. Although their therapeutic and adverse effects are recognized, the underlying mechanisms remain to be elucidated. Recent neuroimaging and post-mortem studies have reported abnormalities in white matter of schizophrenic brains, suggesting the involvement of oligodendrocytes in the etiopathology of schizophrenia. However, there is currently little information available on the response of oligodendrocytes to antipsychotic drugs (APDs), which could be invaluable for corroborating the oligodendrocyte hypothesis. In this study we found: (1) quetiapine (QUE, an atypical APD) treatment in conjunction with addition of growth factors increased the proliferation of neural progenitors isolated from the cerebral cortex of embryonic rats; (2) QUE directed the differentiation of neural progenitors to oligodendrocyte lineage through extracellular signal-related kinases; (3) addition of QUE increased the synthesis of myelin basic protein and facilitated myelination in rat embryonic cortical aggregate cultures; (4) chronic administration of QUE to C57BL/6 mice prevented cortical demyelination and concomitant spatial working memory impairment induced by cuprizone, a neurotoxin. These findings suggest a new neural mechanism of antipsychotic action of QUE, and help to establish a role for oligodendrocytes in the etiopathology and treatment of schizophrenia.

L104

Confocal microscopy of amyloid plaques in prion diseasesBeata Sikorska¹, Pawel P. Liberski¹, Herbert Budka², James W. Ironside³¹Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Lodz, Lodz, Poland²Institute of Neurology, Medical University of Vienna, and Austrian Reference Centre for Human Prion diseases, Vienna, Austria³National Creutzfeldt-Jakob Disease Surveillance Unit, University of Edinburgh, Edinburgh, United KingdomContact: elmo@csk.umed.lodz.pl

Amyloid plaques are a hallmark of transmissible and non-transmissible brain amyloidoses, and in human transmissible spongiform encephalopathies occur in sporadic CJD (sCJD), kuru (both of which may contain kuru plaques), Gerstmann-Sträussler-Scheinker disease (GSS) (multicentric plaques) and variant CJD (vCJD) (florid plaques). Amyloid plaques in 5 cases of variant CJD, 3 cases of GSS, 2 cases of sCJD, 1 case of kuru and 5 cases of AD were studied by immunohistochemistry and confocal laser scanning microscopy and additionally by transmission electron microscopy and ultrastructural image analysis. Antibodies used in this study included antibodies against: HLA-DR, GFAP, APP, CD68, NFP, MAP-tau, SMI31 SMI32, PrP, ubiquitin, p62. Immunohistochemistry showed the presence of microglial cells, astrocytic processes and dystrophic neurites at the periphery of the plaques. Immunoreactivity of AT8 antibody revealed hyperphosphorylated MAP-tau at periphery of the plaques in vCJD. This finding is reminiscent of AD plaques, but in contrast to Alzheimer's disease no PHF were observed in the EM. In our results, contrary to the previous report, minimal immunoreactivity in or around the plaques in kuru, GSS and sCJD was also observed. This difference is probably due to the lack of plaques in sCJD cases studied in previous reports. No co-localization of PrP^{Sc} with MAP-tau or p62 was observed. Conclusions: The structure of florid plaques of vCJD is more reminiscent of neuritic plaques in AD than kuru or multicentric plaques. The reasons for these differences in prion protein plaque structures are poorly understood, but may reflect differences both in the strains of the transmissible agents responsible for these disorders and in host factors such as codon 129 of the *PRNP* gene status. The reasons for the presence of MAP-tau and p62 immunoreactivity around amyloid plaques may be only speculated, the influence of close neighborhood of amyloid fibrils should be taken into consideration.

L105

***In situ* hybridization with quantum dot detection and confocal scanning microscopy for three-dimensional visualization of protein and mRNA: comparison with electron microscopic *in situ* hybridization**Akira Matsuno¹, Johbu Itoh², Susumu Takekoshi², R.Yoshiyuki Osamura²¹Teikyo University Chiba Medical Center, Dept. of Neurosurgery, Chiba, Japan²Tokai University School of Medicine, Kanagawa, JapanContact: akirakun@med.teikyo-u.ac.jp

We have elucidated the intracellular localization of pituitary hormone and its mRNA using electron microscopic *in situ* hybridization and immunohistochemistry [1-3]. This method has very high resolution, and provides two-dimensional images of subcellular localization of pituitary hormone and its mRNA in a pituitary cell. Using preembedding method, growth hormone (GH) messenger RNA are localized as osmium black on RER. GH protein is identified mainly on the secretory granules with colloidal gold particles, and is also identified in the cisternae of RER. However, electron microscopic *in situ* hybridization and immunohistochemistry method cannot tell the localization of mRNA and protein three-dimensionally. Confocal laser scanning microscopy can visualize the localization of protein three-dimensionally. Recently, nanocrystals such as Quantum dot (Qdot) are available for confocal laser scanning microscopic observation. We apply Qdot for the simultaneous detection of intracellular localization of mRNA and its encoded protein in rat pituitary cells. Successfully we can visualize GH and its mRNA (Fig. 1), prolactin and its mRNA with Qdot 605 and 655 [4,5]. This method is useful because it can provide the spatial and three-dimensional relationship between mRNA and protein. Electron microscopic *in situ* hybridization and immunohistochemistry has advantage of high resolution. Meanwhile, *in situ* hybridization and immunohistochemistry using Qdots and confocal laser scanning microscopy has merit that it can provide three dimensional images. Thus, both methods are

useful for understanding the relationship between protein and mRNA simultaneously in two or three dimensions. References: 1. A.Matsuno et al, *Histochem J* 28(1996)703. 2. A.Matsuno et al, *Histochem J* 30(1998)105. 3. A.Matsuno et al, *Acta Histochem Cytochem* 31(1998)259. 4. A.Matsuno et al, *J Histochem Cytochem* 53(2005)833. 5. A.Matsuno et al, *J Acta Histochem Cytochem* 38(2005)253.

L106

Applications of ImageStream system for identification and characterization of murine and human stem cells

Ewa K. Zuba-Surma¹, Magdalena Kucia¹, David A. Basiji², William E. Ortyń², James W. Lillard¹, Mariusz Z. Ratajczak¹

¹Brown Cancer Center, University of Louisville, Louisville, United States

²Amnis Corporation, Seattle, United States

Contact: ekzuba01@louisville.edu

ImageStream system (ISS) is a novel technology combining the capabilities of flow cytometry with high resolution fluorescent microscopy in one platform. The unique capabilities of the system allow the imaging of large populations of cells in suspension and subsequent analysis. Several morphological and fluorescence- features cells can be quantitatively measured including size, shape, texture, and location of probes within, on or between cells. The analyzes of more than 500 image features can be perform on single cell level as well as for selected populations. A growing list of publications is indicative of the multiple possible applications of the system including the evaluation of transcription factor translocation (also in rare subsets of cells), co-localization of molecules in different cellular compartments, protein trafficking, apoptosis quantification including via TUNEL technique, detection of probe binding sites in FISH assays and other several applications (*Clin Lab Med* 2007; 27:653; *Folia Histochem Cytobiol.* 2007; 45:279). In our laboratory, we have found ISS technology to be a valuable method, supporting classical flow cytometry for the identification and morphological characterization of very rear and unique populations of murine and human stem cells such as very small embryonic like stem cells (VSELs). Recently, we identified these primitive cells in adult murine organs including bone marrow (BM) as well as in human cord blood (CB). VSELs i) are CXCR4⁺ Oct-4⁺ SSEA-1⁺ Sca-1⁺ CD45⁻ lin⁻ in mice (*Leukemia* 2006;20:857) and CXCR4⁺ Oct-4⁺ SSEA-4⁺ CD34⁺ AC133⁺ CD45⁻ lin⁻ in humans (*Leukemia* 2006; 21:297); ii) exhibit features of pluripotent stem cells *in vitro*; iii) contain large nuclei with primitive euchromatin and iv) are very small in size (less than 4µm) explaining why VSELs were missed during classical isolation procedures applied in cell sorting (*J Cell Mol Med* 2008; 12:292). Identification of VSELs is difficult and challenging because of the very small size of these cells, which overlaps with cellular debris. By employing ISS, we were able to distinguish VSELs from cellular fragments and falsely positive artifacts which, was especially important for their identification in various tissues after the enzymatic digestion. Moreover, we have characterized morphological features of VSELs isolated from different murine and human tissues that are related to the primitivity of these cells such as size and nuclear to cytoplasmic (N/C) ratio. The ISS is the first technology capable of "decoding the dots" by analyzing the images of the acquired objects which have been visualized until now only as "dots" (events) on the flow cytometric dot-plots. Because of this unique capability, ISS has become a useful tool supporting classical flow cytometry for identification of rare stem cell populations.

L107

New technology and new possibilities in flow cytometry with use of the Cell Lab Quanta SC MPL Beckman Coulter instrument

Roman Pińkowski

Comesa Polska sp. z o.o., Warszawa, Poland

Contact: rpinkowski@comesa.pl

Almost 3 years ago Beckman Coulter introduced to cell analysis new digital flow cytometer type Cell Lab Quanta SC (CLQ). The instrument provides microtitre plate and microtube-based analysis, with 3-color, Coulter Electronic Volume and Side Scatter analysis for unsurpassed precision and time savings. CLQ uses two light source UV 365 nm and Blue 488 nm and gives reliability to work and improve efficiency for a wide array of applications. The Cell Lab Quanta was designed to simultaneously measure

Electronic Cell Volume (EV) and fluorescence (FL). The combination of the EV and FL makes it possible to estimate other parameters like Diameter of cell (µ), Surface of cell (µ²), Mean Cell Volume MCV (µ³), Flow Surface Density FSD (FL/Surface) and Flow Concentration FC (FL/MCV). The number of cell per mL is measured directly without any additional calibration material. EV also helps to discriminate clumps of cells, small dying cells, and debris. Moreover, the Quanta SC MPL provides an unparalleled level of adaptability in applications. With its innovative Multi-Platform Loader (MPL) and AutoSetup capability, the operator can now use sample cups, microcentrifuge tubes and 24-, 96- or 384-well different plates on the same system. Side cup holders contain sample or reagent even for eight different types. Probes are washed automatically. Automatic sample preparation including incubation saves time and eliminates human errors. MPL provides operator many combinations of customized sample preparations including high standardization of sample volume, adding from one to eight reagents' volume, time of the incubation, numbers and intensity of dispensing or shaking etc. The software includes ready to use applications related to apoptosis analysis dual staining, 3-color immunophenotyping, stem cell and stem cell side population analysis, cell cycle and ploidy analyses, distribution of cells into live and dead populations, CFP, GFP, YFP and DsRedFP, phosphorylation of proteins, measurement of bacteria with sensitivity to the size of 0,5 µm, and many other. The optical system and detectors make possible to apply many fluorochromes: DAPI, Hoechst, Cyto, Cascade Yellow, YoPro, FITC, PE, PC5, PC7, PI, Alexa Fluor 488 and many others. CLQ do the compensation automatically in real time and after acquisition. The analysis of the results, the Operator can do in any time with use CLQ workstation or with use own personal computer for example at home. The CLQ software is integrated with Microsoft Office (PPT, Excel) so the Operator saving time can create all document for data calculation and statistic or for presentation and publication.

L108

Publishing and publications in histochemistry: Round Table Symposium

Raymond Coleman

Technion-Israel Institute of Technology, Haifa, Israel

Contact: coleman@tx.technion.ac.il

The adoption of digital technologies has lead to a revolution in scientific publishing. The widespread use of the Internet, specialist databases, search engines, journal citation indices has resulted in commercial publishers of scientific journals needing to adopt new strategies to survive the digital age. Printed copies will almost certainly soon be a thing of the past. Publishers are seeking means to maintain their profitability and we are seeing new approaches including open access versus the more traditional publishing with page charges or fees for downloading papers. Digital publishing has resulted in new strategies including changing the format of manuscripts, allowing supplementary on-line information, video journals with 3-dimensional movies. Scientific libraries are faced with increased selectivity in their purchasing policies. The value of journal impact factors needs to be reassessed as new and improved approaches to evaluation become available. The role of editors is changing, though peer review remains the best method of evaluation. There are continuing problems with authorship and who deserves to be regarded as an author, or even identification of authors when they have very common surnames. The Internet is changing the way we deal with manuscripts and there is considerable suspicion that the traditional manuscript will be supplanted by new forms of information exchange (blogs, commentaries, specialized chat groups).

L109

Measuring journal success: impact factor alternatives

Kevin Roth

Journal of Histochemistry and Cytochemistry, Birmingham, AL, United States

Contact: karoth@uab.edu

A journal's impact factor (IF) is a number calculated annually by Thomson Scientific (Philadelphia, PA; www.isiknowledge.com) based on data collected from approximately 6000 science and technology journals. The annual IF is calculated based on the frequency with which articles published in a given journal over a two year period are cited in the subsequent calendar year. Thus, a journal's 2007 IF equals the number of times that

articles published in that journal in 2005 and 2006 were cited in 2007 divided by the number of articles published in 2005 and 2006. The IF for a particular journal or a group of journals classified by subject category can be found through Thomson Scientific's Journal Citation Reports (JCR). IFs have commonly been used to rank journals relative to one another or as an annual measure of a journal's success. Recently, several authors have called into question the methodology employed to determine IFs and in particular, the utility of IFs in measuring journal quality. In addition to the JCR IF, several other methods for assessing a journal's relative success have recently become available. Among these are the SCImago Journal and Country Rank (www.scimagojr.com) and Eigenfactor (www.eigenfactor.org). Both SCImago JCR and Eigenfactor employ algorithms similar to Google's PageRank to assess not simply the number of citations to articles published in a journal but the relative influence of the citing journals. Thus unlike the JCR IF, a citation from a manuscript published in a prestigious scientific journal carries more "weight" than a citation appearing in a poorly regarded journal. This type of ranking system may provide a more accurate measurement of a journal's prestige and provides an alternative measurement of a journal's success. Although these journal ranking indicators provide useful information, the ultimate success of a journal can only be assessed in the context of the journal's mission. For example, the Journal of Histochemistry and Cytochemistry (JHC) is the official journal of the Histochemical Society (HCS) whose mission is to advance the study of cell and tissue biology with molecular and morphological techniques (www.histochemicalsociety.org). Publication of manuscripts outside of this focused area could potentially improve the JHC's IF but it would not meet the HCS's mission and the "success" of the journal, as defined by the HCS, would decrease. In total, the measurement of a journal's success cannot be distilled to a single number and must take into account both objective, measurable outcomes such as citation frequency and a careful, but potentially subjective, analysis of the journal's overall goals.

L110

Acta Histochemica et Cytochemica, an official Journal of Japan Society of Histochemistry and Cytochemistry: Introduction and recent achievement to modernize it as on-line Journal

Takehiko Koji

Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Contact: tkoji@nagasaki-u.ac.jp

Acta Histochemica et Cytochemica was founded in 1968 and since then each issue was published bimonthly. So far, we published 40 volumes and are proud of our significant contribution to the world of histochemistry and cytochemistry with various developments and improvements of histochemical techniques. Our Journal publishes Regular articles, Reviews, Technical advancements, Notes and Letter to the Editor. We are publishing 19-23 articles per year after 2006. Until now, 6 persons acted as Editor-in-Chief of Acta Histochemica et Cytochemica; late Professor Hideo Takamatsu, late Professor Kazuro Ogawa, Professor Emeritus Setuya Fujita, late Professor Tsukasa Ashihara and Professor Tetsuro Takamatsu. Thus, I am the 6th and have been charged from January 1st, 2008. Now all submitted manuscripts to the Journal have been peer-reviewed at least by one of three Associate Editors; Professor Shoichi Iseki, Professor Norimichi Nemoto and Professor Naoki Saito, and by two of domestic and international Editorial Board members, including ad-hoc referees if necessary. Our current goal is to reduce the time and effort for reviewing process, keeping the quality of the Journal. Our Journal has been cited in ISI, but was not cited in Medline. In fact, the impact factor of our Journal was fluctuated between 0.4-1.0 for a long time. We recognized the lack of citation in a big on-line search engine as one of the major causes for the underestimation. Then we decided to publish our Journal in on-line format as well as print format in 2000 by being in J-Stage as an open access Journal. Consequently, all contents of Acta Histochemica et Cytochemica have become to be cited in PubMed Central from 2006. Therefore, now we believe that scientists, especially non-members of our Society, can have much better access to the contents of our Journal, and we as Editor would like to expect more frequent submissions and citations from all over the world.

L111

Cytometry Part A – the quantitative cell analysis journal

Attila Tarnok

Department of Paediatric Cardiology, Cardiac Centre, University Leipzig, Leipzig, Germany

Contact: tarnok@medizin.uni-leipzig.de

Cytometry Part A embraces the study of the cytochrome, covering all aspects of molecular analysis of cellular systems and publishes original research articles, in-depth reviews, rapid communications of new, novel "hot" topics, and technical innovation articles. Cytometry Part A is the journal of the International Society for Advancement of Cytometry, ISAC www.isac-net.org. "Cytometry Part A covers the scientific discipline of quantitative and stoichiometric single cell analysis. Cytometry Part A is a truly transdisciplinary journal. Research includes cells, tissues, live animals, lower organisms and plants and microbes. Cytometry is by tradition also the technology required to perform these analyses: flow cytometry, quantitative automated imaging, spectral- and hyperspectral analyses, standardization and quality control and mathematical modelling. Because high-throughput and high-content analytical methods are applied, Cytometry Part A is the natural harbor for systemic cell analysis; such as Cytomics and Systems Biology." Topics covered by the journal are: Cytomics and systems biology; Cell biology and microbiology; high-throughput and high-content screening; flow and slide-based cytometry; multimodal imaging; *in vivo* cytometry; innovative cytometric assays and technologies; Molecular array technologies; cell-based spectroscopic analyses; bioinformatics/computational methodologies. Topical coverage includes: – New, novel instrumentation and reagent developments relevant to the measurement of cells or cell constituents. – New techniques or procedures for the analysis of cellular features, constituents, and function. – Novel analysis, statistical approaches, and mathematical modeling techniques relevant to the analysis of analytical cytology and cytomics data. – Application of analytical and cytomics techniques to the areas of cell biology, molecular biology, virology, physiology, pathology, cancer, veterinary science, plant science, genomics, and proteomics. The special features for the journal are: (a) Online manuscript submission and peer review for rapid first decisions. (b) Early View for rapid online publication-articles posted online as soon as they are ready, before the release of the compiled print issues. (c) Rapid review process: ~30days in the mean from 1st submission to 1st decision. (d) Online publication of supplementary material such as data sets and videos. (e) FREE color for all figures in the online editions. (f) ISAC and Clinical Cytometry Society members receive a 50% discount on reprint purchases. The Impact factor for 2006 was 3.293. The editor-in- Chief is Attila Tarnok <http://www.interscience.wiley.com/journal/cytometry>.

L112

Folia Histochemica et Cytobiologica – Official Journal of Polish Histochemical and Cytochemical Society

Lech Chyczewski¹, Lukasz Minarowski²

¹Department of Clinical Molecular Biology, Medical University of Białystok, Poland

²Department of Lung Diseases and Tuberculosis, Medical University of Białystok, Poland

Folia Histochemica et Cytobiologica was established in 1963 under the title: "Folia Histochemica et Cytochemica" by the Polish Histochemical and Cytochemical Society as an international, English-language journal devoted to the rapidly developing fields of histochemistry, cytochemistry and cell biology. In 1984, the profile of the journal was broadened to accommodate papers dealing with cell and tissue biology, and the title was accordingly changed to "Folia Histochemica et Cytobiologica". Currently we publish review articles, original articles, short communications, proceedings of scientific congresses/symposia and book reviews. Since 2007 we implemented several features in order to improve the accessibility of the journals' content. Currently the electronic version published quarterly on-line is of greatest importance for us, but we publish printed version as well. On-line content is available free of charge as open access under Creative Commons license. We believe that open access to high quality publications is essential for development of new scientific ideas.

Lectures



Poster session 1



Poster session 2



Poster session 3



Poster session 4



Disorders of cell structure and function

P1.1

Down-regulation of P2X₇ receptor expression after oxygen and glucose deprivation in rat oligodendrocyte progenitor cells

Wenqin Cai¹, Li-Yan Wang², Peng-hui Chen², Qi-yue Deng²

¹Dept. of Neurobiology, The 3rd Military Medical University, Department of Pediatrics, Xinqiao Hospital, Chongqing, China

²Dept. of Neurobiology, The 3rd Military Medical University, Chongqing, China

Contact: cwenqin@yahoo.com

Oligodendrocyte progenitor cells (OPCs) are the predominant oligodendrocyte lineage stage in the cerebral hemispheres of neonatal rat. Prior studies have shown that OPCs are highly vulnerable to hypoxic-ischemic injury, yet the mechanisms are not well understood. P2X₇ receptor is an ATP-gated ion channel that has unusual properties and plays very complex roles in a variety of neuropathologic conditions. However, little is known about the involvement of P2X₇ receptor in OPCs development and injury. The present study was aimed at examining the presence of P2X₇ receptor in OPCs and evaluating the change of the receptor expression after hypoxia ischemia. Using Immunofluorescence, RT-PCR and western blot analysis, we demonstrated that cultured rat OPCs expressed P2X₇ receptor. The Fura-4-based Ca²⁺-imaging approach was employed to ascertain the existence of functional P2X₇ receptors in OPCs, revealed that ATP triggered raised [Ca²⁺]_i in OPCs with properties characteristic of P2X₇ receptor activation: the increase in [Ca²⁺]_i was more potently activated by 3'-O-(4-benzoyl)benzoyl-ATP (BzATP) than by ATP itself, and blocked by Brilliant Blue G (100nM), which was a selective antagonist for P2X₇ receptor in nanomole range. A decrease in P2X₇ receptor expression was observed in cultured OPCs after exposure to oxygen glucose deprivation (OGD) for 2 hr *in vitro*. By using a neonatal hypoxic-ischemic injury model in postnatal 3 rats, the similar down-regulation was also detected in ischemic cerebral cortex, subcortical white matter and hippocampus compared with sham operation controls. In conclusion, the present data demonstrated that OPCs expressed functional P2X₇ receptor. The post-ischemic down-regulation of P2X₇ receptor suggested a role for this receptor in the pathophysiology of hypoxic-ischemic brain injury. This work was supported by the National Natural Science Foundation of China (No.30670686)

P1.2

Maternal hypothyroidism and its role in the placenta: a morphometric and immunohistochemical study

Candan Ozogul¹, Serap Cilaker², Basak Baykara², Cetin Pekcetin², Alper Bagriyanik², Soner Atmaca², Erdener Ozer³

¹Gazi University Medicine Faculty and Dept Histology and Embryology, Dokuz Eylul University Medical School, Ankara, Izmir, Turkey

²Dept Histology and Embryology, Dokuz Eylul University Medical School, Izmir, Turkey

³Dept Pathology, Dokuz Eylul University Medical School, Izmir, Turkey

Contact: ozogulc@gazi.edu.tr

Introduction: Apoptotic cell death is abnormally accelerated during the pathologic phases leading to clinical hypothyroidism. The aim of this study was to demonstrate histologically the structure of placenta with normal and maternal hypothyroidism and address the mechanism of apoptosis with immunohistochemistry and TUNEL assay. Materials and Methods: Seven mothers with uncomplicated normal pregnancy and eight mothers with hypothyroidism between ages of 26-40 were selected. Placental tissue samples were prepared for light microscopy, immunohistochemistry using Caspase-3, Bcl-2 and Bax antibodies, and TUNEL assay for detection of apoptosis. Results: In the hypothyroid group, Caspase-3 immunostaining was strong in syncytiotrophoblast, cytotrophoblast and mesenchymal cells, whereas Bcl-2 immunostaining was absent in the cytotrophoblast cell, and weak in syncytiotrophoblast cell and focal and weak in the mesenchymal cells. Bax immunostaining was moderate in the syncytiotrophoblast and cytotrophoblast, however it was mild in the mesenchymal cells. In the control group, positive immunostaining for Caspase-3 was moderate in the syncytiotrophoblast cell while it was mild in the cytotrophoblast cell and it

was negative in the mesenchymal cells. Expression of Bcl-2 was moderate in the syncytiotrophoblast, cytotrophoblast and mesenchymal cells. Bax immunostaining was negative in the cytotrophoblast and mesenchymal cells, while immunostaining was mild in the syncytiotrophoblast. Increased TUNEL-positive staining in cytotrophoblast, syncytiotrophoblast and mesenchymal cells was shown in the placenta in the hypothyroid group in comparison to the control group. Mean number of syncytial knots was significantly lower in the control group than in the hypothyroid group ($p < 0.05$). Mean thickness of medium size blood vessels was significantly lower in the hypothyroid group than in the control group ($p < 0.05$). Mean area of stromal fibrosis, demonstrated with Masson trichrom stain, in the hypothyroid group was higher than in the control group ($p < 0.05$). Discussion: We think that significant histological changes might occur in the placentas of hypothyroid mothers with high incidence of apoptotic marker response. Key Words: Hypothyroid, Placenta, Apoptosis

P1.3

Fornix transection alters corticosteroid receptors in the hippocampus and corticotrophin-releasing hormone and vasopressin in the hypothalamic paraventricular nucleus

Fang Han, Hong Liu, YanHui Zhang, YuXiu Shi

China Medical University, ShenYang, China

Contact: hanfang2000@hotmail.com

The hypothalamic paraventricular nucleus (PVN) receives neuronal inputs from the hippocampus via the fornix. Glucocorticoids regulate the hippocampal function through negative feedback. In the present study, we studied the effect of fornix transection (FT) on the gene mediating glucocorticoid feedback on the hippocampus and PVN. We performed immunohistochemistry and western blotting to examine the changes in 2 types of corticosteroid receptors: mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) in the hippocampus as well as the changes in corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) in the PVN at 0, 4, 7, and 10 d after FT. We observed no changes in MR and GR expressions in the hippocampus and in CRH and AVP expressions in the PVN within 4 d after FT. However, beginning from 7 d after FT, the gene expressions were found to differ between the hippocampus and PVN: a significant decrease in the MR and GR expressions in the hippocampus and an increase in the CRH and AVP expressions in the PVN were observed. These results indicated that FT exerts an inhibitory effect on the hippocampus but a stimulatory effect on the PVN at 1 week after FT. This suggests that (i) the corticosterone action mediated by hippocampal corticosteroid receptors indirectly modulates the PVN function, and (ii) FT-induced changes in corticosterone affect the MR and GR function in the hippocampus via negative feedback.

P1.4

Adrenaline and noradrenaline effects on glucose homeostasis and on vasoactive peptide expression in different organs of *Podarcis sicula* lizard

Maria De Falco¹, Claudia Barletta¹, Rosaria Sciarillo², Anna Sellitti³, Sabrina Caliendo³, Salvatore Valiante³, Anna Capaldo³, Flaminia Gay³, Vincenza Laforgia³

¹Dept of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples "Federico II", Naples, Italy

²Dipartimento di Scienze Biologiche e Ambientali, Università degli Studi del Sannio, Benevento, Italy

³Dept of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples "Federico II", Naples, Italy

Contact: madedfalco@unina.it

Catecholamines, both adrenaline and noradrenaline, play an important role in the control of glucose homeostasis of mammals, through feedback mechanisms that regulate synthesis and action of other hormones, such as insulin, ACTH and corticosterone. Moreover, it has been suggested that catecholamines can influence vascular resistances following hyper-

glycemic stress through the control of the synthesis of vasoactive peptides such as endothelin, apelin and vasoactive intestinal peptide (VIP). In this study, we have investigated the effect of adrenaline and noradrenaline administration on glucose homeostasis of lizard *Podarcis sicula* through both biochemical and histochemical assays. Particularly, we have observed that noradrenaline administration was able to increase glucose plasma level stronger than adrenaline. Noradrenaline also increased ACTH release that in turn evoked a strong increase of corticosterone plasma level. This hormonal panel was confirmed observing PAS positivity in *Podarcis sicula* liver; in fact, liver PAS positivity was almost completely absent in the treated specimens due to reduction of hepatic glycogen and subsequent glucose release by hepatocytes. Regarding vasoactive peptides, we have investigated, by immunohistochemistry, the expression of apelin, VIP, endothelin-converting enzymes (ECE-1 and ECE-2), endothelin receptor B (EtB), after catecholamine treatment, in those organs in which the vascular regulation is particularly important for function: heart, lung, liver, stomach, intestine and kidney. Both catecholamines were able to act on such peptides although with different effects depending on target organ. Immunohistochemical analysis suggested that catecholamines increased vasoactive peptide expression in almost all the reptilian tissues considered, suggesting a stimulating effect of catecholamines on the synthesis, release and action of these peptides. Taken together, the obtained results indicate a catecholamine role in the regulation of glucose homeostasis and the whole circulatory system through the activation of various pathways involving both hormones such as insulin, ACTH, corticosterone and neuropeptides such as apelin, VIP and endothelin.

P1.5

Recombinant extracellular matrix protein expressed in a limited number of cells propagates to the target organ throughout the body using its nascent tissue-targeting signal

Yumi Suzuki¹, Mikako Ito¹, Takeshi Okada², Shin'ichi Takeda², Takayasu Fukudome³, Toshiro Yoshimura⁴, Eric Krejci⁵, Kinji Ohno¹

¹Division of Neurogenetics, Center for Neurological Diseases and Cancer, Graduate School of Medicine, Nagoya University, Nagoya, Japan

²Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

³Department of Neurology, Nagasaki Medical Center of Neurology, Nagasaki, Japan

⁴Department of Occupational Therapy, Nagasaki University School of Health Science, Nagasaki, Japan

⁵INSERM U686, Paris, France

Contact: yumi19860602@yahoo.co.jp

Congenital endplate acetylcholinesterase (AChE) deficiency (EAED) causes neuromuscular transmission defects and provokes congenital myasthenia syndrome. Clinical symptoms include muscle weakness, myasthenia, postural scoliosis, and ophthalmoparesis. In many cases, dyspnea and dysphagia lead to death. No effective treatment is currently available. EAED is caused by mutations in *COLQ* encoding collagen Q (ColQ) that makes a helical trimer and anchors the AChE catalytic subunit to the synaptic basal lamina at the neuromuscular junction. A complex comprised of ColQ and AChE is called asymmetric AChE. AAV8 (adeno-associated virus serotype 8) has a tropism for skeletal muscle. In an effort to seek for a therapeutic option for EAED, we administered recombinant AAV8 carrying the human *COLQ* gene (rAAV8-COLQ) to *Colq*-knockout mice and analyzed their effects on AChE expression and motor functions. We injected $\sim 2 \times 10^{12}$ vector genomes of rAAV8-COLQ into the tail vein of *Colq*-knockout mice at postnatal weeks 5 to 11. In two to three weeks after injection, motor symptoms began to ameliorate and some young mice recovered to the level of the wild-type littermates, which lasted at least 4 months after injection without a decline. We observed the presence of asymmetric AChE in skeletal muscles by density gradient ultracentrifugation. We also confirmed anchoring of asymmetric AChE to the neuromuscular junction by anti-ColQ antibody and AChE activity staining along with a-bungarotoxin staining to locate ACh receptors. We additionally traced the distribution of rAAV8 virus in skeletal muscles by injecting rAAV8-LacZ. The analysis disclosed that even when a limited number of skeletal muscles are infected, a large number of neuromuscular junctions throughout the body exhibit expression of asymmetric AChE. Moreover, we injected rAAV8-COLQ into hindlimbs of *Colq*-knockout mice and observed AChE expression in forelimbs. Our current studies imply feasibility of the "protein anchoring therapy" in which a recombinant extracellular matrix molecule expressed in a limited number of cells can be delivered to the target organ throughout the body by exploiting its tissue-targeting signal of the recombinant molecule itself.

P1.6

Expression of galectin-3 in nephrotic syndrome glomerulopathies in children – preliminary report.

Michał Nowicki¹, Beata Kondraciuk¹, Danuta Ostalska-Nowicka², Bogdan Miškowiak²

¹University of Medical Sciences in Poznan, Department of Histology and Embryology, Poznań, Poland

²University of Medical Sciences in Poznan, Department of Pediatric Cardiology and Nephrology, Poznań, Poland

Contact: mnowicki@amp.edu.pl

Background: Galectins are a family of ancient animal carbohydrate binding proteins; the name is from their description as β -galactoside-specific lectins. They have been strongly implicated in inflammation and cancer. Studies of the association of galectins with various aspects of kidney disease in humans are still at an early stage. In line with the above, the aim of the present report was to analyse the immunohistochemical expression of galectin-3 (the only chimera galectin currently identified) in renal biopsy specimens of children with idiopathic nephrotic syndrome (INS). Patients and Methods: Eighteen children with minimal change disease (MCD), 30 with diffuse mesangial proliferation (DMP) and 11 with focal segmental glomerulosclerosis (FSGS) treated between 2003 and 2006 in the Department of Paediatric Cardiology and Nephrology, Poznan University of Medical Sciences. An indirect immunohistochemical protocol using a polyclonal rabbit antibody against human galectin-3 was employed. Results: In the control, MCD and DMP children who responded to steroid therapy anti-galectin-3 reactivity was present both in renal cortex and medulla. It was the strongest within cortical collecting ducts and subjectively less expressed in distal tubules. The total number of galectin-3 positive cortical and medullary segments of collecting ducts was significantly higher in the subjects who did not respond to steroid therapy. These patients revealed also immunohistochemical reactivity of galectin-3 within nuclei of individual glomerular mesangial cells ($p < 0,001$). Conclusions: A suggested galectin-3 authority in mature human glomeruli and tubules during proteinuric glomerulopathies in children may indicate its potent modulatory function and can prognosticate a further glomerular reconstruction leading to FSGS.

P1.7

Plastic changes in the ventral tegmental area-nucleus accumbens feeding circuitry after its partial destruction

Karolina Plucińska, Maciej Jankowski, Bogna Ignatowska-Jankowska, Weronika Trojnar

Department of Animal Physiology, University of Gdansk, Gdańsk, Poland

Contact: karopla@wp.pl

Motivational aspect of food intake seems to be connected with the mesolimbic system originating in the ventral tegmental area (VTA) and involving the nucleus accumbens (Acb) as its main output structure. We found previously that partial unilateral destruction of this system at the level of VTA results in enhanced (by about 20%) reactivity of the contralateral VTA to feeding-inducing electrical stimulation. The effect was immediate and permanent. Such a facilitation of function of the intact VTA coexisted with bilateral enhancement of activity (*c-fos* study) of the VTA output structures including Acb and the nonspecific thalamic nuclei. Here, we present data indicating how changes function of this feeding circuitry after its unilateral destruction at the level of Acb. VTA stimulation-induced feeding was tested in rats implanted with unilateral VTA stimulation electrode and Acb Shell (AcbS) lesion electrode before and after ipsi- or contralateral AcbS lesion (separate groups) using the latency to feed/stimulation frequency curve shift paradigm (procedure designed to test motivational aspect of feeding). It was found that contralateral AcbS lesions impaired (by about 20%) and ipsilateral AcbS lesions facilitated (by about 30%) feeding reaction which manifested as the respective changes in the reaction threshold and a rightward or leftward (respectively) shift of the function relating latency to feed to stimulation frequency. Effective lesions were localized mainly in the posterior Acb including caudomedial part of AcbS. No such effects were found in the control rats subjected to the lesion of the Acb Core. The results obtained indicate that the VTA-AcbS feeding system becomes functionally reorganized after unilateral, partial damage to it, which involves both hemispheres. Ipsilateral AcbS lesion renders VTA more and contralateral AcbS lesion less responsive to stimuli which results in respective changes in feeding behavior.

P1.8

Exendin-4 triggers NGF/P75^{NTR} system and antioxidant defense in diabetic mice pancreas

Selda Gezginci-Oktayoglu, Sehnaz Bolkent

Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey

Contact: selgez@istanbul.edu.tr

Glucagon-like peptide-1 (GLP-1) ameliorates the symptoms of diabetes through stimulation of insulin secretion. We have investigated the effects of exendin-4, a potent GLP-1 receptor agonist, on pancreas of the Streptozotocin (STZ)-induced diabetic mice in this study. BALB/c male mice which have been used in this study were divided into four groups. The first group was given citrate buffer only, the second group was administered exendin-4 alone, the third group received STZ, and the fourth group was given both STZ and exendin-4. Exendin-4 (3 µg/kg) was administered by subcutaneous injection daily for 30 days after the animals were rendered diabetic by single dose (200 mg/kg) administration of STZ. With treatment of exendin-4 to the diabetic mice following results were noted: (i). Blood glucose levels was significantly decreased; (ii). p75 neurotrophin receptor (p75^{NTR}) expression was significantly increased in pancreatic islets; (iii). Pancreatic nerve growth factor (NGF) levels, catalase, superoxide dismutase and glutathione peroxidase activities were significantly increased. These data suggest that the integrity of the NGF/p75^{NTR} system participates in controlling islet cell survival and STZ-induced diabetes. In addition, we concluded that exendin-4 triggers NGF/p75^{NTR} system. Besides, these data also indicate that NGF/p75^{NTR} pathway includes antioxidant defense.

P1.9

HTC hepatoma cells as a model for investigating the effects of low herbicide concentrationsManuela Malatesta¹, Federica Perdoni², Giada Santin², Serafina Battistelli³, Marco Biggiogera⁴, Carlo Pellicciari²¹Dipartimento di Scienze Morfologico-Biomediche, Sezione di Anatomia e Istologia, University of Verona, Verona, Italy²Dipartimento di Biologia Animale, Laboratorio di Biologia Cellulare e Neurobiologia, University of Pavia, Pavia, Italy³Istituto di Istologia e Analisi di Laboratorio, University of Urbino, Urbino, Italy⁴Dipartimento di Biologia Animale, Laboratorio di Biologia Cellulare e Neurobiologia, University of Pavia, and Istituto di Genetica Molecolare del CNR, Pavia, ItalyContact: pelli@unipv.it

Previous studies on mice fed on a genetically modified (GM) soybean demonstrated modifications of the transcription/splicing process as well as morpho-functional changes of mitochondria in hepatocytes [1,2]. The cause(s) of these alterations could not be conclusively established but, since the GM soybean used is resistant to glyphosate [3] and the plants were treated with the glyphosate-containing herbicide Roundup, the possibility exists that the effects observed may be due to herbicide residues. It should be underlined that Roundup undergoes a rapid degradation in the soil, thus making problematic the detection of its residues in the crop by-products. In an attempt to elucidate the potential role of Roundup residues in the occurrence of the cellular modifications found in mice fed on glyphosate-resistant GM soybean, we carried out some experiments on an *in vitro* cell model. HTC cells from a rat hepatoma were incubated with very low concentrations of Roundup (1 and 10 mM) for different time periods (from 1 h to 48 h) and some cellular features were analysed by combining flow cytometry, fluorescence and electron microscopy. Under these experimental conditions, the death rate and the general morphology of HTC cells were not affected, and the cell distribution in the cycle did not change. Most of the cytoplasmic organelles (*i.e.*, rough and smooth endoplasmic reticulum, free ribosomes, Golgi apparatus as well as the actin and tubulin cytoskeleton) did not change even for the longest treatment times. Only lysosomes and mitochondria were affected: in HTC-treated cells, the lysosome density increased and, at the ultrastructural level, the organelles appeared to contain membrane and cytosolic debris, while mitochondria underwent membrane depolarization and a reduction of the inner membrane length which indicate a decline in the respiratory activity. Accordingly, high concentrations of Roundup have been reported to alter the mitochondrial inner membrane [4] and to lower the cell respiratory activity [5]. Moreover, in HTC-treated cells, nuclei underwent morpho-functional modifications suggestive of a decreased transcriptional/splicing activity, consistent with the previously reported transcriptional inhibition induced by Roundup [6,7]. The

striking concordance of the effects induced by low concentrations of Roundup on HTC cells with the effects of a GM soybean-based diet strongly supports the hypothesis that the presence of Roundup residues could be the main factor interfering with multiple metabolic pathways. References: 1. Malatesta M et al. Cell Struct Funct 27:173, 2002. 2. Malatesta M et al. Eur J Histochem 49:237, 2005. 3. Padgett SR et al. Crop Sci 35:1451, 1995. 4. Szarek J et al. Mar Environ Res 50:263, 2000. 5. Peixoto F. Chemosphere 61:1115, 2005. 6. Marc J et al. Chem Res Toxicol 15:326, 2002. 7. Marc J et al. Toxicol Appl Pharmacol 203:1, 2005

P1.10

Alterations in kidney tissue following zinc supplementation to STZ-induced diabetic rats

Ayse Karatug, Engin Kaptan, Ozgur Mutlu, Sehnaz Bolkent, Refiye Yanardag

Istanbul University, Istanbul, Turkey

Contact: akaratug@istanbul.edu.tr

Diabetes mellitus is a chronic disease characterized by anomalies forming in carbohydrate, lipid, protein metabolisms and the incidence of this disease varies widely throughout the world. Zinc is an important element which is essential for life and present in nature. The aim of this study was to determine the effect of zinc supplementation on the kidney structure in streptozotocin (STZ)-induced diabetic rats, microscopically and biochemically. In this study the animals were divided into four groups: Group I: control (untreated) animals; Group II: control animals given zinc sulfate; Group III: diabetic animals; and Group IV: diabetic animals given zinc sulfate. Zinc sulfate was given to the animals by gavage at a daily dose of 100 mg/kg body weight for 60 days. Diabetes was induced by intraperitoneal injection of STZ in a single dose of 65 mg/kg. Kidney tissue was fixed in Bouin's solution and sections stained with Masson's trichrome and Periodic-Acid-Schiff (PAS) for histological determinations. Cell surface alterations were demonstrated by labeling lectins which used as probes for identification of the carbohydrate moieties. Structural alterations in both cortex of kidney tissue and the sugar composition of the apical surface of proximal tubule cells were determined. Very important degenerative changes and a decrease in PAS positive reaction in the kidney tissue of the diabetic animals were observed by light microscope. Zinc sulfate reversed the changes in the diabetic animals. In addition, we detected WGA (wheat germ agglutinin)-binding sites in glomerulus and apical surface of proximal tubular cells compared with density. There is no an important distinction in glomerulus. The binding of WGA was lower in the diabetic animals than the control animals, while it was higher in the diabetic animals given zinc sulfate than the diabetic animals. Serum urea, creatinine and kidney lipid peroxidation (LPO), nonenzymatic glycosylation (NEG) levels were increased in the diabetic group. But kidney glutathione (GSH) levels were decreased in the diabetic group. Administration of the zinc sulfate in the diabetic group caused a decrease in serum urea, creatinine and kidney LPO, NEG levels and an increase in the kidney GSH levels. In conclusion, we can say that zinc sulfate have a curative effect on the kidney tissues in the diabetic animals. In the same time, lectin WGA can be used as a marker in histopathological evaluation of kidney.

P1.11

Quantitative immunofluorescence staining: a tool for detection of spatio-temporal changes of protein molecules alongside nerve fibres following nerve injury

Petr Dubovy

Department of Anatomy, Med. Fac. Masaryk University, Brno, Czech Republic

Contact: pdubovy@med.muni.cz

Nerve injury induces cellular and molecular changes not only in the distal nerve stump but also in associated dorsal root ganglia (DRG) and spinal cord segments to generate neuropathic pain. Signals for the changes remain to be obscure. Recently, little work has been done on cellular and molecular changes in the spinal nerve roots following peripheral nerve injury. Therefore, we have employed quantitative immunofluorescence staining to investigate changes of immunofluorescence staining for laminin-1 and IL-6 cytokine molecules alongside axons, mainly in the dorsal and ventral roots following sciatic nerve ligation. Immunofluorescence (IF) staining

for laminin-1 and IL-6 was compared in the proximal and distal sciatic nerve segments as well as dorsal and ventral roots (L4-L5) of naive rats and those operated for unilateral sciatic nerve ligation (ScNL) for 1, 3, 7, and 14 days. We have optimized indirect IF method for quantitative comparison of laminin-1 and IL-6 immunostaining according to published protocol (Dubovy et al. 2002). The cryostat sections (12 µm) through the sciatic nerve and spinal nerve roots removed from intact and operated rats were cut and incubated simultaneously at the same conditions. An intensity of immunofluorescence staining was compared using the computer-assisted image analysis (Lucia-G v 4.21). Immunostaining for laminin-1 precisely decorated the basal lamina at the surface of nerve fibres, and for IL-6 was present in the Schwann cells of the naive sciatic nerves as well as spinal nerve roots. Unilateral sciatic nerve ligation results in no significant changes of laminin-1 IF in the contralateral sciatic nerve, but it was significantly changed in the nerve stumps and decreased alongside nerve fibres in both dorsal and ventral roots within 7 days after operation. In the same period of survival, the IL-6 IF was increased in the nerve stumps and decreased in the spinal nerve roots. The changes of laminin-1 and IL-6 IF in the spinal nerve roots returned close to intact levels within 2 weeks from operation. The results indicate heterogeneity in distribution of laminin-1 and IL-6 alongside nerve fibers not directly associated with nerve damage. Signals for molecular changes of the spinal nerve roots are probably conveyed by blood flow and cerebrospinal fluid. References: Dubovy et al. 2002. *Histochem Cell Biol* 117, 473-480. Supported by GACR 309/07/0121 and MSM0021622404.

P1.12

Comparison of collagen biomatrix and omentum effectiveness on peripheral nerve regeneration

Berker Cemil¹, Durukan Ture², Banu Cevirgen³, Figen Kaymaz⁴, Memduh Kaymaz⁵

¹Department of Neurosurgery, Numune Education and Research Hospital, Ankara, Turkey

²Departments of Physical Medicine and Rehabilitation, Gazi University School of Medicine, Ankara, Turkey

³Department of Statistics, Middle East Technical University, Ankara, Turkey

⁴Department of Histology and Embryology, Medical faculty, Hacettepe University, Ankara, Turkey

⁵Department of Neurosurgery, Gazi University School of Medicine, Ankara, Turkey

Contact: fkaymaz@hacettepe.edu.tr

Despite the presence of various nerve cooptation materials and techniques, achievement of the functional nerve regeneration is still inadequate. This study was aimed to compare the effectiveness of conduit composed of collagen biomatrix and omentum graft on peripheral nerve regeneration. Thirty-five male Wistar rats were divided into four groups. In Group 1 (control group) the right sciatic nerve was skeletonized from the sciatic notch till the point of bifurcation. In Group 2 (the primary epineurial repair group) the nerve was transected 1 cm proximal to the bifurcation with a sharp pair of micro scissors and then repaired with four epineurial sutures. Group 3, the test group in which the collagen biomatrix was wrapped around the epineurial repaired nerve. Group 4, the test group in which the nonpediculated omentum graft was wrapped around the epineurial repaired nerve. Walking track analysis was performed on 30, 60 and 90th days after procedure. On 90th day electromyographic study was performed. Animals were sacrificed to analyse muscle and nerve histomorphometry. Mann Whitney-U test was used for statistical analysis. There was significant difference in muscle weight ratios between the experimental and control groups. The result indicated that the heaviest group is collagen biomatrix and the lightest group is the omentum grafts ($p < 0.05$). There is no statistically significant difference on mean sciatic function index values in all groups. The result of mean nerve velocity values; collagen biomatrix is higher than all other groups. Subsequently, the experimental and control groups were compared for the average number of myelinated fibers, diameter of myelin, diameter of axons, diameter of nerves and g-ratios. The comparison of the diameter of myelin, diameter of nerves and g-ratios revealed a significant difference between the experimental and control groups. The data clearly show that better recovery was obtained in collagen biomatrix group both functionally and morphologically. Collagen biomatrix wrapped specimens has the best regeneration. The electrophysiological study confirmed the recovery of electrical activity in the regenerated axons.

P1.13

The effect of Quetiapine on the treatment of experimental acute spinal cord injury

Hamit Aytaç¹, Memduh Kaymaz¹, Ertan Ergün¹, Figen Kaymaz², Aydin Pasaoglu¹

¹Gazi University, Faculty of Medicine, Department of Neurosurgery, Ankara, Turkey

²Hacettepe University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Turkey

Contact: fkaymaz@hacettepe.edu.tr

The importance of treatment modalities against damage on spinal cord at secondary phase of spinal cord injury is well known. So, numerous experimental studies have been focused on this phase. Effectiveness of Quetiapine (Serequel®), a 5-HT₂ receptor blocker atypical antipsychotic agent on apoptosis is one of the major mechanisms of secondary damage on spinal cord was researched in our study. 32 female Wistar rats were separated to 4 equal groups. Total laminectomy was performed at T₆ level and spinal cord injury was produced by using Yasargil FT280T temporary aneurysm clip. Each rat was daily injected ip with Quetiapine (10mg/kg/day) at 1st day (D-I) and 7th day (D-II) groups. Control groups of 1st day (K-I) and 7th day (K-II) were performed no treatment. At the end of follow up periods all animals were sacrificed and spinal cords were removed. Apoptotic cells were evaluated by using immunohistochemical technique (TUNEL) (ApopTag Plus Peroksidaz In Situ Apoptosis Detection Kit (Chemicon, USA cat#S7101) in injured spinal cord specimens. Randomly two areas from gray matter and two areas from white matter were selected (x40 magnification each area was 0,0625mm²). Apoptotic cells were counted in all animals and in all groups, in cross sections of spinal cord. Quetiapine had a protective effect against apoptosis of injured D-I and D-II spinal cord groups. All the results were statistically significant. This agent had more protective effect on gray matter cells than white matter cells at 7th day group(D-II). Our findings were suggested that Quetiapine had protective effect on secondary damage of spinal cord injury and it could be a versatile treatment agent by thinking its effectiveness on posttraumatic stress disorder, depression and agitation.

P1.14

Dipeptidyl peptidase IV (DDP IV) /CD 26 in NASH patients

Yasemin Balaban¹, Petek Korkusuz², Halis Simsek³, Hale Gökcan⁴, Gökhan Gedikoglu⁵, Asli Pinar⁶, Gülsen Hascelik⁷, Esin Asan², Erhan Hamalolu⁸, Gonca Tatar³

¹Hacettepe University Faculty of Medicine Dept. Internal Medicine, Division of Gastroenterology, Ankara, Turkey

²Hacettepe University Faculty of Medicine Dept Histology & Embryology, Ankara, Turkey

³Hacettepe University Faculty of Medicine Dept. of Internal Medicine, Division of Gastroenterology, Ankara, Turkey

⁴Baskent University Faculty of Medicine Dept. of Internal Medicine, Division of Gastroenterology, Ankara, Turkey

⁵Hacettepe University Faculty of Medicine Dept. of Pathology, Ankara, Turkey

⁶Hacettepe University Children's Hospital Clinical Chemistry Laboratory, Ankara, Turkey

⁷Hacettepe University Faculty of Medicine Dept of Microbiology and Clinical Microbiology, Ankara, Turkey

⁸Hacettepe University Faculty of Medicine Dept of General Surgery, Ankara, Turkey

Contact: petek@hacettepe.edu.tr

Objectives: Non-alcoholic steatohepatitis (NASH) is a chronic liver disease with unknown etiology. The insulin resistance, immune mechanisms and oxidative stress are the main factors in its pathogenesis. Dipeptidyl peptidase IV (DPPIV) or CD26 is a protein with endocrine and immune functions. This study aimed to elucidate the changes related to DPPIV in NASH patients. Methods: Serum and urinary DPPIV activities were measured in 31 NASH patients and 17 healthy controls. The liver biopsies of 29 patients were immunolabeled for CD26 by indirect immunoperoxidase and immunofluorescent techniques. Results: The mean age of patients was 46 ± 11 years and 14 (45%) of them were female. The serum DPPIV activity was higher in patients (57.3 ± 7.8 U/L) than controls (43.6 ± 10.6 U/L) ($p < 0.0001$), and correlated with the histopathological grade ($p = 0.038$, $r = 0.373$) and hepatosteatosis ($p = 0.018$, $r = 0.423$) but not with stage ($p = 0.286$), class ($p = 0.286$) or CD26 staining ($p = 0.743$). The urinary DPPIV activity was similar in patients (1.52 ± 0.94 U/mmol creatinine) and controls (1.37 ± 0.68 U/mmol creatinine) ($p = 0.861$). Three acinar zones of liver had equal CD26 expression ($p = 0.076$). The intensity of CD26

immunostaining was correlated with histopathological grade ($p = 0.001$) and hepatosteatosis ($p = 0.003$) but no correlation with stage or class could be detected ($p = 0.610$ and 0.956 , respectively). In conclusion: The serum DPPIV activity and the staining intensity of CD26 in the liver are correlated with histopathologic grade of NASH and hepatosteatosis. DPPIV can be proposed as a novel candidate with several potential functions in NASH pathogenesis. Key words: DPPIV, CD26, liver, non-alcoholic steatohepatitis, metabolic syndrome.

P1.15

Expression of serotonin receptors mRNA in the frontal cortex and hippocampus of PTSD rats

Haitao Wang, Fang Han, Yuxiu Shi

Department of Histology and Embryology, College of Basic Medical Sciences, China Medical University, Shenyang, China

Contact: shiyuxiu@163.com

Structural hippocampus' and prefrontal cortex' changes occur in patients with posttraumatic stress disorder (PTSD) that appears to be correlated with cognitive dysfunction. In these brain regions, serotonin (5-HT) plays a prominent role in symptom presentation and treatment of PTSD. Serotonin receptors (5HTRs) have been shown to be affected by stress in experimental animals and related to anxiety and depression in humans. The aim of the present study was to explore the expression of serotonin receptors mRNA in the frontal cortex and hippocampus of PTSD rats. Male Wistar rats were randomly assigned to normal control group and model groups (after SPS 1d, 4d, 7d, 14d and 28d). The PTSD rat model was set up using the SPS-method (single prolonged stress). Total RNA of the frontal cortex and hippocampus was isolated with TRIZOL, and the expression of serotonin receptors' mRNA was assessed by RT-PCR. The results showed that the expression of serotonin type 1A receptors (5-HT1ARs) of PTSD rats was decreased as compared with the control group. No significant differences in other serotonin type receptors were found. We concluded that 5HT1A receptor changes play an important role in the development of PTSD.

P1.15A

The evaluation of megakaryocyte emperipolesis in patients with multiple myeloma

Dorota Lemancewicz¹, Janusz Dzieciol¹, Jaroslaw Piszcz², Agnieszka Lebelt¹

¹Department of Human Anatomy, Medical University of Białystok, Białystok, Poland

²Department of Hematology, Medical University of Białystok, Białystok, Poland

Contact: januszdzeciol@poczta.onet.pl

Background: Multiple myeloma (MM) is a malignant neoplasm characterised by the increase of clonal plasma cells in bone marrow. Bone marrow trephine biopsy (BMT) is used for the assessment of bone marrow involvement by plasma cells. Histopathological examination of bone marrow by BMT makes also possibility for evaluation of the megakaryocyte emperipolesis. The phenomenon of emperipolesis (presence of hematopoietic cells within the cytoplasm of megakaryocytes) is rarely observed in the human bone marrow trephine biopsy specimens. The significance of this phenomenon is still unknown. The aim of the study was an estimation of the influence of thalidomide on emperipolesis. Patients and methods: Megakaryocytic emperipolesis was analyzed in patients before and after 6 cycles of chemotherapy. Studies were carried out on 26 patients, included 15 females and 11 males in age from 36 to 69 years (mean age was 58 years), in compliance with their clinical stage, number of plasma cells in marrow, age and sex. Bone marrow was taken from the postero-superior part of iliac crest using the Jamshidi needle. Bone marrow tissue was carried out in fixative solution and paraffin embedding. Hematoxylin and eosin staining for recognition of cellularity and immunohistochemistry was applied with monoclonal antibodies CD61 for the identification of megakaryocytes and LCA, CD3, CD20 and MPO for identification of cells within them. The quantity of megakaryocytes emperipolesis was estimated. Results: The emperipolesis of single megakaryocytes was found before therapy in 4 patients with third clinical stage of multiple myeloma according to Durie-Salmon. After therapy the emperipolesis was observed in 18 patients. In 16 patients was observed in

two or more megakaryocytes. The lymphocytes were present most often, rarely eosinophilic granulocytes, in the cytoplasm of megakaryocytes. Conclusion: We found the correlation between clinical stage of multiple myeloma and percentage of plasmocytes and higher frequency of megakaryocytic emperipolesis. There was no correlation between emperipolesis and patients age and sex. The therapy caused increasing frequency of emperipolesis and appearance of this phenomenon in cases in which the emperipolesis had been absent before.

P1.16

Effects of cadmium chloride on glial cells of lizard *Podarcis sicula*

Rossana Favorito, Maria Consiglio Grimaldi, Ida Ferrandino

Department of Biological Sciences, Section of Evolutionary and Comparative Biology, University Federico II, Naples, Italy

Contact: rossana.favorito@unina.it

Cadmium (Cd) is a heavy metal, highly toxic and its levels in the environment are increasing due to industrial activities. It is known that an acute exposure to Cd produces toxicity to the lungs, testes and brain, while chronic exposure determines renal dysfunction, anaemia and osteoporosis. It can cause damages to the nervous system, to the immune system and in some cases to have carcinogenic effects. The brain is an organ that concentrates metals, and these metals are often localized in the astroglia, the first cells of the brain parenchyma to encounter metals crossing the blood-brain barrier. In mammals some studies have been carried out on the toxic effect of heavy metals on glia, thanks to which it has been possible to observe that metals like Cd, Tl, Zn and Pb induce morphological changes in the populations of glial cells. The scope of the present study was to observe the possible morphological changes induced by CdCl₂ on the glia of a reptile, the lizard *Podarcis sicula*. The reptilian class of vertebrates has been relatively understudied in the field of ecotoxicology, although it is considered to be suitable as bioindicators of chemical exposure due to their persistence in a variety of habitats. In this work 12 adults specimens of *Podarcis sicula* were exposed to an acute treatment by an intraperitoneal injection of a single and massive dose (2mg/Kg-BW) of CdCl₂ and were sacrificed after 2, 7 and 16 days. Serial sections of the whole brains were processed for routine histological and immunohistochemical staining by ABC technique and the anti-GFAP antiserum at the dilution working of 1/100. GFAP (glial fibrillary acidic protein) is the intermediate filament of glial cells and the most widely accepted marker for glial differentiation. Results: in the brain of the reptile *Podarcis sicula* the general pattern of the GFAP-immunoreactivity was fundamentally represented by long and thick fibres which ran from cell bodies located at the ventricular surface to the meningeal layer. Numerous GFAP-immunopositive structures were observed in the mesencephalon and in the medulla oblongata, but thin fibres GFAP(+) were also present in the telencephalon. The most numerous glial cells observed were of radial glia. In the grey matter of the cerebellum an intense immunoreactivity was observed, while round cells with wide marked cytoplasm were localized in the white matter. After 2 and 7 days the treated lizards showed a decrease of the GFAP-immunopositive structures. After 16 days these reduction of immunostaining was most considerable. The reduction of the presence of the radial glia was most meaningful in the cerebellum and in the optic tectum. This preliminary results show that in the lizards an acute exposition to cadmium provokes decrement of the expression of GFAP marker with possible consequent damage in the function of the glial cells.

P1.17

The protective role of Z-FA.FMK on the lung injury induced by D-GalN/TNF-alpha in mice

Fusun Oztay¹, Selda Gezginci-Oktayoglu¹, Bertan Boran Bayrak², Refiye Yanardag², Sehnaz Bolken³

¹Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey;

²Istanbul University, Faculty of Engineering, Department of Chemistry, Istanbul, Turkey;

³Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey

Contact: fusunoztay@yahoo.com

The study was designed to investigate the acute lung injury occurred with liver injury induced by D-galactosamine and tumour necrosis fac-

tor-alpha (D-GaIN/TNF-a) and to demonstrate whether benzyloxycarbonyl-L-phenylalanyl-alanine-fluoromethylketone (Z-FA.FMK), which is a pharmacological inhibitor of cathepsin B, has a protective role on the lung injury. BALB/c male mice were divided into four groups. The first group received physiologic saline only, the second group was administered Z-FA.FMK alone, the third group received D-GaIN/TNF-a, and the fourth group was given both D-GaIN/TNF-a and Z-FA.FMK. One hour after administration of 8 mg/kg Z-FA.FMK by intravenous injection, D-GaIN (700 mg/kg) and TNF-a (15 mg/kg) were administered by intraperitoneal injection. The D-GaIN/TNF-a-treated mice were characterized by damaged lungs having degenerative structural changes. Treatment with Z-FA.FMK resulted generally in improvement of lung injury in the D-GaIN/TNF-a-treated mice. Despite of the lung injury in D-GaIN/TNF-a-treated mice, the TUNEL method assay showed that the apoptotic index of pneumocytes in mice lung was not different among all experimental groups. However, pneumocytes labelled with proliferating cell nuclear antigen (PCNA) antibodies were numerous in D-GaIN/TNF-a-treated mice receiving Z-FA.FMK when compared to the control mice. In the group given D-GaIN/TNF-a, lipid peroxidation level and lactate dehydrogenase activity were increased but paraoxonase activity was decreased. Treatment with Z-FA.FMK revised these effects. In conclusion, D-GaIN/TNF-a constitutes damage in the lung without the induction of apoptosis. The Z-FA.FMK stimulates especially proliferation of pneumocytes and improves structural and biochemical alterations in injured lung occurred with liver injury induced by D-GaIN/TNF-a in mice.

P1.18

Tight junction molecules in pathogenesis of Barrett's esophagus and adenocarcinoma of the esophagus

Tatiana Demura¹, Evguenia Kogan¹, Ronald Moll²

¹Setchenov Moscow Medical Academy, Moscow, Russia

²Marburg Philipps University, Marburg, Germany

Contact: uursula@yandex.ru

Barrett esophagus (BE) is defined as a columnar metaplasia of the squamous epithelium of the lower esophagus which is a complication of gastroesophageal reflux disease (GERD). BE increases the risk of AC development from 30 to 125 times. Tumor growth is known to influence tight junctions and their constituting molecules. Claudins are the family of plasmalemmal integral proteins known to be the key molecules of tight junctions. In epithelial and endothelial cells claudins regulate diffusion of ions and water, cell polarity, adhesion, differentiation and proliferation. The aim of the study was to investigate pattern of localization of claudins in BE and AC of esophagus using immunohistochemical (IHC) markers. Materials and Methods. The study was performed on 4-quadrant biopsy of the lower esophagus. The specimens were fixed in formalin and embedded in paraffin. From each specimen several slices were made and fixed on the slides. 414 slides were obtained and selected into 5 groups: 1 - GERD squamous epithelium, control group, 2 - GERD gastric metaplasia(GM), 3 - BE intestinal metaplasia without dysplasia (IM), 4 - IM with dysplasia (low and high grade), 5 - AC in patients with BE. Slides were treated with standard IHC protocol with monoclonal antibody claudin 4 (Zymed) and polyclonal antibodies - claudin 1, 2, 3, 5, 7 (Zymed). Results were evaluated with the help of quantitative and semiquantitative methods and statistical analysis. We separately estimated apical (A) and diffuse (D) staining of claudins. Results. Apical staining intensity of claudins 1, 2, 3, 4, 5, 7 decreased from GM and IM (4 points) to dysplasia (2 points) and was minimal or absent in AC (1-0 points), ($p \leq 0,05$), while D staining type of aforementioned claudins was almost absent in GM and IM, increased in dysplasia (4 points) and its level was maximal in AC (6 points), ($p \leq 0,05$). Conclusion. Dysplasia in BE is characterized by both cell atypism and decrease of claudin accumulation in the region of tight junctions as revealed by apical staining. The latter may result in increased of proliferation and disturb differentiation of epithelial cells in BE. Our results showed that IHC detection of claudins 1, 2, 3, 4, 5, 7 can be recommended for differential diagnosis between precancerous state and cancer in BE and in the detection of BE's malignancy potential.

P1.19

MMP-1, MMP-9, TIMP-1, VEGF, Ki-67 and apoptosis in endometrial hyperplasia in premenopausal women: what is new

Tatiana Demura¹, Juliya Zadonskaya², Natalia Litvinova², Juliya Dobrohotova², Gennadij Sukhikh³

¹Setchenov Moscow Medical Academy, Moscow, Russia

²Russian State Medical University, Moscow, Russia

³Research center of Obstetrics, Gynecology and Perinatology, Moscow, Russia

Contact: uursula@yandex.ru

Objective: To evaluate immunohistochemical expression of markers of apoptosis, angiogenesis and extracellular matrix activity in endometrial hyperplasia (EH) without atypia in premenopausal women. Design and methods: The study was performed on endometrial biopsies of 56 patients with endometrial hyperplasia (36, simple EH; 20, complex EH) and 20 with normal endometrium (NE). Standard immunohistochemical reactions were performed with Ki-67 (LabVision, 1:100), APAF-1, (LabVision, 1:100), VEGF (LabVision, 1:200), MMP-1 (LabVision, 1:100), MMP-9 (LabVision, 1:50), and TIMP-1 (LabVision, ready to use, 7 ml). Results were evaluated statistically by quantitative and semiquantitative analyses. Results: Simple EH showed low expression of Ki-67 compared with NE and complex EH ($p < 0,05$). In complex EH Ki-67 was higher than in NE. ($p < 0,05$). APAF-1 expression was significantly lower in EH than NE ($p < 0,05$) and higher in complex EH. The level of VEGF expression was minimal in NE, moderately high in simple EH and the highest in complex EH ($p < 0,05$). Levels of MMP-1 and MMP-9 expression were moderate in simple EH and significantly higher in complex EH ($p < 0,05$), in NE MMP-1 was minimal and MMP-9 was not determined. The expression of TIMP-1 in NE was significantly higher than in EH ($p < 0,05$). Conclusion: EH is characterized by increased levels of proliferation, apoptosis, angiogenesis and MMPs activity and decreased level of TIMP-1.

P1.20

Immunohistochemical approach to adenomyosis

Evguenia Kogan, Ara Unanyan, Tatiana Demura, Olga Grechukhina

Setchenov Moscow Medical Academy, Moscow, Russia

Contact: uursula@yandex.ru

Background: Adenomyosis is one of the most common diseases which is found in women of 20-40 years old. The precise pathogenesis and developmental events leading to this disease remain unknown. Aim of the study: The aim of our study was to investigate molecular aspects of pathogenesis of adenomyosis with different histological activity and clinical manifestation. Design: The study was performed on biopsy samples of uterus with adenomyosis taken from 33 women of reproductive age. Immunohistochemical detection of TIMP-1, TIMP-4, E-cadherin (Lab Vision), PCNA (DAKO), Apo, EGFR, ER, PR (Novocastra) was used. Results were evaluated with the help of quantitative and semi quantitative methods and statistical analysis. Results: Molecular analysis revealed two types of foci: active foci (expressed high levels of MMPs, GFs, PR) and non-active (increased production of TIMPs and E-cadherin). In active foci proliferation prevailed over apoptosis, in non-active - vice versa. Fibroblasts and endothelium showed higher levels of MMP2 (2.8 ± 0.1 and 2.5 ± 0.12 for active foci and 1.7 ± 0.2 and 1.6 ± 0.1 for non-active) and MMP9 (2.4 ± 0.12 and 2.7 ± 0.17 for active foci and 1.4 ± 0.1 and 1.7 ± 0.12 for non-active in stroma and endothelium, respectively) in adenomyosis. Epithelium produced high levels of MMP7 (3.7 ± 0.2 and 2.2 ± 0.15 in active and non-active foci), TIMP4 (4.2 ± 0.2 and 6 ± 0.1 in active and non-active, respectively), E-cadherin (1.8 ± 0.12 in active type and 2 ± 0.15 in non-active type of the disease), EGFR and PR. Adenomyosis and basal layer of eutopic endometrium, especially stromal component, had similar levels of expression of MMP1, 2, 7, 9, TIMP-4, EGFR, PR. Endometrium functionalis had different molecular features. Conclusion: According to immunohistochemical pattern (expression of markers of proliferation, invasion, neoangiogenesis) foci of adenomyosis may be classified as active and silent. The obtained data also suggests that adenomyosis may rather result from the invasion of endometrium into the myometrium which occurs due to the activity of MMPs in stroma and increased angiogenesis than from the activity of glandular epithelial cells.

P1.21

Early morphological and physiological changes of canine hepatocytes after alpha-amanitin exposition

Aleksandra Piotrowska¹, Jan Magdalan², Ilona Iżykowska¹, Alina Ostrowska³, Barbara Dolińska-Krajewska¹, Marzena Podhorska-Okolów¹, Piotr Dziegiel¹

¹Department of Histology and Embryology, Wrocław Medical University, Wrocław, Poland

²Department of Pharmacology, Wrocław Medical University, Wrocław, Poland

³XenoBiotic Laboratories, Inc., Plainsboro, New Jersey, United States

Contact: ola@hist.am.wroc.pl

High mortality rate in the toadstool death cap (*Amanita phalloides*) intoxications principally results from acute liver failure following significant hepatocyte damage due to hepatocellular uptake of the toadstool toxin, amanitin. However, our knowledge about the morphological and physiological changes during the toadstool intoxication is still insufficient because of late manifestation of first clinical symptoms. The objective of our study was to evaluate early morphological and physiological alterations in hepatocytes exposed to different concentrations (1, 5, 10, 20 and 40 µM) of α -amanitin (α -AMA). Experiment was performed on cultured canine hepatocytes which were isolated from the left lobe of canine liver by a modified two-step perfusion technique. The cells were suspended in L-15 plating medium with 10% FBS, gentamicin and amphotericin B and then dispensed into 96- and 24-well collagen-coated plates. After 4 hrs of initial incubation, the plating medium was substituted with defined culture medium (combination of EBSS and Waymouth's 752/1 supplemented with 10% FBS). Different concentrations of α -AMA were used to determine the sensitivity of hepatocytes to the toxin and to assess accompanying morphological and physiological changes. MTT test was employed to determine the energetic and metabolic activity of amanitin-exposed cultured cells after 6, 12, 24 and 48 hrs. Ultrastructure of hepatocytes was studied in the same time course by transmission electron microscopy (TEM). Differences between values of MTT were analyzed by individual comparison with Mann-Whitney U-test. Statistical analysis was carried out using Statistica 6.0 Software. A significant decrease of hepatocytes viability was noted after 6 hr-long incubation with α -AMA at concentrations 10, 20 and 40 µM, after 12 hr of α -AMA exposition at concentrations 5, 10, 20 and 40 µM and after 24 and 48 hr at all concentration used. In TEM hepatocytes displayed ultrastructural changes, such as pale spaces in cytoplasm, and condensation and marginalization of nuclear chromatin after 6 hr of incubation with α -AMA at concentration 1 or 5 µM, and after 12 hr incubation at concentration 1 µM. In the remaining studied groups were observed apoptotic bodies and/or necrotic cells. The obtained results suggest that α -AMA induced early ultrastructural changes in cultured canine hepatocytes. The longer was time of α -AMA exposition, the more intensive morphological changes in cultured cells were observed. It seems that the toxic influence of α -AMA on hepatocytes depends on its concentration and exposition time.

P1.22

The role of insulin-like growth factor-I and polyamines in developing rat small intestine

Omar Karabulut-Bulan, Sehnaz Bolkent

Istanbul University, Istanbul, Turkey

Contact: omurbulan@yahoo.com

Insulin like growth factor-I (IGF-I) is a multi-functional polypeptide which organizes the proliferation and differentiation of various cell types and has a metabolic activity similar to insulin. It is known that IGF-I receptors are localized in the gastrointestinal tract. Polyamines are important mediators in cell differentiation and in the synthesis of macromolecules such as DNA, RNA and proteins. Polyamines play an important role in the gastrointestinal growth which is connected with IGF-I. α -difluoromethylornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase (ODC), the rate-limiting enzyme in the synthesis of polyamines. In the IGF-I-promoted growth the role of polyamines was mainly investigated in *in vitro* systems, however, *in vivo* studies are nec-

essary to clarify the subject. Our aim in this study was to examine *in vivo* role of IGF-I as a stimulant in the growth of developing rat's small intestine, and to clarify the role of polyamines in the period of growth promoted by IGF-I. In this study, animals were divided into 2 groups. DFMO (500 mg/kg/day) was administered for 10 days to 9 rats forming the experimental group. Sterile 0.9% NaCl was injected at the same volume as for the experimental animals into 8 rats forming the control group. Tissue samples were taken from the rats for histological, immunohistochemical and biochemical analyses on the 10th day. In the group given DFMO, expansion and compression of the villi, as well as a decrease in the PAS-positive reaction intensity of brush border and goblet cells, and a decrease in the number of goblet cells were observed. Immunohistochemical assessments showed that the number of PCNA, ODC and IGF-I positive cells in the DFMO-treated group decreased significantly, compared with the control group. Biochemical investigations showed that DNA values in the small intestinal tissue decreased insignificantly in the DFMO treated group as compared to the control one. We conclude that application of DFMO in the developing rat small intestine inhibited polyamine synthesis and IGF-I synthesis and partially prevented growth of the intestine.

P1.23

Wolfram expression in different tissues of rats subjected to hyperlipidic diet

Maria De Falco¹, Simona Capodanno¹, Anna Sellitti¹, Lucrezia Manente², Raffaella Crescenzo³, Italia Falcone³, Salvatore Valiante¹, Antonio De Luca², Vincenza Laforgia¹

¹Dept. of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples "Federico II", Naples, Italy;

²Dept. of Medicine and Public Health, Section of Anatomy, Second University of Naples, Naples, Italy;

³Dept. of Biological Sciences, Section of Physiology, University of Naples "Federico II", Naples, Italy

Contact: madefalco@unina.it

Wolfram, a transmembrane glycoprotein of endoplasmic reticulum, consisting of 890 amino acids, is encoded by WSF1 gene which mutations are well correlated to Wolfram syndrome, also called DIDMOAD, a rare autosomal recessive disorder characterized by juvenile-onset diabetes mellitus, optic atrophy, diabetes insipidus and sensorineural deafness. It has been demonstrated that wolfram is directly involved in insulin release and that disruption of the WFS1 gene in mice causes progressive β -cell loss in the pancreas and impaired stimulus-secretion coupling in insulin secretion. However, the physiological function of this protein remains totally unknown. We have investigated the pattern of expression of wolfram in different organs of diet induced obesity (DIO) rats compared to controls by biochemical and immunohistochemical assays. We have demonstrated that wolfram was modulated in all examined organs although with a different degree among controls and DIO rats. In particular, we have observed a strong decrease of wolfram expression in intestine of DIO rats compared to controls, together with a moderate and low decrease of the protein in adrenal glands and testis, respectively, of DIO rats compared to controls. On the contrary, we have observed an increase of wolfram expression in the pancreas of DIO rats compared to controls. Since DIO rats develop resistance to insulin following hyper-lipidic diet, we have hypothesized that wolfram could be involved in the continuous induction of insulin release influencing different target organs. Specifically, the decrease of wolfram expression in intestine, adrenal glands and testis, could induce an absent endocellular fat assimilation and the formation of ectopic fat that all together were able to worsen insulin resistance leading to diabetes and metabolic syndrome. Moreover, hyperlipidic diet induced an increase of wolfram expression in pancreas of DIO rats that probably determined a rise of insulin secretion as demonstrated by Crescenzo et al. [1]. Taken together, these results indicating a modulation of wolfram expression among DIO rats and controls, seem to suggest that wolfram may be involved in the onset and/or in the worsening of metabolic syndrome. Reference: 1. Crescenzo R. et al. (2008) Alterations in hepatic mitochondrial compartment in a model of obesity and insulin resistance. Obesity doi:10.1038/oby.2008.10.

P1.24

The effects of retinoic acid and selenium on human neuroblastoma cells in two and three dimensional cell culture models

Leyla Tapul, Sevilcan Tuna, Betül Bozkurt, Selcuk Erdem, Ayhan Bilir

Istanbul University, Istanbul Faculty of Medicine, The Department of Histology and Embryology, Istanbul, Turkey

Contact: tapul@istanbul.edu.tr

Introduction: The drug effects on tumour cells *in vivo* is known to be reflected better in three dimensional cell culture models [1,2]. In this study, we have examined the effects of selenium, an antioxidant, and retinoic acid, an antineoplastic drug on human neuroblastoma cell line (SH-SY5Y) using two and three dimensional cell culture models [3,4,5]. **Materials and Methods:** 13-cis retinoic acid (10 µM) and selenium (10 µM) was administered for 48 and 72 hours to human SH-SY5Y cells grown in two and three dimensional cell culture. S-fase fraction was determined by the bromodeoxy-uridine (BrdU) labeling. One hour before the termination of experiments, cells were incubated with BrdU. Bromodeoxy-uridine labeling index (BrdU-LI, a proliferation marker) was determined immunohistochemically using anti-BrdU antibody. **Results:** In two dimensional cell culture model, the proliferation index was significantly decreased by retinoic acid and/or selenium when compared to control at 48 and 72 hours ($p < 0,0083$). In addition, the decrease in proliferation index by retinoic acid plus selenium was significantly more than retinoic acid and selenium at 48 hours ($p < 0,0083$). At 72 hours, proliferation index was observed to decrease significantly more than retinoic acid ($p < 0,0083$). In three dimensional cell culture model, retinoic acid and/or selenium treatments decrease proliferation index significantly when compared to control ($p < 0,0083$), while no significant decrease in proliferation index by retinoic acid plus selenium was observed when compared retinoic acid and selenium ($p > 0,0083$). **Conclusion:** The contradictory results obtained in two and three dimensional cell culture models were discussed. **References:** 1. Kunz-Suchughart LA, Freyer JP, Hofstaedter F, Ebner R: The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. *J. Biomol. Screen.* 2004; 9: 273-285. 2. Mueller-Klieser W: Three-dimensional cell cultures: from molecular mechanism to clinical applications. *Am. J. Physiol.* 1997; 273: C1109-C1123. 3. Brtko J., Filipcik P., Hudecova S., Brtkova A., Bransova J.: Nuclear all-trans retinoic acid receptors: *in vitro* effects of selenium. *Biol. Trace Elem. Res.* 1998 62(1-2): 43-50. 4. Combs GF Jr.: Current evidence and research needs to support a health claim for selenium and cancer prevention. *J. Nutr.* 2005; 135(2): 343-347. 5. Ponthan F, Borgstrom P, Hassan M, Wassberg E, Redfern CP, Kogner P.: The vitamin A analogues: 13-cis retinoic acid, 9-cis retinoic acid, and Ro 13-6307 inhibit neuroblastoma tumour growth *in vivo*. *Med Pediatr Oncol.* 2001; 36(1): 127-31. This work was supported by Research Fund of the Istanbul University. Project number: 546/05052006.

P1.25

Immunohistochemical study of 8-OHdG and its relationship with OGG1, G6PD, and some cancer related proteins in cutaneous melanoma

Daniela Murtas¹, Franca Piras¹, Luigi Minerba², Jorge Ugalde³, Carlo Floris⁴, Cristina Maxia¹, Paolo Demurtas¹, Maria Teresa Perra¹, Paola Sirigu³

¹Department of Cytomorphology, University of Cagliari, Monserrato, Cagliari, Italy;

²Department of Public Health, University of Cagliari, Monserrato, Cagliari, Italy;

³Department of Pathology, Cancer Institute, Solca, Ecuador; Department of Cytomorphology, University of Cagliari, Monserrato, Cagliari, Italy;

⁴Oncologic Hospital "Businco", Cagliari, Italy

Contact: murtas@unica.it

UV-induced oxidative DNA damage is believed to be implicated in the pathogenesis of many tumors, including cutaneous melanoma. 8-hydroxydeoxyguanosine (8-OHdG) is one of the most common mutagenic oxidative modifications induced in DNA and is considered a reliable marker of

DNA damage induced by oxidative stress. Various enzymes, such as 8-oxoguanine DNA-glycosylase 1 (OGG1) and glucose-6-phosphate dehydrogenase (G6PD), function as protection against oxidative stress. OGG1 is involved in the repair and prevention of 8-OHdG-derived mutations and may be up-regulated by oxidative stress. G6PD is indispensable to reductive metabolism and maintenance of cellular redox homeostasis. Previous studies have suggested an involvement of oxidative stress in the UV activation of the tumor suppressor gene *p53*, whose product plays an important role in the regulation of cellular response to DNA damage. Up-regulation of wild-type *p53* results in a transient G1 arrest, allowing cells to repair the DNA damage before resuming the cell cycle. Wild-type *p53* has also been suggested to repress transcription of the *survivin* gene. *Survivin* is a member of the inhibitor of apoptosis protein family, undetectable in most differentiated normal tissues, but strongly expressed in embryonic and fetal organs. It is overexpressed in many human malignancies and such overexpression is associated with poor prognosis. *Survivin* has been implicated in cell division, prevention of apoptosis, cellular stress response, and checkpoint mechanisms of genomic integrity. Although *survivin* and *p53* are both critical modulators of the opposing cellular processes of proliferation and apoptosis, there is currently no evidence of interaction between mechanisms regulating the expression of each. In this study, 8-OHdG, OGG1, G6PD, *p53*, and *survivin* expression was determined by immunohistochemistry and the relationship among these factors in primary cutaneous melanoma was analyzed. Accumulation of impaired 8-OHdG could lead to increased genomic instability that in turn could lead to a more malignant phenotypic behaviour of tumors. Low activity of enzymes such as OGG1 and G6PD has been consistently associated with increased risk for various tumors. Therefore, the effects of 8-OHdG, OGG1, and G6PD on survival in melanoma patients was evaluated. The results will be discussed.

P1.26

Relationship between cyclooxygenase 2 and survivin expression in primary pterygium

Paolo Demurtas¹, Cristina Maxia¹, Maria Teresa Perra¹, Luigi Minerba², Daniela Murtas¹, Diego Carpio³, Romano Colombari⁴, Franca Piras¹, Paola Sirigu⁵

¹Department of Cytomorphology, University of Cagliari, Cagliari, Italy;

²Department of Public Health, University of Cagliari, Cagliari, Italy;

³Department of Ophthalmology, University of Cuenca, Cuenca, Ecuador;

⁴Pathologic Anatomy, Hospital "San Bonifacio", San Bonifacio (VR), Italy;

⁵Instituto del Cancer "Solca", Cuenca, Ecuador; Department of Cytomorphology, University of Cagliari, Cagliari, Italy

Contact: pdemurtas@unica.it

Pterygium is a conjunctival lesion exhibiting degenerative and hyperplastic changes as well as proliferative, inflammatory features and a rich vasculature. It has long been considered to be a chronic degenerative condition, but, following the discovery of an abnormal expression of *p53* in the epithelium in pterygium, some researchers feel that pterygium is an ultraviolet (UV) related-tumor rather than a degenerative disease. UV irradiation has a key role the formation of radical oxygen species (ROS), which have been associated with initiation and progression of tumors. ROS can induce cyclooxygenase 2 (COX-2) formation via activation of the NF-κB signaling pathway. COX-2 expression is known to increase with the effect of agents such as cytokines, growth factors, and mitogens, in fact COX-2 gene has been associated with cellular growth and differentiation and it also plays an important role in inflammation. Both ROS and COX-2 were found to play the most important role in UV-related cutaneous carcinogenesis. Studies by Subbaramaiah et al. (1999) reported that normal *p53* suppresses COX-2 expression, suggesting that loss of *p53* function may result in the induction of COX-2 expression. Recent report by Aziz et al. (2004) demonstrated that UV-B exposure leads to a significant activation of *survivin*, a member of the inhibitor of apoptosis protein family (IAPs), overexpressed in most human malignancies and implicated in the cellular stress response. It is well-known that the functional loss of wild-type *p53* is associated with up-regulation of *survivin* expression in several types of tumors. In our recent study we demonstrated in pterygium a significant association between *survivin* expression and oxidative stress and *p53*. Since recent studies demonstrated a correlation

between COX-2 and survivin expression in lung and breast cancer (Krysan et al., 2004; Barnes et al., 2006), the aim of the present study was to investigate, by immunohistochemistry, the expression of COX-2 in a group of 93 Ecuadorian primary pterygia, and to evaluate a possible association between COX-2 and survivin. The staining for both COX-2 and survivin was localized in the epithelial layers. When analyzed by Fisher's exact test, the expression of COX-2 showed a strong significant correlation with survivin ($P=0.001$). This is the first report of the significant correlation between COX-2 and survivin in primary pterygium. Our results will be discussed. References: Subbaramaiah K et al., J Biol Chem. 1999;274(16):10911-5. Aziz MH et al., Photochem Photobiol. 2004; 80: 602-8. Krysan K et al., Faseb J, 2004;18:206-8. Barnes N et al., Br J Cancer, 2006;94:253-8.

P1.27

Application of FISH method in karyotype analysis of radiation induced mouse lymphomas - models of human lymphoid neoplasms

Joanna Piskorska¹, Beata Grygalewicz², Hanna Szymańska¹, Elżbieta Krysiak¹, Barbara Pińkowska-Grela²

¹Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Genetics and Animal Breeding, Warsaw, Poland

²Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Cytogenetic Laboratory, Warsaw, Poland

Contact: jolechowska@interia.pl

Large number of chromosomal aberrations are found in many human and mouse lymphoid neoplasms. Certain chromosomal aberrations, mainly translocations, are responsible for the activation of proto-oncogenes resulting in development of lymphomas. The studies of mouse chromosomal changes have been more limited due to difficulties in reliable chromosomal identification related to acrocentric nature and similar sizes of mouse chromosomes. The FISH (Fluorescence in Situ Hybridization) method is much more effective in identifying chromosomal abnormalities than the sole application of karyotyping used in routine cytogenetic analysis. FISH can be performed on dividing as well as nondividing cells. 45 radiation induced lymphomas developed in backcross (CcS17xCcS2)xCcS2 mice obtained from two lines among CcS/Dem recombinant strains were evaluated to designate chromosomal aberrations. The lymphomas were classified according to *Bethesda proposals for classification of lymphoid neoplasms in mice* (Morse et al. 2002), generated by the Hematopathology Committee of Mouse Models of Human Cancer Consortium. The characteristics of the lymphoma types were based on histopathological, immunohistochemical and flow cytometry analyses. Cytogenetic analysis was performed on 33 lymphoma cases. The karyotypes were described according to The International Committee on Standardized Genetic Nomenclature for Mice: Rules for Nomenclature of Chromosome Aberrations and documented with Lucia imaging System. Aberrations found in G-banded chromosomes in metaphases were subsequently confirmed using FISH method with adequate DNA probes (QBIOGENE). Hybridization signals were analyzed with Nikon fluorescence microscope. We detected non-random numerical and structural chromosomal changes in 19 cases out of 33 analysed lymphomas. Fourteen lymphomas showed diploid karyotype without visible cytogenetic changes. A polyploid main lines were found in both T-cell and B-cell lymphomas (4 and 5 cases, respectively). The trisomies of individual chromosomes, detected in T-cell lymphomas exclusively, concerned chromosomes 14 and 16. Ts14 were seen once while the trisomy of chromosome 16 was a consequence of the Robertsonian translocation. Rb(16.16) was found in two cases. In one case of T-cell lymphoma the deletion of distal part of the chromosome X - Del(X)- was detected additionally to Ts14. Among B-cell lymphoma cases we found 4 hyperdiploid cases (41-42 chr/cell) with supernumerary, unidentified markers. Structural aberrations involved chromosome 2 (two cases) and 12 (one case). In one case the material of chromosome 2 was involved in non-reciprocal translocation with chromosome 12 and undefined chromosome T(2;12;?). One case showed amplification chromosome 2 material, observable as additional, derivative chromosome 2 copy. The cytogenetic characteristics of the examined lymphomas could permit to recommend them as mouse models of human lymphoid neoplasms.

P1.28

Annexin A5 expression up-regulated in the uterine cervical squamous cell carcinoma

Fulu Gao

Hebei Normal University, Shijiazhuang, China

Contact: gaofulu@yahoo.com.cn

Objective: To determine whether the expression of annexin A5 (ANXA5) is different between uterine cervical squamous cell carcinoma and normal uterine cervical tissue so as to study the mechanism of the development of uterine cervical carcinoma. Methods: 1 Specimen collection: Twenty-five fresh tissues of uterine cervical squamous cell carcinoma and fifteen fresh normal uterine cervical tissues were collected from the affiliated hospital of Chengde Medical College, also from which twenty-six carcinoma and fifteen normal paraffin imbedded specimen were obtained. 2 Western blotting and immunohistochemistry were employed to examine the annexin A5 expression difference on the protein level between the tumor group and the normal group. 3 In situ hybridization was used to detect the annexin A5 expression difference between the tumor group and the normal group on the RNA level. 4 Western blotting and immunohistochemistry were used to examine the annexin A5 expression in the uterine cervical carcinoma cell lines. Results: 1 The annexin A5 expression in the tumor group is much higher than that of the normal group on both the protein level and the RNA level ($p<0.05$). 2 With the differentiation of the carcinoma from well to poor, the annexin A5 expression increased. The difference between well-differentiated and moderately-differentiated group has no difference ($p>0.05$) while the annexin A5 expression of both the two groups have significant difference when compared with that of the poor-differentiated group ($p<0.05$). 3 Annexin A5 protein expressed in the uterine cervical carcinoma cell lines Hela and SiHa cells, which is in accordance with that of the carcinoma tissue. Conclusion: There exists some relation between annexin A5 and the development of uterine cervical squamous cell carcinoma.

P1.29

p53 and Bcl-2 expression in malignant melanomas and spitz nevi

Selma Yilmazer¹, Fatma Kaya Dagistanli¹, Gultekin Kaner²

¹Istanbul University Cerrahpasa Medical Faculty Department of Medical Biology, Istanbul, Turkey

²Istanbul University Cerrahpasa Medical Faculty Department of Pathology, Istanbul, Turkey

Contact: selmayilmazer@myynet.com

Introduction: The etiology of malignant and benign melanocytic neoplasms is still not fully understood. To provide further molecular basis for understanding the progression of melanoma we aimed to study the expression pattern of p53 which is a key apoptotic regulator and Bcl-2, an inhibitor of apoptosis in malignant melanomas (MM) and spitz nevi (SN). Material and Method: In this study the expression of p53 and Bcl-2 was examined immunohistochemically in 12 cases with cutaneous MM, 2 cases with metastatic MM and 5 cases with SN. All tissue samples were stained with hematoxylin/eosin and dermatopathological diagnosis were performed by two independent dermatopathologists. Results: Expression of p53 which was detected in the nuclei of tumor cells, was found in 10 of 12 MM cases and in all of the cases with metastatic MM and SN. Although p53 positive nuclei were detected in the cases with SN, the number of the (+) nuclei was very low. Bcl-2 immunoreactivity which was seen as nuclear membrane staining, was observed in 9/12 cases with MM, 3/5 cases with SN and was not seen in metastatic MM. Conclusion: This study showed that expression of p53 was higher in malignant melanocytic lesions when compared with benign melanocytic lesions. Bcl-2 expression was seen in approximately equal percentage of MM and melanocytic nevi cases suggesting that Bcl-2 expression could be a common finding in cutaneous melanocytic lesions.

P1.30

Implication of a cancer stem cell marker CD133 in the phenotypes of lung cancers

Natsumi Hata, Noriyo Tokuda, Yuji Kondo, Zhang Qing, Koichi Furukawa

Nagoya University, Nagoya, Japan

Contact: bamukerokarochan@mbp.nifty.com

CD133 is a five-transmembrane protein. It has eight N-glycosylations and two big loops in the extracellular domain. This protein is frequently used as a cancer stem cell marker in colon cancers, gliomas, prostate cancers and others. However, actual roles of CD133 have never been elucidated so far. We analyzed expression levels of CD133 in lung cancer cell lines and tried to clarify its functions in the cancer cells with focus on its role in the stemness. Among lung cancer cell lines, majority of small cell lung cancers (SCLCs) showed positive patterns in flow cytometry. On the other hand, adenocarcinomas, squamous cell carcinomas and large cell lung cancers (NSCLCs) scarcely express CD133 with some exception. RT-PCR with RNA from these cell lines revealed similar expression patterns to those shown in flow cytometry. Immunoblotting also showed similar results. To analyze the roles of CD133 in cancer cells, LC-1 cells (adenocarcinomas) expressing a low level of CD133 were separated into high level CD133-expressing cells (CD133-H) and low level CD133-expressing cells (CD133-L) by flow cytometry/sorter. Then, we examined proliferating and invasive abilities of these cells in MTT assay and in Boyden chamber assay, respectively. Consequently, CD133-H showed increased cell growth and invasion. These results corresponded with the natures of SCLCs and NSCLCs, i.e. SCLCs are more aggressive and show poorer prognosis than NSCLCs, and suggested that CD133 is involved in the malignant properties of lung cancers.

P1.31

Correlation between hormone receptors, HER-2 expression status and biological features of early advanced breast cancer

Beata Biesaga¹, Joanna Niemiec¹, Marek Ziobro², Joanna Wysocka³, Anna Kruczak³

¹Dept. of Applied Radiobiology, Centre of Oncology, Krakow, Poland;

²Dept. of Medical Oncology, Centre of Oncology, Krakow, Poland;

³Dept. of Pathology, Center of Oncology, Krakow, Poland

Contact: z5biesag@cyf-kr.edu.pl

Background: Triple-negative breast cancer (TN: with lack of estrogen and progesterone receptors (ER, PgR) and absence of HER-2 overexpression) is associated with high risk of recurrence and poor outcome compared to other hormone dependent breast cancer subtypes. Therefore, the aim of this study was to analyze correlation between clinico-pathological and biological features of different breast cancer subgroups, according to hormonal receptors and HER-2 status. Material and methods: The study was performed in the group of 94 breast cancer patients (mean age: 50.5 years; range: 27 - 69; clinical stage: T1-33 patients, T2-58; N1-84, N2-8, M0; histological grade: G1-5, G2-33, G3-31). Hormone receptors and HER-2 expression status was evaluated using immunohistochemistry (IHC). Biological features of studied tumours: proliferation rate (labelling index of Ki-67- Ki-67LI), expressions of P53 (labelling index of P53- P53LI), topoisomerase II alpha (TOPOIIA labelling index-TOPOIIALI) and microvessel density (MVD) were also assessed by IHC. Results: Among 94 studied tumours, 83.9% were positive for ER, 82.8% expressed PgR and 31.9% demonstrated overexpression of HER-2. According to the status of these parameters, all tumours were divided into three subgroups: (1) triple positive-TP, n=16, (2) cancers with positive expression of hormone receptors and absence of HER-2 overexpression-DP, n=38 and (3) triple negative-TN, n=7. All TN tumours had histological grade G3, whereas in the other two subgroups, tumours with lower histological grade were significantly more frequent (p=0.003). The mean values of Ki-67LI, P53LI, TOPOIIALI and MVD were 22.8%±1.3 (SE), 9.6%±3.4, 5.4±1.1 and 163.5 vessels/mm²±6.6, respectively. There was no correlation between clinico-pathological features (TNM stage, histological grade, patient age) and the assessed biological parameters. Higher proliferation rate was significantly associated with higher expression of P53 (p=0.016) and TOPOIIA (p=0.01). TN tumours were characterized by significantly higher proliferation rate (p=0.04) and higher expression of P53 protein (p=0.02) compared to other subgroups. No signif-

icant differences in expression of TOPOIIA and MVD between TN, DP and TP subtypes were found. Conclusion: The data presented here indicate that triple negative breast cancers, which are known to have more aggressive course of disease and worse prognosis, are also characterized by rapid proliferation and high expression of P53 protein. This study is supported by Polish Ministry of Education and Science; grant number N401 173 31/3808.

P1.32

The effects of gemcitabine and vinorelbine on inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) distribution of MCF-7 breast cancer cell line

N. Dilara Zeybek¹, Sevinc Inan², Nuran Ekerbicer³, Seda Vatanserver², Jale Karakaya⁴, Sevdia F. Muftuoglu⁵

¹Hacettepe University Faculty of Medicine Department of Histology & Embryology, Ankara, Turkey;

²Celal Bayar University Faculty of Medicine Department of Histology & Embryology, Manisa, Turkey;

³Celal Bayar University Faculty of Medicine Department of Physiology, Manisa, Turkey;

⁴Hacettepe University Faculty of Medicine Department of Biostatistics, Ankara, Turkey;

⁵Hacettepe University Faculty of Medicine Department of Histology and Embryology, Ankara, Turkey

Contact: smuftuog@hacettepe.edu.tr

Gemcitabine and Vinorelbine are two agents that have demonstrated good anti-tumor activity and favorable toxicity profiles as single-agent therapy for advanced breast cancer. Nitric oxide generated by nitric oxide synthase acts as a messenger in diverse functions including anti-tumor and anti-pathogenic activities. In this study, we aimed to examine the distribution of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) on MCF-7 breast cancer cell line in response to treatment with Gemcitabine (G), Vinorelbine (V) and combination of Gemcitabine and Vinorelbine (G+V). MCF-7 breast cancer cell line was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ-Germany). The expressions of iNOS and eNOS were determined by indirect immunoperoxidase or immunofluorescence methods and cytoELISA. Cells were cultured in RPMI 1640 containing 10% FCS, 1% L-glutamine and 1% penicillin/streptomycin. MCF-7 breast cancer cell lines were grown on coverslips for immunohistochemistry, and 96 well-plates for cytoELISA, then incubated with Gemcitabine (Gemzar; Eli Lilly and Company) and Vinorelbine (Navelbine, Pierre-Fabre-Medicament) for 24, 48 and 72 hours. Cells were fixed and then incubated with anti-eNOS (Labvision, CA-USA) and anti-iNOS (Labvision, CA-USA) primary antibodies for one hour at room temperature in humidified chamber. After removal of primary antibodies, they were washed with PBS and incubated with peroxidase or FITC labelled secondary antibody for half an hour at room temperature. Cells were counterstained with hematoxylin or propidium iodide. The immunolabelling was analyzed using cytoELISA and presented as the mean ± SD of at least 3 separate experiments. Statistical significance was determined by Friedman and Kruskal-Wallis tests. Significance was defined as p<0.05 for both tests. Significant increase of eNOS immunostaining on MCF-7 cells treated with G and G+V were observed however the immunostaining of eNOS was similar when compared to control and Vinorelbine groups. Apoptotic cells were also detected morphologically in G, V and G+V treated MCF-7 cells. The immunostaining of iNOS was detected in all groups but this immunoreactivity was not different among the groups. In conclusion, while Gemcitabine treatment, induces S-phase arrest, triggered the NOS pathway after treatment of MCF-7 cells, Vinorelbine treatment, arrests microtubular organization, NOS pathway was not changed, because of using different mechanisms. Detection of increased eNOS immunostaining and apoptosis after Gemcitabine treatment on MCF-7 cells could be important to the treatment of human breast cancer.

P1.33

Enhanced B-Raf protein expression is independent of V600E mutant status in thyroid carcinomas

Tetsuo Kondo, Nakazawa Tadao, Kawasaki Tomonori, Nakamura Nobuki, Yamane Tetsu, Katoh Ryohei

Department of Pathology, University of Yamanashi, Yamanashi, Japan

Contact: ktetsuo@yamanashi.ac.jp

BRAF (7q24) encodes a serine/threonine protein kinase, and its expression level varies in different tissues. Although a high prevalence of BRAF mutation has been suggested as an important event in thyroid tumorigenesis, lit-

tle is known about the expression pattern of B-Raf in the thyroid. Thus, we examined the expression of B-Raf in various neoplastic and nonneoplastic thyroid tissues and compared it with BRAF mutational status. Normal and hyperplastic thyroid tissues showed focal and faint immunoreactivity for B-Raf, especially in cuboidal follicular cells of small follicles. In contrast, diffuse expression of B-Raf was observed in follicular adenomas and well-differentiated carcinomas. The missense point mutation BRAF(V600E) was identified in 42% (13/31 cases) of papillary carcinomas and 33% (5/15 cases) of undifferentiated carcinomas but not in normal thyroid tissues, nodular hyperplasia, follicular adenomas, or follicular carcinomas. The immunohistochemical expression of B-Raf did not correlate with BRAF mutational status. Moreover, Western blotting revealed that B-Raf expression in thyroid carcinoma cell lines was also independent of BRAF mutation. Serum or fibroblast growth factor-1 stimulation further activates ERK1/2 in heterozygous BRAF(V600E)-positive carcinoma cells as well as BRAF(V600E) mutation-negative carcinoma cells. In conclusion, heterogeneous focal expression of wild-type B-Raf in nonneoplastic tissues may play a role in the growth or functional activity of thyroid follicular cells. In contrast, diffuse expression of wild-type and/or mutant B-Raf may be involved in the tumorigenic process resulting in activation of the mitogen-activated protein kinase signaling pathway in cooperation with other genetic abnormalities and activation of ligand-receptor signaling pathways.

P1.34

Heme oxygenase-1 expression in stromal cells protects against tumor induction and progression

Halina Waś¹, Aleksandra Sierpniewska², Małgorzata Sokolowska¹, Tomasz Cichoń³, Ryszard Smolarczyk³, Paweł Dominik¹, Klaudia Skrzypek¹, Anna Ratajska⁴, Bożena Lackowska⁵, Agnieszka Mazur⁶, Krzysztof Lemke⁷, Stanisław Szala³, Józef Dulak¹, Alicja Józkowicz¹

¹Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Medical Biotechnology, Kraków, Poland;

²Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Biophysics, Kraków, Poland;

³Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Molecular Biology, Gliwice, Poland;

⁴Medical University of Warsaw, Department of Pathological Anatomy, Warsaw, Poland;

⁵Oncology Center, Department of Tumor Pathology, Kraków, Poland;

⁶Jagiellonian University, Institute of Zoology, Department of Evolutionary Immunobiology, Kraków, Poland;

⁷ADAMED Sp. z o.o., Pieńków, Poland

Contact: washalina@gazeta.pl

Heme oxygenase-1 (HO-1), a cytoprotective, proangiogenic and anti-inflammatory enzyme, has been shown to facilitate the progression of many types of tumors. In our previous experiments we demonstrated that overexpression of HO-1 in tumor cells led to decrease in survival time of melanoma-bearing mice, inhibition of inflammatory reaction, as assessed by hematoxylin & eosin staining and FACS analysis using anti-CD45 antibodies, stronger vascularization revealed by immunohistochemical staining using anti-CD31 antibodies, and number of metastases in lungs. In present study our aim was to elucidate the effects of HO-1 expressed in stromal cells on induction and progression of murine skin cancers. Using mice of different genotypes (HO-1^{+/+}, HO-1^{+/-}, and HO-1^{-/-}) we investigated the role of HO-1 on tumor initiation. We showed that lower expression of HO-1 shortened survival of animals and made them more sensitive to induction and progression of squamous cell carcinoma (SCC). Furthermore, reduced expression of HO-1 was accompanied by higher levels of proinflammatory (G-CSF, VEGFR-1, and IL-6) and proangiogenic cytokines (KC, and VEGF), whereas production of pleiotropic antitumor factors (IL-12, and TNF) was strongly inhibited. Finally, expressions of antioxidative enzymes (SOD1, SOD2, and GSTA1) were slightly elevated in livers of mice with lower HO-1 expression. In the growing tumors, lack of HO-1 in stromal cells did not influence significantly the host survival, as demonstrated after intracutaneous and intravenous inoculation of mice with B16(F10) melanoma labeled with luciferase. Nevertheless, we showed that males with lower HO-1 expression grew bigger tumors and formed more lung metastases as shown by measurement of luciferase activity using IVIS® imaging system, whereas females of all genotypes rejected tumors. Moreover, some HO-1^{+/+} males showed the kidney and spleen micrometastases, while some HO-1^{-/-} males formed the liver and blood micrometastases.

Additionally, reduced level of HO-1 in host cells correlated with attenuated leukocytes infiltration of primary tumors as evaluated by FACS analysis and alterations in level of inflammatory (IL-6, IL-10, IL-12, IFN γ , and MCP-1) and angiogenic cytokines (VEGF, and KC). Interestingly, tumors in males were mainly invaded by granulocytes, whereas in females by lymphocytes. Finally, HO-1 expression as well as tumor progression changed hematological parameters (WBC, RBC, and platelets). In conclusion, HO-1 could protect healthy tissues against chemically-induced carcinogenesis and melanoma progression, and level of its expression might be suggested as a prognostic marker in cancer prevention. This work was partially supported by grant from the Polish Ministry for Education and Science 2296/B/P01/2007/33. H.W. is the Adam Krzyżanowski and The START (from Foundation for Polish Science) scholarships holder. A.J. is the International Senior Research Fellow of The Wellcome Trust.

P1.35

Immunohistochemical localization of nucleostemin in some human tumour cells

Sheng-hong Liu¹, Ye-Qing Tong¹, Hong-hui Jiang², Yan Wu¹, Xiao-li Wang¹, Liu Zhi-long³

¹Department of Histology and Embryology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China;

²Department of Surgery, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China;

³Reproductive Medical Center, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Contact: liush01@mails.tjmu.edu.cn

Nucleostemin is a novel protein which can bind G-protein in the nuclei of stem cells or tumour cells, and play an important role in regulating cell proliferation. To understand the clinical significance of nucleostemin in the diagnosis and classification of tumours, in the present study, we examined the characteristic feature of nucleostemin localization in some human tumour cells. Tissues from breast carcinoma and colorectal cancer were fixed in 4% polyformaldehyde, dehydrated in ethanol and embedded in paraffin. ABC and immunofluorescent methods were used to label nucleostemin on paraffin sections of the tumour tissues. It was found that the localization of nucleostemin in different tumour cells varied. For example, the positive immunostain of nucleostemin was observed in the nucleolus or nucleoplasm of some cells of colorectal cancer, and positive immunostain of nucleostemin in the cytoplasm was found in some breast carcinoma cells. The results suggest that the characteristic feature of nucleostemin localization in tumour cells may be related with the origin or differentiation of some tumours.

P1.36

Morphological and cellular alternations of HepG2 cells overexpressing CYP3A4 after treatment with antitumor imidazoacridinone derivative C-1311

Ewa Augustin, Joanna Koprowska, Monika Demusiak, Jerzy Konopa

Gdańsk University of Technology, Chemical Faculty, Department of Pharmaceutical Technology and Biochemistry, Gdańsk, Poland

Contact: koprowska@epf.pl

The imidazoacridinone derivative C-1311 is potent antitumor agent currently undergoing Phase II clinical trials. Our previous studies have shown that metabolic activation of C-1311 was crucial for biological activity of this drug. Furthermore, studies with rat and human microsomal enzymes revealed that CYP3A4 isoenzyme was suspected to be responsible for this metabolism. It is well known that expression level of enzymes responsible for drug metabolism altered cell cycle progression and cellular response induced by anticancer drug. Therefore, here we elucidate the cellular response to C-1311 in hepatocellular carcinoma cell line HepG2 overexpressing CYP3A4 (Hep3A4) compared to empty vector-transfected cells (HepC34). All experiments were performed at biological relevant dose of the drug corresponding to its EC₅₀ value (Hep3A4 = 1,7 mM, HepC34 = 0,6 mM) and for different time periods varying from 3 to 144 h. Cell cycle analysis revealed that both hepatocellular carcinoma cell lines starting from 12 h underwent stable G2/M arrest after C-1311 treatment. This cell cycle effect was accompanied by the appearance of sub-G1 DNA content which reached about 20% after

prolonged incubation with the drug. Morphological changes of hepatocellular carcinoma cells in response to C-1311 treatment were assessed by fluorescence microscopy. DAPI staining revealed that starting from 12 h of C-1311 treatment, both studied hepatocellular carcinoma cell lines exhibited condensed chromatin and apoptotic-body like structures concomitantly with the appearance of the enlarged cells with multiple micronuclei, typical for mitotic cell death. However, starting from 48 h, C-1311-treated cells developed features of senescence with flattened, enlarged morphology and increasing degree of SA-b-galactosidase (SA-b-gal) staining. After 144 h of incubation with drug the number of SA-b-gal-positive cells increased to about 100%. To conclude, obtained results suggest that in both hepatocellular carcinoma cells senescence-like arrest appears to be a major cellular response to C-1311 treatment. Moreover, various expression level of cytochrome P450 isoenzyme CYP3A4 does not have significant influence on the type of the cellular response induced by C-1311 in hepatoma cells.

P1.37

Expression of luminal and basal cytokeratins in node negative breast cancer patients

Joanna Niemiec¹, Krzysztof Malecki², Andrzej Komorowski³, Kaja Niesiolowska¹, Janusz Ryś⁴

¹Department of Radiation Biology, Cracow, Poland;

²Breast and Thoracic Cancer Unit, Cracow, Poland;

³Department of Surgical Oncology, Cracow, Poland;

⁴Department of Pathology, Cracow, Poland

Contact: joanna@eikon.pl

In the early breast cancer, the decision about adjuvant chemotherapy (ChT) is made on the basis of clinico-pathological parameters. Emerging data demonstrate that stratification of breast cancers by gene expression profiles and other techniques into basal and luminal subtypes, may have clinical implication. Hence, we tried to specify the group of early breast cancer patients with no risk of progression, on the basis of expression of cytokeratins 5/6 (basal) and 8/18 (luminal) and/or other biological parameters. Materials and method: Eighty eight ductal breast cancer patients (T1-T2 N0 M0), operated on in the Centre of Oncology between 1993-5, not treated with adjuvant ChT, were included into the study. Mean age of patients was 57.4±1.3 years. The expression of the biological parameters was assessed on formalin fixed and paraffin embedded tissue sections using immunocytochemistry. The expression of P-cadherin, Ep-CAM, HER-2, cytokeratins 5/6 (CK5/6), CK8/18, was assessed in tumour cells, using semi-quantitative criteria. Microvascular density (MVD) was assessed on the basis of CD-34 expression. Proliferative potential was expressed as percentage of MIB-1 positive cells (MIB-1LI). Results: 14.7% of tumours showed expression of CK5/6 (CK5/6+). Cytoplasmic expression of CK8/18 (CK8/18+) was observed in 76.2% of tumours, while lack of cytoplasmic expression (CK8/18-) in 23.8%. Tumours were classified into 3 immunophenotypes on the basis of CK5/6 and CK8/18 expression. There was 16.1% tumours with basal (CK5/6+ and CK8/18-), 12.5% with mixed (CK5/6+ or CK8/18-) and 71.4% with luminal (CK5/6- and CK8/18+) immunophenotype. The basal phenotype presented significantly higher MVD than mixed and luminal phenotype (p=0.034). MIB-1LI was higher in basal than in mixed (p=0.055) or luminal immunophenotype (p=0.000). There was significant relation between basal immunophenotype and high tumour grade (p=0.023), ER negativity (p=0.000) and HER-2 negativity (not significant). Additionally significant relation was found between luminal phenotype and P-cadherin negativity (p=0.007). Double negative tumours (ER-/HER-2-) had higher MIB-1LI than ER+ and/or HER-2+ tumours (p=0.000). Disease free survival (DFS) estimated using Kaplan-Meier method was 71%. DFS was not related to: G, ER, HER-2, P-cadherin, Ep-CAM. All patients with tumours presenting the mixed immunophenotype survived without progression, while DFS in the group of women with luminal or basal phenotype of tumour, was 64%. Conclusion: The preliminary data indicate that assessment of tumour immunophenotype might be helpful in stratifying of the early breast cancer patients into groups characterized by low and high risk of progression. Acknowledgment: The study was supported by Polish Ministry of Education and Science; grant number N401 2344 33.

P1.38

HER2 expression in neuroblastic tumors - the preliminary study

Ewa Izycka-Świeszewska¹, Grażyna Kobierska-Gulida¹, Elżbieta Drożyńska², Wiesława Grajkowska³, Bożena Dembowska⁴, Sylwia Koltan⁵, Teresa Klepacka⁶, Danuta Perek⁴, Anna Balcerska²

¹Dept. of Pathomorphology, Medical University of Gdansk, Gdańsk, Poland;

²Dept. of Pediatrics, Hematology, Oncology and Endocrinology, Medical University of Gdańsk, Gdańsk, Poland;

³Dept. of Pathology, Children's Memorial Health Institute Warsaw, Warszawa, Poland;

⁴Dept. of Oncology, Children's Memorial Health Institute Warsaw, Warszawa, Poland;

⁵Dept. of Pediatrics, Hematology and Oncology Collegium Medicum in Bydgoszcz the Nicolaus Copernicus University, Bydgoszcz, Poland;

⁶Dept. of Pathology, National Research Institute of Mother and Child, Warsaw, Poland

Contact: eczis@wp.pl

108 cases of neuroblastic tumors from four Polish centers were evaluated for HER2 expression in routinely processed tissue material. The analyzed data included: age, stage, primary tumor localization, histological diagnosis, NMYC status and patients' survival. The series included 70 tumors untreated before sampling and 38 after inductive chemotherapy, composed of 6 undifferentiated, 38 poorly differentiated, 34 differentiating neuroblastomas, 19 ganglioneuroblastoma, 11 ganglioneuroma. The children's age ranged from 1 to 192 months. 33 patients died of disease. There were 52 suprarenal, 28 abdominal, 16 mediastinal, 5 neck, 4 paravertebral and 3 sacral tumors, with staging: 8-I, 18-II, 33-III, 45-IV, 4-IVs. NMYC amplification characterized 27 cases. Tumor slides were stained with cerb-b2 antibody with EnVision method (DAKO). The mean HER2 expression score (MS) was assessed based on intensity of reaction and percentage of immunopositive cells. HER2 labeling concerning less than 1% of neoplastic cells was treated as negative. MS HER2 was graded as 1, 2, 3. HER2 expression was membranous, cytoplasmic or mixed, depending on the maturation of neuroblastic cells. In 20 cases MS HER2 was negative, in 88 MS HER2 was positive. MS 1 was found in 32 cases, MS 2 characterized 30 tumors and MS 3-26. Statistical analysis showed the significant relation of HER2 negativity with mortality, higher tumor stage and NMYC amplification. HER2 expression was connected to tumor histology with a strong labeling and prevalent MS 2 and MS 3 in differentiating neuroblastoma and ganglioneuroblastomas. The relation of higher HER2 and tumor differentiation with inductive chemotherapy was also found. Our analysis show the relation of HER2 expression with some patho-clinical characteristics of neuroblastic tumors. HER2 expression in neuroblastic tumors seems to depend mainly on the status of differentiation of neuroblastic cells.

P1.39

Histochemical and immunohistochemical techniques as a tool to classify mouse hematopoietic neoplasms - counterparts of human diseases

Hanna Szymańska, Joanna Piskorowska, Elżbieta Krysiak, Marek Woszczyński

Department of Genetics and Laboratory Animal Breeding, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland

Contact: maczym@yaho.com

Hematopoietic neoplasms can develop spontaneously in mice of certain strains, in GEM mice or follow the induction with: irradiation, exposure to chemicals or exogenous infection with mouse leukemia viruses (MuLVs). The Hematopathology Subcommittee of Mouse Models of Human Cancer Consortium proposed the classification of mouse hematopoietic neoplasms (2002) compared with the human WHO classification. As contribution to the proposed classification we evaluated spontaneously developed hematopoietic neoplasms in 201 mice of five inbred strains: AKR/W, C57BL/6W, C57BL/10W, BALB/cW and 129/SvW. We used two immunohistochemical IHC techniques: ABCComplex/HPR and the Vector[®] M.O.M[™] (Mouse-on-Mouse) Peroxidase Kit. ABC systems are based on pre-established detection complexes consisting of peroxidase, avidin and biotin. M.O.M is immunodetection kit to detect mouse primary antibodies on mouse tissues. When using of histochemical staining-ASD, chloroacetate esterase activity demonstrated granulocytic leukemia. The processed material of affected organs included: paraffin and cryostat sections, air-dried imprints, bone marrow and blood smears. The antigen retrieval method was used for paraffin sections by incubating them with citric acid solution in a microwave oven. The beginning IHC panel included antibodies against B-cell lymphomas (B220) and T-cell lym-

phomas (Thy1.1/Thy1.2 and CD3) or granulocytic leukaemia Gr-1. Based on beginning IHC and morphology if there was alteration of B-cell areas antibodies against κ , γ , IgM, IgD and CD5 were used. When T-cell lymphoma was considered antibodies against CD4, CD8, TCR $\alpha\beta$ were used. Simultaneously, the immunophenotyping was done by flow cytometry. In AKR/W mice 88% of lymphoid neoplasms were T-cell while this type accounted for only 7% in BALB/cW mice, 4% in C57BL/6W, 2% in C57BL/10W and 1% in 129/SvW mice. T-cell lymphoma appeared earlier in AKR/W mice (271 days) than in the other strains (median age 628-866 days). The incidence of B-cell lymphomas was the highest in C57BL/10W-30%; 17% in BALB/cW, 14% in C57BL/6W, 10% in 129/SvW mice and only 0.8% in AKR/W mice. B-cell lymphomas developed only in aging mice (median age 787 days). The T-cell lymphomas were histological diagnosed as Precursor T-cell lymphoblastic lymphomas but demonstrated five different immunophenotypes. B-cell lymphomas were recognized as Follicular B-cell lymphoma or Diffuse large B-cell lymphoma but showed only one immunophenotype. The incidence of granulocytic leukaemia was 13 cases - 6% of all hematopoietic neoplasms in the examined strains. The hematopoietic neoplasms developed in these common mouse strains could be useful as models for human hematopoietic neoplasms.

P1.40

Immunohistochemical expression of Olig2 in gliomas focused on tumor morphology and biological behavior

Taku Homma, Makoto Sano, Yukari Obana, Tetsuyo Maeda, Toshinori Oinuma, Masahiko Sugitani, Norimichi Nemoto

Department of Pathology, Nihon University School of Medicine, Tokyo, Japan

Contact: homma@med.nihon-u.ac.jp

To clarify the relationship between the biological behavior of gliomas and the expression of Olig2, a transcription factor in cell differentiation of oligodendroglial lineage, immunohistochemical analysis was done. Materials and methods: Of 27 glial neoplasms, 12 high grade astrocytomas (2 anaplastic astrocytomas and 10 glioblastomas), 6 low grade astrocytomas (1 pilocytic astrocytoma and 5 diffuse astrocytomas), and 9 oligodendroglial neoplasms (3 oligoastrocytomas and 6 anaplastic oligoastrocytomas) were used in this study. All the tumor tissues were fixed in 10% formalin and embedded in paraffin. Five micrometer thick tissue sections were immersed in citrate buffer, pH 6.0, and autoclaved for antigen retrieval. Then processed for immunostaining using anti-Olig2 antibody (ImunoBiological Lab. Takasaki Gunma, Japan) and ENVISION system (DAKO). Results: In astrocytic neoplasms, the immunoreactivity against Olig2 was more frequently observed in the low grade group (except for pilocytic astrocytoma) than the high grade one. Particularly in glioblastomas, the immunoreactivity of Olig2 was quite low. On the other hand, no significant difference of Olig2 immunoreactivity was observed in both oligoastrocytoma and anaplastic oligoastrocytoma groups. Discussion and Conclusion: It has been well known that oligodendroglial tumors are more sensitive against chemotherapies and show better prognosis than astrocytic tumors. In addition, several studies indicated that glioblastomas with oligodendroglial component show better outcome than conventional glioblastomas. The diagnostic criteria for anaplastic oligoastrocytoma and glioblastoma with oligodendroglial component are obscure, though the prognosis and the sensitivity against chemotherapies are much different between both the groups. In this study, a low immunoreactivity against Olig2 was observed in the tumor groups with more heterogeneous histology, such as pilocytic astrocytoma, oligoastrocytomas and glioblastomas. As a result, the expression of Olig2 might be related to the tumor histopathology and the polymorphism of growth pattern in the gliomas.

P1.41

Carbohydrate-binding profile of mammary carcinoma and lymphoma cells

Olga Kurmyshkina, Eugenia Rapoport, Ekaterina Moiseeva, Galina Pazynina, Nikolai Bovin

Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia

Contact: studioza@mail.ru

Beta-galactoside-binding lectins (galectins) are expressed on normal and tumor cells and involved in cell growth, cell-cell and cell-matrix adhesion through their binding to glycoproteins. Expression of galectins and other lectins have been shown to correlate with cancer progression that makes them attractive marker for cancer diagnostics and prognostics. We have

studied the ability of carbohydrate probes Glyc-PAA-fluo (where Glyc is a ligand for galectin, PAA is soluble hydrophilic polymer, fluo is fluorescein label) to bind tumor and normal cells. The next cells were probed: cell lines of epithelial and lymphoid origin, cells obtained from spontaneous and transplanted mouse mammary carcinomas and T-cell leukemic lymphomas, cells from surgically obtained human breast carcinoma samples. Cells obtained from the lung, kidney and thymus of intact mice were used as negative control. It was shown that: 1) several Glyc-PAA probes bound to mammary carcinoma cells (murine and human), whereas cells of lymphoid origin and normal cells did not display any binding; 2) 6OSuLacdiNAc-PAA-fluo was the most potent probe for all tested carcinoma cells, totally 18 carbohydrate probes were used. Our data suggest an accumulation of 6OSuLacdiNAc-binding lectin in mammary carcinoma cells to be promising histochemical marker. The work is supported by the grant of Russian Foundation for Basic Research No. 07-04-00969.

P1.42

Effect of MG-132 proteasome inhibitor on viability and apoptosis of Bomirski amelanotic melanoma cells

Justyna Sidor-Kaczmarek¹, Sławomir Wójcik¹, Mirosława Cichorek², Jan H. Spodnik¹, Janusz Morys¹

¹Department of Anatomy and Neurobiology, Medical University, Gdańsk, Poland

²Department of Embryology, Medical University, Gdańsk, Poland, Gdańsk, Poland

Contact: j.sidor@amg.gda.pl

Melanoma is a neoplastic malignancy of cutaneous origin characterized by high metastatic potential and resistance to conventional treatment like chemotherapy or radiotherapy. It has been proposed that proteasome inhibitors may be evaluated as a new class of new antitumor agents. The proteasome is a multiprotein complex of various activities, which controls eukaryotic protein degradation. In this way it affects the cell cycle, DNA transcription and replication, and inflammatory responses. It is known that proteasome inhibitors promote apoptosis in many types of tumor cells *in vitro* and exert the antitumor effect *in vivo*. One compound, bortezomib, entered the phase of clinical trials and has been approved for the treatment of multiple myeloma. The first discovered and best known class of the proteasome inhibitors represent peptide aldehydes, e.g. MG-132 that reversibly inhibits the chemotrypsin-like activity of proteasomes. The aim of our study was to analyze the biological effects of proteasome inhibitor MG-132 on Bomirski amelanotic (Ab) melanoma cells. Amelanotic melanoma is a hamster transplantable melanoma that serves as a convenient animal model for the studies on antitumor agents. We used cells derived from amelanotic melanoma because of their higher growth rate comparing to melanotic ones. The cells were isolated from the solid tumor and grown in the appropriate conditions in presence of MG-132. Cell viability was assayed with the use of XTT test. Induction of apoptosis was examined by Western blot detection of caspase-3 active subunit and apoptosis-inducing factor (AIF) as well as visualizing mitochondria and cell nuclei. The viability of amelanotic melanoma cells started to decrease after 12 hours of incubation with MG-132 reaching over 98% of dead cells following 48 h of incubation. Evident morphological changes in the mitochondria accompanied the cell death. Release of AIF from the mitochondria and its accumulation in the cytoplasm and evidence of caspase-3 cleavage preceded cell death. Our findings confirm the antitumor effect of MG-132 on Ab Bomirski melanoma cells and indicate apoptosis as the major way of cell death with activation of both caspase-dependent and caspase-independent apoptotic pathways. This study supports the position of proteasome inhibitors as potent selective cancer therapeutics.

P1.43

Presence of intestinal nervous system neuropeptides VIP, NPY, CGRP, and GAL in carcinoma of the human large intestine

Janusz Godlewski¹, Mirosław Łakomy², Zenon Pidsudko², Waldemar Sienkiewicz²

¹Department of Histology and Embryology, Faculty of Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

²Division of Animal Anatomy, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Contact: janusz350@poczta.onet.pl

Although the presence of neuropeptides in components of enteric nervous

system in parts of colon wall invaded by cancer has been recently demonstrated by microscopy, no data exist concerning the quantitative aspects of their occurrence. The aim of this study was to determine the presence of intestinal nervous system neuropeptides: vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), and galanin (GAL) in the tissue of cancerous tumors of the large intestine, to assess the content of these neuropeptides in tissue, and to compare these results with the quantity of neuropeptides in a pathologically unchanged (control) sections of the intestine wall. The study was carried out on tissue samples from the full thickness of the intestinal wall affected by colon cancer, and from unchanged colon. The material was obtained from 15 patients operated on cancer of the sigmoidum and rectum by the resection of the sigmoid colon and anterior resection of the rectum. The concentrations of neuropeptides was determined by specific human ELISA tests. The study was approved by the the Bioethical Commission of the Warmia and Mazury District Medical Association, permit no. 107/2004/II. The results showed presence of VIP at an average level of 28.9 ng/g, NPY at 8.7 ng/g and GAL at 9.4 ng/g in cancerous tissue of the sigmoid colon and rectum. These values were significantly lower comparison to the average levels for unchanged tissue of the intestinal wall: VIP 83.9 ng/g, NPY 19.5 ng/g, and GAL 12.3 ng/g. The content of CGRP was similar in the cancerous (13.3 ng/g) and in the control, unchanged tissue (13.4 ng/g). The decrease in the concentration of VIP, NPY and GAL in the cancerous tissue in comparison to the unchanged one may suggest motoric dysfunction of the part of colon wall invaded by cancer. Similar concentration of CGRP may indicate persistent level of pain perception in affected part of intestine.

P1.44

Quercetin as a P-glycoprotein inhibitor in resistant gastric cell lines

Sylvia Borska, Małgorzata Drag-Zalesińska, Teresa Wysocka, Maciej Zabel, Piotr Dzięgiel

Department of Histology and Embryology, Medical University, Wrocław, Poland
Contact: borska@hist.am.wroc.pl

Multidrug resistance (MDR) is still a significant obstacle in providing effective chemotherapy. The classic MDR is associated with the overexpression of P-glycoprotein (P-gp), ATP-dependent membrane transporter encoded by *ABC1* gene. MDR results in increased efflux of chemotherapeutic drugs from cancer cells. Inhibition P-gp as a way of reversing MDR has been extensively studied for over 30 years. Many agents that modulate P-gp function were identified but most of them are associated with unacceptably serious side effects. Biopolyphenols, including quercetin (Q), are known to have anti-cancerogenic properties due to their antiproliferative and proapoptotic activity. It has been recently reported that some of them may also act as selective and safe modulators of P-gp function. The aim of the study was to determine if quercetin exerts significant influence on P-gp presence in cell membrane and on transport inhibition in the cells of daunorubicin-resistant gastric cancer line. We also assessed the effect of quercetin on the expression of *ABC1* gene in studied cells. The investigations were performed *in vitro* on two tumour cell lines, daunorubicin-resistant gastric cell line EPG85-257RDB and its sensitive variant EPG85-257P as a control. The effect of quercetin was tested at three concentrations of 3, 6 and 12 μ M chosen after initial cytotoxicity tests. P-gp expression was determined by immunocytochemical analysis with C-219 monoclonal antibodies. Transport functions were checked by measuring calcein accumulation. Changes in *ABC1* expression after quercetin exposure were analyzed using real-time PCR technique. Quercetin was found to inhibit P-gp expression and increase calcein efflux in gastric cancer resistant cells at the concentration of 12 μ M. Moreover, treatment of the cells with studied biopolyphenol significantly decreased expression of *ABC1*. The results obtained in our study are promising, showing the possibility of quercetin supplementation as a way to increase cancer cells sensitivity and the efficacy of chemotherapy.

P1.45

Expression of mRNA for metallothionein isoforms in ductal breast cancer

Agnieszka Gomulkiewicz¹, Piotr Dzięgiel¹, Marzena Podhorska-Okołów¹, Andrzej Wojnar², Maciej Ugorski³, Maciej Zabel¹

¹Department of Histology and Embryology, Medical University, Wrocław, Poland; ²Lower Silesia Centre of Oncology, Wrocław, Poland;

³Chair of Biochemistry, Pharmacology and Toxicology, University of Natural Sciences, Wrocław, Poland

Contact: agom@hist.am.wroc.pl

Metallothioneins (MTs) are a family of low molecular weight (6-7 kDa) intracellular proteins, characterized by a high affinity for heavy metal ions. In humans there are at least 10 functional isoforms of this protein. MTs are involved in many pathophysiological processes, including cell proliferation, differentiation and apoptosis. In recent years variety of studies have shown an increased expression of MTs in certain malignant tumors, including breast cancer. However, the prognostic significance of this protein remains unclear. Moreover, no convincing data are available on expression of individual MT isoforms. The aim of this study was to examine the relationship between expression of mRNA for selected MT isoforms and some clinicopathological indices: histological grade (G), proliferative potential (Ki-67 antigen), estrogen receptors (ER) and progesterone receptors (PgR) in breast cancer cases. Material for the studies involved 54 cases of invasive ductal breast cancer of G1, G2, G3 histological grade. In paraffin sections of the tumors immunohistochemical reactions were performed using monoclonal antibodies directed against MT, Ki-67, ER, PgR (Dako). Expression of MT was determined using the IRS scale according to Remmele, considering number of positive cells and intensity of colour reaction. Expression of Ki-67, ER and PgR was evaluated using a scale taking into account number of positive cells. Expression of mRNA for metallothionein isoforms: MT-1E, MT-1F, MT-1X, MT-2A and MT-3 was assessed by real-time PCR (7900HT Fast Real-Time PCR System, Applied Biosystems). Among the studied MT isoforms the highest expression of mRNA in ductal breast cancer was noted for MT-3. The level of mRNA for MT-3 positively correlated with expression of Ki-67 antigen. Moreover, the level of MT-3 was inversely related to estrogen and progesterone receptor (ER, PgR) expression.

P1.46

Heat shock protein 70 (HSP 70), prostatespecific antigen (PSA), E-cadherin and racemase in the differential diagnosis and prognosis of prostate intraepithelial neoplasia and prostate cancer

Evguenia Kogan, N. Kovalenko, Anna Markova, D. Zdanov, O. Dobrovolskaya, A. Vinarov

Setchenov Moscow Medical Academy, Moscow, Russia

Contact: uursula@yandex.ru

The goal of the study was to investigate HSP 70, PSA, E-cadherin and racemase on the different studies of prostate cancer (PCa) progression and in prostate intraepithelial neoplasia (PIN). Design. Prostate biopsies and surgical material from 35 patients and 3 control patients (normal prostate tissue) were used. Immunohistochemistry on paraffin sections with microwave pretreatment was performed with antibodies to HSP 70 (self-made polyclonal antibodies), PSA, E-cadherin, racemase (DAKO Cytomation). Results were evaluated with the help of semiquantitative method and statistical analysis was performed by the nonparametric Mann-Whitney test. Results. HSP 70 production was found in epithelial cells of the majority patients with PIN and was absent in PCa ($p < 0.05$). The four PIN patients with negative staining to HSP 70 were associated with the subsequent PCa development in three year period. PSA appeared to give low information in differential diagnosis of PCa and PIN. The level of E-cadherin expression was significantly higher in PIN than in localized PCa and in PCa with extracapsular extension or metastases ($p < 0.05$). The level of racemase expression was low in PIN ($p < 0.05$) and related to the stage of PCa progression ($p < 0.05$). Conclusion. HSP 70, E-cadherin and racemase can be proposed for differential diagnosis of PCa and PIN, while PSA presence turned out to be non-specific for this case. Racemase can be a valuable marker of PCa prognosis. Thus, the use of the immunohistochemical method increases the precision of differential diagnosis and makes possible the individual prognosis of carcinoma development.

P1.47

The effect of betulinic acid on the proliferation and apoptosis induction in human sensitive and resistant cancer cell lines**Malgorzata Drąg-Zalesińska¹, Teresa Wysocka¹, Urszula Ciesielska¹, Ewa Jagoda¹, Piotr Dzięgiel¹, Maciej Zabel²**¹Department of Histology and Embriology, University School of Medicine, Wrocław, Poland;²Department of Histology and Embriology, University School of Medicine, Poznań, PolandContact: jarekzal@onet.eu

The phenomenon of multi-drug resistance is still the main cause of failure in anticancer treatment. The best know mechanism for this resistance is the action of membrane transporter proteins, which results from an increased efflux of chemotherapeutic drugs from cancer cells. Inhibited sensitivity of cancer cells for anticancer agents is connected with their apoptosis resistance. This is the reason for the search for apoptosis-inducing agents, which

could support currently applied cancer therapies. Previously performed studies show that betulinic acid is a promising tool to achieve this goal. Betulinic acid is a plant-derived pentacyclic lupiene-type triterpene, which was discovered in a National Cancer Institute drug screening program of natural plant extracts, and has been recognized to possess potent pharmacological properties. Betulinic acid is present in some kinds of birch trees. Triterpenoids are well know for their anticancer specificity. The aim of the study was to estimate the influence of betulinic acid on sensitive and resistant tumor cell lines. The studies were performed *in vitro* on two human gastric tumor lines: EPG 257/85P (sensitive line), EPG 257/85RDB (daunorubicin-resistance line) and two human pancreas tumor lines: EPP 181/85P (sensitive line) and EPP 181/85RDB (daunorubicin-resistance line). For each studied line after drug exposure (betulinic acid and daunorubicin) the cytotoxicity tests were performed using the cytochrometric SRB assay. Apoptosis was detected by TUNEL and immunohistochemical estimation of caspase-3 expression. The study showed that betulinic acid is a promising anticancer agent with an activity against drug-sensitive and drug-resistant human cancer cells. Betulinic acid induces proliferation and evokes apoptosis in the studied cancer cells lines.

Histochemistry of endocrine cells

P1.48

Expression of beta-chemokine RANTES and its receptors in human epididymis

Zhen Li, Zhijian Sun, Jie Zhao, Yuanqiang Zhang

Department of Histology and Embryology, The fourth Military Medical University, Xi'an, China

Contact: lizhenhe@fmmu.edu.cn

RANTES (regulated upon activation normal T cell expressed and secreted; CCL5) is a member of the -CC- subfamily of chemokines, which induces leukocyte migration by binding to specific receptors in the seven-transmembrane G-protein-coupled receptor (GPCR) family, namely CCR1, CCR3, CCR4 and CCR5. The presence of RANTES has been detected in the seminal plasma of adult man. However, the source of RANTES in the tissues of genital tract is not well defined. To investigate the expression and cellular distribution of RANTES and its receptors in testis and epididymis of adult man, RT-PCR, immunohistochemical staining, as well as immunofluorescence staining was employed. Total RNA was prepared from human testis and epididymis tissue. cDNA fragments encoding human RANTES, CCR1, CCR3, CCR4 and CCR5 were amplified by RT-PCR using specific primers. Meanwhile, tissues of testis and epididymis were fixed and prepared for immunohistochemical and immunofluorescence staining. We did not detect mRNA for RANTES in human testis. In contrast, the epididymis produced high amount of RANTES transcripts. Pronounced RANTES immunochemical staining was highly restricted to the proximal caput epididymal epithelium, mainly in the cytoplasm near the lumen of high/low columnar cells in the efferent duct. There was no definitive RANTES-positive staining within the distal caput, corpus and cauda of epididymis. Immunofluorescence studies also revealed that the expression pattern of RANTES in the epididymis was obviously segment-specific. CCR3 is absent from the epididymis whereas CCR1, CCR4 and CCR5 are present on the apical surfaces of epithelial cells lining the efferent ducts and in the intermediate zone, proximal caput, and corpus and cauda regions of the epididymis. Taken together, these data indicate that RANTES protein is synthesized in most of the principle cells in the initial segment and it can be secreted into the lumen of epididymis. Thus, we speculate that RANTES may bind to its high affinity receptors CCR1 and CCR5, as well as its low affinity receptor CCR4 to initiate a chemotactic effect on human sperm. The exact physiologic, pathophysiologic and molecular mechanisms involved in this process require further study.

P1.49

Calcitonin gene-related peptide: an experimental study in hypertension rat gastric endocrine cells

Irena Kasacka¹, Halina Ostrowska²

¹Zakład Histologii i Embriologii Uniwersytetu Medycznego, Białystok, Poland

²Zakład Biologii Uniwersytetu Medycznego, Białystok, Poland

Contact: kasacka@amb.edu.pl

Hypertension is the most common cardiovascular disease in humans. Most patients with essential hypertension have abnormalities in myocardium, vascular smooth muscle or endothelium, and in physiological regulatory system for blood pressure, including neurotransmitters and humoral factors. Calcitonin gene-related peptide (CGRP) plays an important role in regulating vascular resistance and regional organ blood flow, both under normal physiological conditions and in pathophysiology of hypertension. For this reason, authors decided to investigate the behaviour of CGRP-producing cells of the stomach in the "two kidney, one clip" (2K,1C) renovascular hypertension model in rats. The aim of the present study was to examine the number and distribution of CGRP-containing cells in the pylorus of rats with hypertension. Male Wistar rats weighing 160-180 g were used. All rats were anaesthetized with pentobarbital. A 0.2- mm clip was placed on the left renal artery in 10 rats and the right kidney was left untouched. The 5 control, sham operated rats underwent identical surgical procedures, except that a clip was not

applied to the renal artery. Measurement of systolic blood pressure was performed by the tail-cuff method. Most (7) of the clipping rats were hypertensive when killed (systolic blood pressure >160 mmHg). Three clipped rats were not hypertensive and were excluded from the study. The systolic blood pressure of the sham-operated rats remained normal at 120±6 mmHg. Segments of the gastric pylorus were collected 6 weeks after the renal artery clipping procedure. They were fixed in Bouin's fluid, and processed routinely for embedding in paraffin. Sections were cut at 4 µm in thickness, and stained by H+E for general histological examination, and by Grimelius' method revealing neuroendocrine cells, following the impregnation of their cytoplasmic granules with silver salts. To identify the neuroendocrine cells, an immunohistochemical reaction was performed with a specific antibody against CGRP. It was revealed that the number of CGRP-immunoreactive cells in the stomach of hypertension animals considerably increased in the pyloric mucosa of hypertension animals as compared with the values in the control rats. The results can be regarded as a morphological manifestation of the hyperreaction of CGRP-producing endocrine cells in the rat gastric mucosa in hypertension.

P1.50

Atrial natriuretic peptide-immunoreactive cells in gastric mucosa of hypertensive rats

Irena Kasacka¹, Halina Ostrowska²

¹Zakład Histologii i Embriologii Uniwersytetu Medycznego, Białystok, Poland

²Zakład Biologii Uniwersytetu Medycznego, Białystok, Poland

Contact: kasacka@amb.edu.pl

Atrial Natriuretic Peptide (ANP) is the predominant member of a family of at least three structurally and functionally related peptide hormones that exert a wide array of effects on cardiovascular and renal functions. It has been found immunohistochemically that EC cells in the antral gastric mucosa, besides being serotonin positive, also manufactured ANP. Since evidence of the actual behaviour ANP-producing cells in the stomach is rather scarce, and the pathogenesis of digestive tract disorders in hypertension not fully explained, it seemed interesting to study the distribution and occurrence of these cells in the stomach in the "two kidney, one clip" (2K,1C) renovascular hypertension model of rats. The aim of the present study was to examine the number and distribution of ANP-containing cells in the pylorus of rats with hypertension. Male Wistar rats weighing 160-180 g were used. All rats were anaesthetized with pentobarbital. A 0.2 mm clip was placed on the left renal artery in 10 rats and the right kidney was left untouched. The 5 control, sham operated rats underwent identical surgical procedures, except that a clip was not applied to the renal artery. Measurement of systolic blood pressure was performed by the tail-cuff method. Most (7) of the clipping rats were hypertensive when killed (systolic blood pressure >160 mmHg). Three clipped rats were not hypertensive and were excluded from the study. The systolic blood pressure of the sham-operated rats remained normal at 120±6 mmHg. Segments of the gastric pylorus were collected 6 weeks after the renal artery clipping procedure. They were fixed in Bouin's fluid and processed routinely for embedding in paraffin. Sections were cut at 4 µm in thickness, and stained by H+E for general histological examination, and by the Grimelius' method revealing neuroendocrine cells, following the impregnation of their cytoplasmic granules with silver salts. To identify the neuroendocrine cells, an immunohistochemical reaction was performed with a specific antibody against ANP. It was found that the number of ANP-immunoreactive cells in the stomach of hypertensive rats significantly decreased as compared to control animals. The decrease in the total number of ANP-positive cells in the hypertensive rats may have been associated with either impaired synthesis or a heightened demand for the hormone resulting from the disorders in the internal environment caused by renovascular hypertension.

P1.51

The influence of moderate exposure to cadmium on the thyroid parafollicular cells of female ratsBarbara Piłat-Marcinkiewicz¹, Małgorzata M. Brzóska², Bogusław Sawicki¹¹Department of Histology and Embryology, Medical University of Białystok, Białystok, Poland²Department of Toxicology, Medical University of Białystok, Białystok, PolandContact: bmarcink@umwb.edu.pl

Introduction: The thyroid C-cells, via calcitonin (CT) secretion and presence of numerous regulatory peptides, play an important role in the maintenance of body homeostasis. Previously, we have reported that chronic exposure to relatively high doses of cadmium (Cd) affects the structure and function of the thyroid gland. The aim of the present study was to investigate the effect of Cd on the ultrastructure and secretory function of the thyroid C-cells in a rat model corresponding to moderate human (especially smokers') exposure that may take place under environmental or occupational conditions. **Materials and methods:** Young female Wistar rats were exposed to Cd in drinking water at the concentration of 5 mg Cd/dm³, for up to 9 months. Control animals drank water free of Cd. Both thyroid lobes were collected for analysis after 3, 6 and 9 months of the experiment. CT was detected immunohistochemically (PAP technique) in the C-cells with the use of specific rabbit antibodies. The reaction was performed on paraffin sections of the thyroid fixed in Bouin's fluid. Moreover, the average optical density was analysed in parafollicular cells expressing CT using Olympus D-soft 3.2 version image analysis computer system. **Ultrastructural evaluation of the C-cells** was performed with a transmission electron microscope (Opton 900PC). For this purpose, thyroid sections, fixed in 3.6% glutaraldehyde and refixed in 2% osmium tetroxide, after embedding in Epon 812 were cut into ultrathin sections. **Results:** The exposure to Cd resulted in a weakening in the immunohistochemical reaction for CT of the C-cells with a simultaneous increase in the average optical density of the reaction. In the ultrastructure of these cells, changes within the nucleus (irregular shape, small and weakly visible nucleoli, numerous empty spaces in the nuclear matrix) and cytoplasm (swollen mitochondria, dilated ergastoplasmic reticulum with small number of ribosomes and many free ribosomes, arranged in rosettes) have been observed. The immunohistochemical and ultrastructural changes were noted already after 3 months of exposure to Cd and intensified with its duration. After 9 months, the number of endocrine (secretory) granules was clearly diminished and their electron opacity was rarefied as well. **Conclusions:** The results indicate that exposure to Cd results in changes in the thyroid C-cells ultrastructure. The weakening of the immunohistochemical reaction for CT in C-cells and changes in their ultrastructure may suggest that Cd does not affect the secretory ability of the thyroid C-cells and can inhibit CT synthesis.

P1.52

Effects of GLP-1 receptor agonist on pancreatic β cell neogenesis and islet organization in STZ treated neonatal rats

Fatma Kaya Dagistanli, Melek Ozturk

Istanbul University Cerrahpasa Medical Faculty Medical Biology Department, Istanbul, Turkey

Contact: fkaya@istanbul.edu.tr

The aims of this study are to determine the β cell neogenesis in the early stages of the neonatal STZ (n-STZ) diabetic model and investigate the effects of exendin-4 treatment on β cell regeneration. On the second day after birth 100 mg/kg STZ was given i.p. to two groups of newborn rats. The first group was the neonatal streptozotocin-treated (nSTZ) group. To the second group, starting from third day, 3 μ g/kg/day exendin-4 (Ex4) was given for 5 days. The third group was a healthy control group. On tissue sections, in situ hybridization with an insulin probe and double immunostaining for insulin and BrdU were carried out. The sections were also immunostained with insulin, glucagon, somatostatin, synaptophysin and pdx-1 antibodies. There was a marked increase in BrdU/insulin double immunostained cells in Ex-4 treated yd2-STZ diabetic group as compared to healthy controls. When compared with the control group, we determined that the number of glucagon cells was increased in the islets of the nSTZ

and Ex4 treated groups. Somatostatin immunopositive cells were hypertrophic in the treated and untreated nSTZ. Although pdx-1 immunopositive cells were decreased in the untreated STZ diabetic group as compared to the control group, the exendin-4 treated group was similar to the control group. Synaptophysin expression was detected in all the endocrine cells of islets in all groups. Numerous synaptophysin cells were detected in the lining of duct epithelium as well as exocrine tissue in the untreated and Ex4 treated STZ diabetic groups. The results show that the Ex4 treatment causes production of new beta cell clusters by inducing beta cell proliferation.

P1.53

Alterations in ghrelin cells induced by short and long-term omeprazole treatment in the rat fundus mucosa

Sema Bolkent, Selma Yilmazer, Neslihan Türk

Department of Medical Biology, Faculty of Cerrahpasa Medicine, Istanbul University, Istanbul, Turkey

Contact: semabolkent@yahoo.com

Ghrelin is a novel 28 amino acid gastric peptide, which controls the acid release and is produced abundantly in fundus glands. Omeprazole is a potent inhibitor of gastric acid secretion by inhibiting H⁺,K⁺-ATPase. In this study, we aimed to examine the alterations in ghrelin cells and fundus mucosa of rats in acid inhibition provided by omeprazole of different duration and dose. Female Sprague-Dawley rats, weighing 200-250 g, were divided into six groups, each group containing 6 rats. The first group was the control group for 4 days. The second group received 100mg/kg/day omeprazole as intragastric instillations for 4 days. The third group was given 20mg/kg/day omeprazole as in the second group. The fourth group was the control group for 2 months. The fifth group received 100mg/kg/day omeprazole as intragastric instillations for a period of 2 months. The sixth group was given 20mg/kg/day omeprazole as in the fifth group. Gastric fundus sections were examined for histopathological changes and by immunohistochemistry for expression of ghrelin. In the rats treated with omeprazole, slightly degenerative changes in the fundus mucosa were observed as compared to the controls. The mean number of immunoreactive ghrelin cells in the groups treated with omeprazole showed a statistically significant increase ($p < 0.001$) as compared to the control groups. These results suggest that inhibition of acid secretion stimulates ghrelin production in mucosa of rat gastric fundus.

P1.54

Exendin-4 exerts its insulinotropic effect through decreasing somatostatin expression in diabetic mice pancreas

Sehnaz Bolkent, Selda Gezginci-Oktayoglu

Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey

Contact: sbolkent@istanbul.edu.tr

Exendin-4 is an antidiabetic drug that acts through the glucagon-like peptide 1 receptor and has recently been approved for the treatment of type 2 diabetes mellitus. Exendin-4 also has been shown to affect beta cell gene expression and increase beta cell mass in rodent models of type 1 diabetes mellitus, but the mechanisms are not fully understood. We therefore analyzed the effect of exendin-4 on islet cell proliferation, insulin and somatostatin expression in diabetic and non-diabetic mice. BALB/c male mice which were used in this study were divided into four groups. The first group was given citrate buffer only, the second group was administered exendin-4 alone, the third group received Streptozotocin (STZ), and the fourth group was given both STZ and exendin-4. Exendin-4 (3 μ g/kg) was administered by subcutaneous injections daily for 30 days after the animals were rendered diabetic by single dose (200 mg/kg) administration of STZ. With treatment of exendin-4 of the diabetic mice the following results were noted: (i). Blood glucose levels and somatostatin expression was significantly decreased in pancreatic islets; (ii). Insulin expression was significantly increased in pancreatic islets; (iii). There was no alteration in the number of proliferated cell nuclear antigen positive cells in pancreatic islets. These data suggest that exendin-4 shows insulinotropic effect through decreasing somatostatin expression in diabetic mice pancreas. In addition, we concluded that exendin-4 has no effect on islet cell proliferation.

P1.55

Expression of estrogen receptors in porcine granulosa cells: the effect of phytoestrogen genisteinAnna Nynca¹, Olga Kraszewska¹, Brian Petroff², Renata Ciereszko¹¹Department of Animal Physiology, University of Warmia and Mazury, Olsztyn, Poland²Center for Reproductive Sciences, University of Kansas Medical Center, Kansas City, United StatesContact: anna.nynca@uwm.edu.pl

The phytoestrogen genistein affects female reproductive functions on several regulatory levels including that of the ovary. Genistein's mechanism of action may involve activation of estrogen receptors (ER α and ER β). The objectives of this study were to determine the effect of genistein on ER α and ER β mRNA and protein expression in granulosa cells harvested from medium (3 - 6 mm) and large (> 8 mm) porcine follicles. Granulosa cells were first pre-cultured for 48 or 72 h (37°C, 95% air, 5% CO₂) in Eagle medium and then cultured for subsequent 48 h with genistein (RT-PCR: 0.5 and 5 μ M; immunocytochemistry: 0.05, 0.5, 5 and 50 μ M). The expression of ER α and ER β mRNA in granulosa cells was evaluated by semi-quantitative RT-PCR. ER β protein expression (the percentage of the ER β positively stained granulosa cells and the intensity of cell nuclei immunostaining) was determined by immunocytochemistry using primary mouse monoclonal antibodies against ER β (Serotec, Great Britain) at a dilution of 1:20 and secondary biotinylated horse anti-mouse antibody (Vector Laboratories, USA) at a 1:300 dilution. Genistein did not affect the expression of ER α mRNA in the granulosa cells isolated from medium or large porcine follicles. The lower dose of genistein (0.5 μ M) significantly ($p < 0.05$) increased and the higher dose (5 μ M) tended to increase ($p = 0.06$) ER β mRNA expression in cells harvested from medium follicles. In granulosa cells from large follicles, the lower dose of genistein (0.5 μ M) tended to increase ($p = 0.07$) and the higher dose (5 μ M) did significantly ($p < 0.05$) increase ER β mRNA expression. The intensity of ER β immunostaining in the granulosa cells isolated from medium follicles ($n = 4$) was significantly enhanced ($p < 0.05$) by genistein. In granulosa cells harvested from large porcine follicles, genistein did not affect the ER β protein expression. In conclusion, genistein did not affect the expression of ER α . The expression of both ER β mRNA and ER β protein was increased by genistein in porcine granulosa cells. This study was supported by The State Committee for Scientific Research in Poland: KBN 2 P06D 010 29, PBZ-KBN-084/P06/2002.5.9, UWM 522.0206.0206 and European Social Fund (for AN).

P1.56

Prolactin receptor distribution in the dorsal skin of non-hibernating and hibernating frog (*Rana ridibunda*)

Meliha Sengezer Inceli, Serap Sancar Bas, Engin Kaptan

Istanbul University, Faculty of Science, Istanbul, Turkey

Contact: ssancar@istanbul.edu.tr

The aim of this study was to investigate prolactin receptor (PRLR) distribution in the skin of hibernating and non-hibernating frogs (*Rana ridibunda*) and to discuss its possible functions. Prolactin has several biological functions including promotion of growth and development, metamorphosis, osmoregulation, and reproduction in amphibians. Frog skin is an excretory organ active in the regulation of water and electrolyte balance. Changes in the environmental temperature and humidity during the annual or seasonal cycle affect some important physiological phenomena of the skin, such as respiration and water and ion transport. In our study, the immunoreactivity of prolactin receptor was detected by streptavidin-biotin complex technique. We detected prolactin receptor immunoreactivity in epidermal layers except for epidermal stratum corneum, myoepithelial cells and secretory epithelium of poison glands in both groups. However, in hibernating frogs PRLR immunoreactivity was stronger than in non-hibernating ones. The results have shown that prolactin may contribute to the adaptation of the skin to environmental changes during periods of hibernations.

P1.57

The use of prostaglandin E (1) for the prevention of the lung injury occurred with renal failure induced by renal ischemia-reperfusion in ratsRefiye Yanardag¹, Fusun Oztay², Beyhan Kara¹, Nurcan Orhan³, Sehnaz Bolkent²¹Istanbul University, Faculty of Engineering, Department of Chemistry, Istanbul, Turkey²Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey³Istanbul University, Institute for Experimental Medical Research and Application, Istanbul, TurkeyContact: refiyeyanardag@yahoo.com

In this study, a possible protective effect of prostaglandin E (1) (PGE1) on the lung injury occurred with renal failure induced by renal ischemia-reperfusion was investigated. Sprague Dawley male rats were divided into four groups: (I) control rats given physiologic saline; (II) rats given PGE1 (20mg/kg); (III) rats with renal ischemia-reperfusion model; and (IV) rats with renal ischemia-reperfusion model given PGE1 30 min prior to ischemia and just before the reperfusion period, respectively. In the experimental model, rats were unilaterally nephrectomized and subjected to 60 min of renal pedicle occlusion followed by 60 min of reperfusion. Although rats with renal ischemia-reperfusion did not exhibit heavy degenerative alterations in the lung structure, they possessed some necrotic areas and interstitial edema in the lung. The receiving of PGE1 by rats with renal ischemia-reperfusion resulted in improvement of lung structure, while edema was still permanent. Furthermore, the number of pneumocytes labelled with proliferating cell nuclear antigen (PCNA) antibodies was increased in all experimental groups when compared to the control rats, but this increase was not statistically significant between group III and group IV. Lung glutathione (GSH) levels were decreased in rats with renal ischemia-reperfusion applied. Also, lung lipid peroxidation (LPO) levels in the rats with renal ischemia-reperfusion were increased compared to the control group. Administration of PGE1 caused a significant increase in the lung GSH levels in rats with renal ischemia-reperfusion applied. Whereas lung LPO levels were decreased after administration of PGE1 in rats with renal ischemia-reperfusion. As a result, PGE1 may be useful to prevent the structural and biochemical alterations of the lung injury occurred with renal failure induced by renal ischemia-reperfusion in rats, except for edema.

P1.58

The effect of season on histology and morphometry of domestic gander testis

Anna Leska, Ewa Banachowska, Marek Opalka, Barbara Kamińska, Luiza Dusza

Department of Animal Physiology, University of Warmia and Mazury, Olsztyn, Poland

Contact: anna.leska@uwm.edu.pl

The length of photoperiod is the main factor which affects seasonal morphological and functional changes within the avian gonads. To our knowledge seasonal changes in the histology of testis have been reported only in wild Canada goose and there is lack of data about domestic goose, which is characterized by the most definite seasonality in reproduction within farm poultry. The objective of the study was to investigate the effect of season on histological and morphometric parameters within testis of domestic gander. The study was performed on White Kofuda ganders. Birds were maintained under standard feed and lighting regimes on breeding farm. Testes were collected in three characteristic phases of geese annual reproductive cycle: the peak of reproductive activity (March, $n = 6$), summer photorefractoriness (July, $n = 6$) and fall sexual reactivation (November, $n = 6$). Animals were transcardially perfused with 4% paraformaldehyde in phosphate-buffer saline. Testes were removed, postfixed, embedded in paraffin, cut into 7 μ m thick cross sections and stained with the hematoxylin-eosin technique. The analysis of testis morphometric characteristics concerns area and diameter of the seminiferous tubules, thickness of the germinal epithelium and area of interstitial cells. The morphology of active testis (March) was characterized by complete spermatogenesis process with spermatozoa in the seminiferous tubules and the tubular lumen and by narrow interstitium. In contrast, the inactive testis (July) displayed an involution of seminiferous epithelium, absence of spermatogenesis, a lot of germinal cells in the seminiferous lumen and large area of interstitial cells.

The fall sexual reactivation phase (November) was not clearly shown in the testis morphology. There was observed no recovery of seminiferous epithelium and present of degenerative cells in some tubular lumen. The impact of changing photoperiod on gander testis was reflected in striking variations in all examined morphometric parameters. The highest values of 1) area of seminiferous tubules 2) diameter of seminiferous tubules and 3) thickness of the germinative epithelium were in the breeding season which was followed by significant reduction of 1) 8; 2) 2,9; and 3) 2,6-fold in photorefractoriness. In the fall sexual reactivation modest but not significant 1) 1,7; 2) 1,2; and 3) 1,4-fold increase in comparison with photorefractoriness was noticed. The interstitial cells area (shown as percent of interstitial cells area per seminiferous tubule area) was minimum in reproductive phase and then significantly increased 4,3-fold in non-breeding season and was similar during fall sexual reactivation. In conclusion, the morphometric analysis of gander testis shows evidence for distinct testicular seasonal cycle. This study was supported by The State Committee for Scientific Research in Poland (Grants: KBN N N311 098034, UWM 528.0206.0805) and by European Social Found for AL.

P1.59

Seasonal changes of luteinizing hormone (LH), prolactin (PRL) and thyrotropin (TSH) protein expression in the pituitary gland of domestic gander

Anna Leska, Monika Budzyńska, Luiza Dusza

Department of Animal Physiology, University of Warmia and Mazury, Olsztyn, Poland

Contact: anna.leska@uwm.edu.pl

Luteinizing hormone (LH), prolactin (PRL) and thyrotropin (TSH), hormones synthesized by anterior lobe of the pituitary gland, play critical role in the regulation of seasonal reproduction in birds. Plasma levels of geese pituitary hormones were extensively examined and showed characteristic seasonal variation. However, there is lack of data concerning seasonal changes in LH, PRL and TSH pituitary hormones content. The objective of the study was to localize LH, PRL and TSH secreting cells and examine the effect of season on pituitary level of these hormones in domestic gander (*Anser anser f. domestica*). Domestic goose displays the most definite seasonality in reproduction within farm poultry. Experiments were carried out on White Koluda ganders. Birds were reared under standard feed and lighting regimes on a breeding farm. The study included three characteristic phases of geese annual reproductive cycle: the peak of reproductive activity (March, n=5), summer photorefractoriness (July, n=5) and fall sexual reactivation (November, n=5). Animals were transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline. Pituitaries were dissected, postfixed, embedded in paraplast and cut into 4 µm thick sagittal sections. Expression of antigens examined was evaluated by immunohistochemistry. Detection of LH, PRL and TSH was conducted with rabbit polyclonal anti-LH, anti-PRL and anti-TSH primary antibodies and biotinylated swine secondary antibody. The immunoreactive area (% area) was measured by cell D Soft Imaging System (Olympus). The cytoplasmic localization of LH, PRL and TSH in the pituitary cells of domestic gander was observed. LH and TSH cells were widely distributed throughout the pars distalis, while PRL cells concentrated mainly in the anterior part of the pars distalis. The mean immunoreactive area of LH cells in successive phases throughout the season was 11,41%; 9,49% and 12,17%, that of PRL 6,5%; 5,05% and 1,89% and that of TSH 8,18%; 6,64% and 8,65%. Seasonal changes in the percentage of immunoreactive area was noted for all peptides examined. There was a significant decline in LH and TSH protein expression during the non-breeding season in comparison with two other phases. The level of PRL pituitary protein content decreased from the peak of the reproductive activity towards its end and reached minimum during sexual reactivation. Dynamic seasonal variation in pituitary LH, PRL and TSH synthesis provides proof of the involvement of these peptides in the regulation of domestic geese seasonal reproduction. It was found that pituitary content of LH, PRL and TSH is not always parallel to its plasma concentration what indicates that the mechanism controlling the storage and release of pituitary hormones plays an important role in geese seasonal reproduction. This study was supported by the grants: KBN N N311 098034, UWM 528.0206.0805 and by European Social Found for AL.

P1.60

Expression of the StAR protein in ovarian and testicular cells of the pig and bank vole

M. Slomczyńska, M. Duda, J. Galas, B. Bilińska

Department of Endocrinology and Tissue Culture, Institute of Zoology, Jagiellonian University, Kraków, Poland

Contact: slom@zukunft.edu.pl

Recent advances in the knowledge of the acute regulatory mechanisms during steroidogenesis, concern the biochemical and molecular characteristics of steroidogenic acute regulatory protein (StAR), which plays a key role in the mitochondrial step of steroid hormone biosynthesis. This protein is responsible for cholesterol transport from the outer to the inner mitochondrial membrane of the steroidogenic cell. Synthesis of steroid hormones is regulated by signals from the anterior pituitary gland that act on specific steroidogenic cells in adrenals, ovaries, and testes. In response to these signals the StAR is synthesized, playing an essential role in adrenal and gonadal steroidogenesis. The aim of this study was to show the expression of the StAR protein in porcine granulosa cells and in bank vole Leydig cells *in vitro*. To examine the influence of insulin and LH on porcine granulosa cells, progesterone production and the StAR accumulation within cells were studied. LH and insulin alone exerted a small effect on progesterone secretion, however it depended on the size of follicles used as the source of granulosa cells. In contrast, insulin in combination with LH increased both progesterone secretion and StAR expression as shown by RIA, immunocytochemistry and Western blot using a specific antibody against the StAR protein (a gift from Dr. D.M. Stocco). The *in vitro* study indicates that insulin and LH may contribute to steroidogenic differentiation during follicular maturation. To show whether the StAR protein is co-localized with mitochondria a selective staining of mitochondria in living Leydig cells was performed using a Mito Tracker[®] Red CMXRos probe. Superimposed images from double-fluorescence staining showed a remarkable degree of similarity in the distribution of the StAR protein and mitochondria, indicating mitochondrial localization of the StAR in Leydig cells *in vitro*. Immunofluorescent double-staining seems to be a good technique for visualization of the StAR protein within cell mitochondria. Supported by DS/BiNoZ/IZ/770/2008

P1.61

Connexin 43 expression in human and mouse testes with impaired spermatogenesis

Malgorzata Kotula-Balak, Anna Hejmej, Jolanta Sadowska, Barbara Bilińska

Department of Endocrinology and Tissue Culture, Institute of Zoology, Jagiellonian University, Kraków, Poland

Contact: bbili@zukunft.edu.pl

Gap junctions are intercellular protein channels that provide a pathway for exchange of ions, second messengers, and small molecules. This exchange allows electrical and metabolic coupling of cells as well as coordinated responses of coupled cells to hormones and growth factors. Connexins (Cx) are the principal protein components of gap junctions. Although the presence of several connexin proteins has been reported in various tissues including testes, Cx43 was found to be the most abundantly expressed gap junction protein in testicular cells. The aim of this study was to examine the expression of Cx43 in the testis of a patient with Klinefelter's syndrome and of mice with the *mosaic* mutation (*Atp7a^{mo-ms}*) and a partial deletion in the long arm of the Y chromosome (B10. BR-Y^{del}). These genetic disorders are characterized by the presence of numerous degenerated seminiferous tubules and impaired spermatogenesis. In mouse testes, the expression and presence of Cx43 were detected by means of immunohistochemistry and Western blot analysis, respectively. In testes of Klinefelter's patient only immunoeexpression of Cx43 was detected. Regardless of the species Cx43 protein was ubiquitously distributed in testes of reproductively normal males, whereas in those with testicular disorders either a weak intensity of staining or no staining within the seminiferous tubules was observed. Moderate to strong or very strong staining was confined to the interstitial tissue. In an immunoblot analysis of testicular homogenates Cx43 appeared as one major band of approximately 43 kDa. Our study adds three more examples of pathological gonads in which the absence or apparent decrease of Cx43 expression within the seminiferous tubules was found. A positive

correlation between severe spermatogenic impairment and loss of Cx43 immunoreactivity observed in this study supports previous data that gap junctions play a crucial role in spermatogenesis. Strong Cx43 expression detected mostly in the interstitial tissue of the Klinefelter's patient may presumably be of importance in sustaining Leydig cell metabolic activity. However, the role of gap junction communication in the control of Leydig cell function seems to be more complex than originally thought. The authors kindly acknowledge a generous donation of mice and human testicular biopsy by Professors Józefa Styrna from the Department of Genetics and Evolution of the Jagiellonian University and Leszek Bablok from the Department of Andrology, Institute of Obstetrics and Gynecology, Warsaw Medical University. This work was supported by DS/BiNoZ/IZ/770/2008.

P1.62

The distribution of chromokinesin KIF4A in human decidua during early pregnancy

Sati Leyla, Yasemin Seval, Ramazan Demir

University, Antalya, Turkey

Contact: akademisyen07@yahoo.com

Objectives: Kinesin superfamily proteins (KIFs), such as KIF4A, are microtubule based motor proteins that generate directional movement along microtubules. KIFs are key players or central proteins in the intracellular transport system, which is essential for cellular function and morphology, including cell division. A large portion of human KIF4A is associated with the nuclear matrix during the interphase, whereas a small portion of them is found in the cytoplasm. In this study, we aimed to identify the presence and cellular localization of KIF4A in human deciduas from early pregnancies. Materials and Methods: Tissue samples from human decidua (22-28 days) were embedded in paraffin for immunohistochemistry method. KIF4A antibody was applied on paraffin embedded tissue sections to demonstrate the expression patterns. Semi-quantitative analysis was performed according to staining intensity. Results: Immunohistochemical analysis revealed that endothelial cells and leucocytes were moderately immunopositive with Kif4A in contrast to strong staining intensity in the glands, particularly membranous, and decidual cells. In addition, syncytial knots and extravascular trophoblast cells in the implantation areas showed a moderate Kif4A immunolocalization pattern even though there was a weak immunoreaction in the decidual cells. Finally, there was a strong staining intensity in the placental trophoblast cells. Conclusions: In the present study, we identified the presence of Kif4A in human decidual tissues for the first time in the literature. The strong membranous expression in glandular cells may suggest that KIF4A might play role as a part of cargo system. KIF4 is mainly localized in the nucleus and that nuclear KIF4 is predominantly associated with the nuclear matrix. However, the biological significance of cytoplasmic localization as observed in our tissue sections and association with the nuclear matrix remains obscure. Therefore, human decidual tissues might serve as an interesting model to study as yet unidentified functions of human KIF4A such as its precise molecular and pathological connection with human cancers.

P1.63

Expression of ghrelin, its receptors and transcripts in rat thyroid and their putative functional role. An immunohistochemical and *in situ* hybridisation study

Marek Ruchala¹, Andrzej Łukaszyk², Ludmila Rafińska², Marcin Ruciński², Piotr Sawiński², Aldona Kasprzak², Karolina Olejniczak², Jerzy Sowiński¹

¹Department of Endocrinology, Metabolism and Internal Medicine, Poznań University of Medical Sciences, Poznań, Poland;

²Department of Histology and Embryology, Poznań University of Medical Sciences, Poznań, Poland

Contact: anluk@ump.edu.pl

Some results of our previous studies indicate modulatory effects of ghrelin on secretory function of rat and porcine thyroids and evidence an uptake of 125I-ghrelin from systemic circulation to the gland of male. *In vivo* opposite effects could be recorded in relation to serum concentration of ghrelin and T3 and T4 following short-term starvation in rat. In an experiment performed on the porcine thyroid slices *in vitro* ghrelin, when added to incubation medium together with somatostatin, diminished the effect of

somatostatin which alone up-regulated the TSH-stimulated T3 secretion. Because of controversial opinions on the thyroid cells producing ghrelin and responding to this neuropeptide as the objectives we intend to take part in the discussion with results of the presented study. Expression of ghrelin and its receptors in thyroids was demonstrated immunohistochemically in paraffin sections fixed in Bouin fluid or formaldehyde, using antibodies (anti-ghrelin and anti-GHSR1a, Phoenix-Pharmaceuticals); peroxidase and alkaline phosphatase were used as markers of the immune reaction. For positive control the expression of hormone in ghrelin cells of gastric mucosa and other organs of the same subjects was accepted and for the negative control IgG and complete rabbit serum were used replacing antibodies. Expression of genes for ghrelin and their receptors in rat thyroids was demonstrated in formaldehyde-fixed sections applying RT-PCR technique and also *in situ* hybridisation technique using digoxigenin-labelled oligoprobes (5'-TTA GCT GGC GCC TCT TTG ACC TCT TC-3') specific for rat ghrelin and (5'-ACA CCA CCA CAG CAA GCA TCT TCA C-3') for rat ghrelin receptor mRNA; ovine anti-digoxigenin monoclonal antibodies (Fab fragments) were used to visualize the reaction site immunohistochemically. The immunohistochemical expression of ghrelin in parafollicular and follicular thyroid cells of adolescent and adult rats of both sexes was documented, and also expression of gene encoding ghrelin (mRNA) was demonstrated by RT-PCR in thyroid and by hybridocytochemistry in parafollicular and follicular thyroid cells. With RT-PCR the gene expression for GHSR1a and GHSR1b was identified and with *in situ* hybridisation technique the mRNA for GHSR1a was shown in follicular cells. In follicular cells expression of the type 1a ghrelin receptor could also be demonstrated immunohistochemically. A confrontation of the results and the effects of uptake of 125I-ghrelin from systemic circulation allow to conclude that rat thyroid may respond to both the exogenous ghrelin and that produced locally, and that besides paracrine/autocrine an intracrine action of ghrelin in thyroid follicular cells can not be excluded. Supported by funds from Ministry of Science and Higher Education (3951/P01/2006/31)

P1.64

Ghrelin signaling system in rat seminiferous epithelium: an immunohistochemical and hybridocytochemical study

Andrzej Łukaszyk¹, Ludmila Rafińska¹, Piotr Sawiński¹, Aldona Kasprzak¹, Karolina Olejniczak¹, Marcin Ruciński¹, Marek Ruchala², Jerzy Sowiński²

¹Department of Histology and Embryology, Poznań University of Medical Sciences, Poznań, Poland;

²Department of Endocrinology, Metabolism and Internal Medicine, Poznań University of Medical Sciences, Poznań, Poland

Contact: anluk@ump.edu.pl

There are growing evidences for the expression of ghrelin and its regulatory effects in male gonads especially in relation to proliferation and functional differentiation of Leydig cell. The objectives of the study was an analysis of distribution of immunohistochemical and hybridocytochemical reaction for ghrelin, its receptor and their transcripts in seminiferous epithelium of rat taking into account the cycle of the epithelium and phases of spermatogenesis. Ghrelin and its receptors in the rat male gonads was demonstrated immunohistochemically, in paraffin sections fixed in Bouin fluid or formaldehyde, using antibodies (anti-ghrelin and anti-GHSR1a Phoenix-Pharmaceuticals); peroxidase and alkaline phosphatase were used as markers of the immune reaction. For positive control an expression of the hormone in ghrelin cells of stomach of the same subjects was chosen and for the negative control IgG and complete rabbit serum were used instead of antibodies. Expression of genes for ghrelin and their receptors in rat testis was demonstrated with RT-PCR technique and also with *in situ* hybridisation technique in formaldehyde fixed sections using digoxigenin-labelled oligoprobes (5'-TTA GCT GGC GCC TCT TTG ACC TCT TC-3') specific for rat ghrelin and (5'-ACA CCA CCA CAG CAA GCA TCT TCA C-3') for rat ghrelin receptor mRNA; ovine anti-digoxigenin monoclonal antibodies (Fab fragments) were used to visualize the reaction site immunohistochemically. It was found that in testis, in contrast to other organs, the expression of ghrelin, its receptors and their transcripts may be seen at site of nuclei of spermatogenic cells and specifically at particular steps of spermatogenesis. Expression of ghrelin and its receptor occurs in some spermatogonia and leptotenes, disappears in pachytene and reappears in spermatids at advanced acrosome step of spermiogenesis. Finally, expression of these proteins declines in maturation phase of the process and disappears again when elongation of spermatids was progressed. In turn, the transcripts for ghrelin and its receptor were found to be expressed in

some spermatogonia and from meiotic pachytenes up to acrosome phase of spermiogenesis. The results seem to suggest the presence of ghrelin signaling system, neuropeptide and its functional receptor, in the rat seminiferous epithelium as a member of the local regulatory mechanism for spermatogenesis interacting, presumably, with c-kit signaling system. Supported by funds from Ministry of Science and Higher Education (3951/P01/2006/31).

P1.65

Immunochemical and morphological properties of mammalian pineal cells in the monolayer culture

Bogdan Lewczuk, Barbara Przybylska-Gornowicz, Michał Bulc

Division of Histology and Embryology, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

Contact: lewczukb@uwm.edu.pl

A technique of monolayer culture is commonly used in the studies on various aspects of the pineal biology including the regulation of melatonin secretion and cell differentiation. The primary culture derived from the pineal gland contains a variety of cell types including pinealocytes, glial cells, fibroblasts, endothelial cells and pigment cells. The morphology of cells changes during the culture due to adhesion to the growth surface, mitotic activity, formation of processes and adaptation. An important, although difficult, step in many experiments conducted in the monolayer culture is the identification of cells. For example, it enables to perform microinjections into specific cells and assures correct interpretation of the results. No doubt, immunocytochemical staining is the most convenient method of cell identification. The aim of the study was to compare immunochemical and morphological properties of cells in primary monolayer cultures obtained from the pineal glands of various mammalian species: rat, goat, pig and fox. The cultures were fixed at successive days of incubation and subjected to immunocytochemical stainings. The antibodies against enzymes of the melatonin synthesis pathway (tryptophan hydroxylase, hydroxyindole-O-methyltransferase - HIOMT), serotonin, antigen S - photoreceptor specific protein, neuronal markers (synaptophysin, PGP 9.5), glial fibrillary acidic protein - GFAP and fibronectin were used. The antibody-antigen complexes were visualized using secondary antibodies coupled to fluorescence dyes or biotin. Morphology of the cells was studied using techniques of phase and relief contrasts. Pinealocytes of all investigated mammals showed positive reactions with antibodies against tryptophan hydroxylase, HIOMT, serotonin, synaptophysin and PGP 9.5, however the intensity of staining with anti-serotonin antibodies varied considerably (from negative to extremely strong) between cells. Positive staining with the antibody against S-antigen was found in rat, goat and fox pinealocytes. Pinealocytes were found to lie single or in small groups and formed long processes in all cultures. The shape of their cell bodies differed between species, being usually flat and polygonal in rat, oval in pig and goat, oval or polygonal in fox pinealocytes. It was possible to recognize (with a large degree of convenience) pinealocytes among other cells in living cultures. GFAP-positive cells were flat and polygonal in all studied species. They could not be identified in the unstained cultures. Fibroblasts, characterized by the presence of fibronectin on their surface, were the predominant cell type in all studied cultures.

P1.66

Immunohistochemical studies on the post-hatching development of the turkey pineal gland

Barbara Przybylska-Gornowicz, Bogdan Lewczuk, Michał Bulc, Magdalena Prusik

Division of Histology and Embryology, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

Contact: przybylb@gmail.com

Our previous study has shown the existence of crucial alternations in the morphology of the turkey pineal organ during the post-hatching life. The developmental changes affect many aspects of the pineal morphology including the organ size, attachment of the pineal stalk to the intercommisural region and the presence of calcium concretions. However, the most important alternations seem to occur in the structure of follicles forming the parenchyma. The aim of the present study was to provide a detailed description of changes in the composition of the follicular wall during the post-hatching development of the turkey pineal gland. The glands were col-

lected from turkeys at the age of 1 day, 2, 8, 14, 56 weeks, fixed and cut using cryostat. The sections were subjected to double immunofluorescence staining with the use of antibodies against hydroxyindole-O-methyltransferase (HIOMT) - a highly specific marker of pinealocytes and glial fibrillary acid protein (GFAP) - a marker of glial cells. Immunoreactive (IR) cells were exclusively observed in the walls of the follicles. Generally two types of HIOMT-IR cells were distinguished - elongated cells bordering the follicular cavities and round cells located in the outer layer of the follicular wall. Comparison of the present data with the results of ultrastructural studies allowed to suspect that these cells were the rudimentary receptor pinealocytes and the secretory pinealocytes, respectively. In the pineals of turkeys at the age of 1 day and 2 weeks, HIOMT immunoreactivity was found mainly in the elongated cells. They were endowed with immunopositive bulbous prolongations projecting into the follicular cavities. The HIOMT-IR round cells were only sporadically observed. In the birds aged 8 and 14 weeks, the elongated HIOMT-IR cells were very large and formed prominent basal processes. A clear increase in the number of HIOMT-IR round cells was also noted. In the follicular wall of 56-week-old turkeys the round HIOMT-IR cells were the predominating components. They formed long varicose processes. The elongated cells were small and usually had no apical prolongations. The intensity of staining with anti-GFAP antibody was very low in the pineals of 1-day- and 2-week-old turkeys and markedly higher in the older birds. There were two types of GFAP-IR cells. The first type included columnar cells, which were localized between the elongated HIOMT-IR cells and probably correspond to the ependymal-like supporting cells, known from ultrastructural studies. The second type was represented by round (astrocyte-like) cells localized in the basal part of the follicular wall. Their number increased significantly with the animal age.

P1.67

Expression of estrogen receptor beta (ESR2) in adult European bison (*Bison bonasus*) testis - a preliminary study

Elżbieta Czykier¹, Grzegorz Zalewski²

¹Department of Histology and Embryology, Medical University of Białystok, Białystok, Poland;

²Department of Clinical Molecular Biology, Medical University of Białystok, Białystok, Poland

Contact: gregzal@wp.pl

The study was carried out to document the expression of estrogen receptor β (ESR2) in the testes of sexually mature European bison *Bison bonasus* (Linnaeus, 1758). Testicular samples of seven adult European bison males (aged 4-7 years) were analysed. Immunocytochemical reactions were performed using the streptavidin-biotinylated peroxidase. Specific polyclonal rabbit antibodies to ESR2 (H-150): sc-8974, raised against a recombinant protein corresponding to amino acids 1-150 mapping at the amino terminus of ESR2 of human origin (Santa Cruz Biotechnology, Inc, USA) were applied. The main finding was that the expression of ESR2 in the testis of the adult European bison was localized to the late spermatids. In some of these cells, the reaction was evenly distributed in the nucleus and the nuclear staining was weak. Sertoli cells, Leydig cells and peritubular cells were immunonegative.

P1.68

Ultrastructural changes of the mitochondria and immunocytochemically localized SCC enzymes within the zona glomerulosa region of the adrenal cortex of the wild type (+/+) and mutant mice (+/- and L/L) due to sodium and potassium chloride administration

Chanchal Kumar Manna

Endocrinology Laboratory, Department of Zoology, University of Kalyani, Kalyani, India

Contact: manna_kly@sify.com

The clear demarcation of the various regions of the adrenal cortex of mammals has attracted the attention of endocrinologists to correlate structure with function. While zona glomerulosa secretes the most important mineralocorticoid hormone, aldosterone, and zona fasciculata the main glucocorticoid hormone, cortisol, the function of the inner zone, zona reticularis, is still not clear, although its cells secrete sex hormones. The aim of the study

was to assess in adrenal cortex ultrastructure of mitochondria and the expression pattern of SCC (cytochrome P450 side chain cleavage) enzymes under various experimental conditions in the wild type (+/+) and SCC mutant (+/- and L/L) mice in steroid hormone biosynthesis. The mice were generated by Dr. Bon-Chu-Chung et co. (*Molec Endocrinol*, 2008, 22:915-923) and characterized before experiments by genomic PCR. Adult male mice (6 in each group) aged 8 weeks were used in each experiment. When wild type mice animals were treated with low dose of NaCl in the diet (LSD), high dose of NaCl (4%), and high dose of KCl (4%) in drinking water important changes in mitochondrial ultrastructure were noticed. Broad and continuous type cristae were observed in the high dose of NaCl treated animals. However, in the LSD and KCl-treated animals numerous small cristae and newly formed mitochondria were seen. Similar features

were also observed in the mutant (+/- and L/L) mice. Remarkable changes in the SCC enzymes in the zona glomerulosa region of the adrenal cortex were noticed after the administration of low NaCl and high dose of KCl. No reaction in the zona glomerulosa region was observed due to high dose of sodium chloride treatment. The expression pattern of CYP11A1 protein in the adrenal gland of these animals also showed similar features. Increased amounts of aldosterone were also noticed in the LSD and KCl treated animals but high dose of NaCl decreased the production of aldosterone. These observations in the wild type (+/+) and mutant (+/- and L/L) mice show that the zona glomerulosa region of the adrenal gland is regulated by some still unknown factors. It seems that some signaling molecules are involved in its activation. Key words: immunocytochemistry, (SCC) side chain cleavage, mitochondria, mineralocorticoid, glucocorticoid.

Lectures



Poster session 1



Poster session 2



Poster session 3



Poster session 4



Histochemistry of immune system cells

P2.1

Foxp3+ regulatory T cells are increased in the untreated coeliac mucosa and are expanded by gliadin in the *in vitro* cultured treated mucosa

Giuseppe Mazzarella, Rosita Stefanile

Immunomorphology, National Council Research, Avellino, Italy

Contact: gmazzarella@isa.cnr.it

Introduction. Celiac disease is characterized by a dysregulated HLA-DQ2/8-restricted Th1 response to gliadin, responsible for intestinal mucosa remodeling. It is unclear if a deficit of immunoregulation contributes to such hyper-responsiveness. The transcription factor Foxp3 has been identified as a marker of CD4+ regulatory T cells (T_R). The organ culture of small intestine is a valuable model to study the immunological events occurring in the coeliac mucosa following contact with gliadin. The aim of this study was to investigate the presence of Foxp3+ cells in the coeliac small intestinal mucosa and their correlation with disease state and gluten stimulation. **Methods:** The expression of Foxp3, CD4, CD8 and CD25 were analyzed by immunohistochemistry in duodenal biopsies, taken from patients with treated CD (n=15; age 34 yrs), with untreated CD (n=15; age 24 yrs) and from non-CD controls (n=15, age 38 yrs), as well as *in vitro* cultured treated CD biopsies upon challenge with gliadin (n=6). **Results:** In untreated CD the number/mm² of lamina propria CD4+Foxp3+ (median, range: 91, 60-142) was significantly higher ($p < 0.05$) in comparison to treated CD (8, 5-13) and controls (6, 2-12); density in treated CD was not statistically different from controls. Lamina propria Foxp3+ cells in biopsies from treated CD cultured with gliadin (16, 4-32) were significantly higher ($p < 0.05$) than in biopsies cultured with medium alone (6, 1-15). Interestingly, the density of Foxp3 T_R exceeded the density of CD4+CD25+ suggesting that Foxp3 is expressed also by CD4+CD25- T cells. **Conclusion.** CD4+ Foxp3+ T_R cells are more frequent in untreated intestinal mucosa compared to non-inflamed mucosa from either treated or control subjects. The significant increase in treated CD mucosa following *in vitro* challenge with gliadin suggests *in situ* induction of Foxp3. Taken together, these results suggest that in CD there is no basic defect of Foxp3+ T_R cells, but an expansion of this subset as an attempt to counteract the Th1-skewed ongoing mucosal immune response.

P2.2

Mast cells' development and density in fetal sheep skin

Suzan Daglioglu, Abit Aktas

Istanbul, Turkey

Contact: suzandag@istanbul.edu.tr

Mast cells (MC) have important regulatory functions in allergic and inflammatory disorders. MCs derived from the bone marrow migrate to tissues and there complete their differentiation [1,2,3]. In this study, histological and histochemical properties and distribution of mast cells were investigated in fetal sheep skin. **Materials and Methods:** The ages of the sheep fetuses were determined by the Crown-Rump Length technique. Skin samples were taken from 35 fetuses aged between 50- and 150-day-old. The samples were fixed in neutral buffered formaline and the paraffin sections were cut to a thickness of 5 μ m. Tissues were stained with haematoxylin and eosin to confirm the identity of tissue constituents, with toluidine blue (TB) and alcian blue/safranin O (AB/SO) to demonstrate the mast cells' granules. MC numbers were determined in a test area (0.6 mm²). Significant control of differences of the obtained values for groups was conducted by the use of unidirectional variance analysis (Anova). Also, tissue samples were prepared for electron microscopic (EM) investigation. **Results:** There were no mast cells observed in the 1st group (56-75 days). They were first histochemically detected in the skin of 83-day-old fetuses. In TB stained slides, MC density in the 3rd group's fetal skin (75-85 days) was detected as 1.40 ± 0.87 . A highly significant increase in MC density in the 4th group was observed. MC numbers in the 4th group (85-100 days) were 6.20 ± 2.035 , in the 5th group (100-110 days) 11.40 ± 2.015 , in the 6th group (110-120 days)

13.00 ± 1.049 and the 7th group (120-145 days) 13.60 ± 0.245 . The significance between first groups and last groups were statistically important ($P < 0.001$). MCs were observed around the blood vessels and hair follicles in dermis. MC granules were stained as AB(+) in the 3rd and 4th group. Also, granules were stained as SO(+) in the 5th, 6th and 7th group. EM investigation showed that MCs contained different types of electron dense granules. MC granules were increased in cells belonging to progressive days. MCs were spherical. The nuclei appeared to have acquired the shape of the cells. Heterochromatin was in the periphery of the nucleus. These studies suggest that MC density increased and the chemical properties of mast cells granules changed during skin development in sheep fetuses. **References:** 1. Kube, P. at all. (1998): *Histochem Cell Biol.* 110(2):129-35. 2. Holgte, S.T. (2000): *Clin. Exp. Allergy* 30S(1), 28-32. 3. Tomimory, Y. at all. (2002): *Biochem. And Biophysical Research Com.* 290.1478-1482.

P2.3

Lymphocyte populations following high frequency stimulation of the subthalamic nucleus in rats

Beata Grembecka, Pawel Ledóchowski, Wojciech Glac, Danuta Wrona

Department of Animal Physiology, Gdańsk University, Gdańsk, Poland

Contact: beatagrem@wp.pl

Immune abnormalities in brain and/or peripheral blood have been observed in Parkinson disease (PD). It is also known that high frequency stimulation (HFS) of the subthalamic nucleus (STN) is effective treatment for alleviating the motor symptoms of parkinsonian patients. However, the influence of HFS on the immune response remain unknown. In this study, we analyzed the effects of HFS (pulse width: 60 μ s, frequency: 130 Hz, stimulation intensity: 30-125 μ A, during a 1 hr stimulation period) on the percentage of blood and spleen leukocytes, lymphocytes and their subsets in freely moving rats. Blood and spleen lymphocyte populations were determined by flow cytometry using three-color immunofluorescent antibody staining procedure: CD3-FITC/CD45RA-PC7/CD161A-APC and CD3-FITC/CD4-PC7/CD8-APC for determination of T/B/NK and T cells subpopulations (CD3+CD4+, CD3+CD8+), respectively. As compared to the sham controls, HFS of STN significantly ($P < 0.05$) increased NK cell percentage number (17.87 ± 6.6 % vs. 28.66 ± 9.0 %) in peripheral blood. However, there was no significant effect of HFS of STN on splenocytes (10.00 ± 3.7 % vs. 8.31 ± 2.6 %). In addition, there were no significant differences in both blood and spleen percentage of T or B lymphocytes between stimulated and control groups (47.11 ± 6.82 % vs. 42.40 ± 10.6 %, 20.57 ± 4.4 % vs. 17.06 ± 3.3 % and 66.46 ± 4.5 % vs. 65.07 ± 8.4 %, 17.50 ± 5.3 % vs. 20.71 ± 6.6 %). The results obtained indicate that HFS of STN enhances blood innate antitumor and antiviral immune defense in rats.

P2.4

Innervation of particular hematopoietic cell populations in the bone marrow of the rat

Zbigniew Tabarowski¹, Mariusz Gajda²

¹Department of Experimental Hematology, Institute of Zoology, Jagiellonian University, Kraków, Poland;

²Department of Histology, Jagiellonian University, Medical College, Kraków, Poland

Contact: tab@zukunft.uj.edu.pl

Mammalian bone marrow is richly innervated. Various cell populations residing the bone marrow are not randomly distributed. They preferentially localize with a certain relationship to the blood vessels. The majority of nerve fibers are usually wrapped around vessels. Some nerve fibers project into the bone marrow, release neurotransmitters and thus influence target cells equipped with appropriate receptors. Therefore, the aim of the present study was to examine whether several bone marrow cell populations are located in a defined relation to the nerve fibers. The study was performed

on Wistar rats. Animals were perfusion fixed and excised hindlimbs were postfixed in 4% buffered paraformaldehyde, demineralized in 10% EDTA and immersed in 25% sucrose. Tissue blocks were snap-frozen and cut into 16 µm-thick cryosections. For double immunohistochemical staining the sections were incubated with primary antibodies raised against a general neural marker (anti-PGP 9.5), sensory fibers (anti-CGRP), sympathetic fibers (anti-NPY) and against markers of certain marrow cell lines: mononuclear phagocytes (anti-IC7), B lymphocytes (anti-CD45R), T-lymphocytes (anti-CD6), erythroid cells (anti-HIS49), and megakaryocytic cells (anti-CD42d), respectively. After a rinse in PBS, sections were incubated with appropriate secondary antibodies conjugated with fluorochromes (DTAF and Cy3). Finally, they were examined under an epifluorescence microscope. Although marrow cells can be cultured *in vitro* without any neural influence, in the *in vivo* setting neurotransmitters can finely tune their growth and differentiation. The actual role of marrow innervation is still debated but recent observations provided evidence that sympathetic nerves are involved in the regulation of stem cell egress from the marrow niche. The possible functional role of sensory endings was also suggested. The obtained results have shown spatial relationship between hematopoietic cell populations and nerve fibers. Neurotransmitters released by nerve terminals may influence the location and function of cells in the bone marrow cavity. Our data extend the current knowledge on the location of several cell populations in the bone marrow. However, regarding the vast spectrum of neurotransmitters/neuromodulators found in the peripheral nerves and various populations of marrow cells at different stages of development we are still far from understanding functional implications of such cooperation.

P2.5

Some equivocality of "markers" used for identification of infiltration cells in myositic muscles

Hanna Gendek-Kubiak¹, Ewa Gendek²

¹Department of Cytophysiology, Histology and Embryology, Medical University of Łódź, Łódź, Poland

²Department of Chemistry and Clinical Biochemistry, Medical University of Łódź, Łódź, Poland

Contact: h_kubiak@hotmail.com

Dendritic cells belong to newly appreciated morphotic elements of infiltration arising in muscles affected with idiopathic inflammatory myopathies (IIM). Applying antibodies against some newly discovered dendritic cells' antigens surprisingly allowed to reveal substantial participation of these cells in the infiltration. The discovery was unexpected, since no information on unknown or unidentified infiltration cells used to be reported and detailed numbers of different infiltration cell kinds were given based on immunostainings of antigens characteristic of T cells, B cells and macrophages. Thus, it has been shown that CD4, CD8 and CD68 antigens are not so specific as they used to be considered. On the other hand, one of the markers of mature dendritic cells appears to be fascin – an actin-bundling protein, which may also be expressed in myogenic cells. Another protein, NCAM (CD56) was considered to be a marker of satellite cells and of regenerating myofibers. Intensive search for stem cells, also in skeletal muscles, resulted in finding a pool of the so called "side population" cells in endomysium – the cells able to regenerate myofibers, just like satellite cells do, and which are also NCAM positive. In special circumstances, bone marrow stem cells – potentially myogenic, but indicating co-expression of the myogenic and endothelial cell markers (CD56, CD34, CD144), and thus being myoendothelial cells – may migrate to muscles and accompany the above-mentioned two myogenic cell kinds. Moreover, the presence of blood vessels, which in IIM are subjected to damage and also regenerate, has finally been appreciated. Thus angiogenic cells might be present among the infiltration cells, too. All kinds of neurofibers used to be identified immunohistochemically by applying an antibody against the "pan-neuronal marker" – antigen PGP9.5, however some kinds of dendritic cells have recently been shown to give a positive reaction to this antigen. Additionally, the regenerating myofibers indicate positive reaction to this "marker". Thus, it is possible to take myogenic cells for dendritic cells or even for neurofibers. Experiments aimed to induce IIM in animals are being conducted, but they haven't been very successful, so far. The equivocality of cell kinds being detected using antibodies will probably be much more pronounced during immunostainings in experimental animals. This work has been supported by the Medical University of Łódź (grant numbers: 502-18-558 and 502-17-555).

P2.6

Immune component of human umbilical cord tissue: an immunohistochemical study

Yesim Ugur¹, Aysegul Nazikoglu¹, Pergin Atilla¹, Sinan Beksac², A Nur Cakar¹

¹Hacettepe University Faculty of Medicine, Department of Histology and Embryology, Ankara, Turkey

²Hacettepe University Faculty of Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey

Contact: patilla@hacettepe.edu.tr

The umbilical cord is vital for the growth and well being of fetus but there is almost no data about the possible intrinsic or migratory immune component of it. In the current study, we searched for any expression of CD44 molecule, which is involved in the adhesion, migration, invasion, survival and activation of especially immune response-related cells, within the umbilical cord tissues of healthy and auto-immune disease-bearing mothers. In addition we investigated the existence of CD68 molecule which is predominantly expressed on primary dendritic cell macrophages. These two molecules are also reported to be functionally related. We have previously shown the increase in endothelial marker expressions of auto-immune disease-bearing mothers' umbilical cord tissues when compared to healthy ones. Endothelium is proven to be highly involved in the initiation and maintenance of immune reactions. So our previous data led us to investigate the existence of immune response-related cells within the umbilical cord tissue. The control group consisted of healthy term mothers' umbilical cord tissue samples (n=6). The diseased group consisted of auto-immune disease-bearing mothers' umbilical cord tissue samples (n=15). Fresh tissue samples were frozen in liquid nitrogen, 6-8 mm thick cryostat sections were cut, air-dried, fixed in acetone and stained by indirect immunoperoxidase technique. Immunoreactive cells were counted separately by two histologists under the light microscope and mean values were recorded. The Kruskal Wallis test was used for statistical analysis and p<0.05 was accepted as significant. We found a statistically significant increase in the number of CD44(+) cells in disease-bearing mothers when compared to healthy ones. Worthy of attention, we observed barrier-constructing CD44(+) cells in the diseased group not far away from the umbilical vessels (especially the umbilical vein). The statistically significant increase was also observed regarding CD68(+) cells. Our attention was dragged to the fact that CD68(+) cells were especially accumulated within the wall of or immediately around the umbilical vessels. This raised questions about the origin of these cells – whether they migrated from umbilical cord blood or are the resident cells of cord tissue. Some of the CD68(+) cells resembled neutrophils. As a summary, this study put forward basic data declaring the existence and active role of an immune component within the umbilical cord tissue.

P2.7

Effect of experimental *Brachyspira hyodysenteriae* infection on the concentrations of selected inflammation-associated neuropeptides (SP and galanin) and on CD2+, CD5+ and CD21+ lymphocyte subpopulations in ileum and ileal lymph nodes in the pig

Krzysztof Wasowicz¹, Anna Winnicka², Waldemar Sienkiewicz¹, Piotr Podlasz¹, Jerzy Kaleczyc¹, Mirosław Lakomy¹

¹Division of Animal Anatomy, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

²Department of Clinical Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland

Contact: krzysztof.wasowicz@uwm.edu.pl

The aim of the study was to examine changes in the number of CD2+, CD5+ and CD21+ lymphocytes as well as the concentrations of Substance P (SP) and galanin (Gal) in the ileal lymphatic plate and ileal lymph nodes in the course of experimental infection with *Brachyspira hyodysenteriae* in pigs. Eleven gilts aged 4 months were used in the study. Six clinically healthy animals were inoculated with a *B. hyodysenteriae* culture through a gastric catheter. 5 animals were left as controls. After the specific symptoms of the disease developed, all animals (infected and control) were deeply anaesthetized, exsanguinated and samples of the ileum with lymphatic plate and ileal lymph nodes were excised. For lymphocyte phenotyping the lymph nodes and ileum samples were finely chopped and vigor-

usly shaken with PBS. The lymphocyte suspensions were incubated with specific primary antibodies for CD2, CD5 and CD21 antigens and secondary antibodies. The cells were analysed with FACScalibur and CellQuest. For determination of tissue concentrations of SP and Gal the tissues were homogenised and extracts were assayed with commercial ELISA kits. The results were read in a microplate reader. As regards the lymphocyte subpopulations, in ileal lymph nodes of control animals antigens CD2, CD5 and CD21 were expressed by 50.0, 63.7 and 52.7% of lymphocytes, respectively. In the ileum antigens CD2, CD5 and CD21 were expressed by 30.8, 55.0 and 62.2% of lymphocytes, respectively. In animals suffering from swine dysentery there were statistically significant changes regarding only the CD21+ lymphocyte subpopulation. In the lymph nodes of sick animals 25.8% of lymphocytes expressed antigen CD21, and in the ileum the respective percentage was 43.9%. As regards the tissue concentration of Gal and SP, in the ileum of control animals concentrations (expressed per gram of wet tissue) of Gal and SP were 14.39 and 1.84 ng, respectively. In case of the lymph nodes the respective figures for Gal and SP were 12.04 ng and 5.5 ng. In animals suffering from swine dysentery profound changes in tissue concentrations of the studied neuropeptides were detected. In the ileum of sick animals concentrations of Gal and SP were 23.35 ng and 3.03 ng, respectively. In case of the lymph nodes the respective figures for Gal and SP were 24.8 ng and 5.3 ng. All differences were found to be statistically significant.

P2.8

Immunomorphological analysis of neuroblastic tumours with opsoclonus-myoclonus-ataxia syndrome

Ewa Iżycka-Świeszewska¹, Joanna Stefanowicz², Elżbieta Drożyńska², Ewa Bień², Jerzy Pienczk³, Wiesława Grajkowska⁴, Edyta Szurowska⁵

¹Dept. of Pathomorphology, Medical University of Gdańsk, Gdańsk, Poland;

²Dept. of Pediatrics, Hematology, Oncology and Endocrinology, Medical University of Gdańsk, Gdańsk, Poland;

³Dept. of Developmental Neurology, Medical University of Gdańsk, Gdańsk, Poland;

⁴Dept. of Pathology, Children's Memorial Health Institute Warsaw, Warszawa, Poland;

⁵Dept. of Radiology, Gdańsk, Poland

Contact: eczis@wp.pl

Tumours linked to neurological paraneoplastic syndromes express onco-neuronal antigens, which activate the immunological reaction focused on the neoplastic tissue and CNS neurons. Paraneoplastic opsoclonus-myoclonus-ataxia syndrome (OMA) in children is connected to neuroblastic tumours

(Kinsbourne'a encefalopathy). The oncological prognosis of patients is good, however their neurobehavioral prognosis is uncertain. We examined four cases of neuroblastic tumours with OMA in children treated in Medical University of Gdańsk. The neurological symptoms occurred between 1 m – 6 yrs before the suprarenal gland tumour detection. Histopathological examination was performed on paraffin-embedded tumour sections. Immunohistochemical analysis was made based on antibodies against LCA, CD3, CD20, CD56, CD4, CD8, TNF α , CD68, HLA-DR, CD21, MHC-1 (Dako, Denmark). Histologically differentiating neuroblastoma, 2 ganglioneuroblastoma and maturing ganglioneuroma were diagnosed. In all cases intense inflammatory reactions in the form of diffuse parenchymal, perivascular infiltrates and/or lymphadenoplastic foci were encountered. These infiltrates were composed mainly of T lymphocytes (CD3+), CD56+ NK cells and a smaller population of B cells, dendritic cells (CD21+) and macrophages (CD68+). The T cell infiltrates consisted mainly of cytotoxic CD8+ lymphocytes, which with CD56+ cells invaded and destroyed neuroblastic and gangliocytic cells. Perivascular infiltration consisted of mixed populations of lymphocytes T and B. HLA-DR expression was observed within germinal centers of lymphoid follicles. TNF α labeling was found in the stromal cells – macrophages and endothelial cells. The neuroblastic and ganglion cells expressed MHC-1 but did not express HLA-DR antigen. In the examined neuroblastic tumours with OMA we found intense inflammatory reactions against neuroblastic lineage cells. The immunological reactions were predominantly of a cellular type.

P2.9

Ghrelin stimulates enterocyte apoptosis and autophagy in the small intestine of pig neonates

Anna Kotunia¹, Piotr Pietrzak², Ikuo Kato³, Atsukazu Kuwahara⁴, Michal M. Godlewski⁵, Joanna Bierla⁵, Romuald Zabielski⁵

¹The Kielanowski Institute of Animal Physiology and Nutrition, Jablonna, Poland

²Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warszawa, Poland

³Yanaihara Institute, Fujinomiya, Japan

⁴Laboratory of Physiology, University of Shizuoka, Shizuoka, Japan

⁵Department of Physiological Sciences, Warsaw University of Life Sciences, Warszawa, Poland

Contact: anka.kot@wp.pl

Ghrelin is a 28 amino acid acylated gastric hormone which links control of food intake, gastric motility and growth hormone secretion through the gut-brain interactions. Previous studies in neonatal rats showed that, in contrast to adult animals, exogenous ghrelin may inhibit the development of the small intestine. Abundance of ghrelin and ghrelin receptor expression was observed in the neonatal gastrointestinal tissues. Active ghrelin was found in sow colostrum and milk. The present study aimed to clarify the role of ghrelin in the intestinal epithelium proliferation, apoptosis and autophagy in pig neonates. One day old animals were installed in artificial sow system, and randomly divided into 5 groups. The piglets were treated for 6 d with ghrelin (Yanaihara Inst.) and/or ghrelin receptor antagonist (100 nmol/kg body weight (b. wt.), [D-Lys-3]GHRP-6, Peptide Int.), respectively. Ghrelin, 0, 7.5, and 15 µg/kg b. wt. was administered every 8 h by gastric gavage, and the antagonist was administered either in combination with 15 µg/kg b. wt. ghrelin or alone. Seven d old piglets were killed, mid jejunum segments were fixed in embedding medium and frozen. Frozen slices were labeled with specific antibodies: for mitosis analysis, anti-Ki-67-FITC-conjugated antibodies (BD Pharmingen), for apoptosis, anti-Cpp32 fragment of active caspase 3 (DAKO) and secondary chicken anti-rabbit Alexa Fluor 488 antibodies (Molecular Probes), and for autophagy, goat anti-MAP1LC3 (Santa Cruz) and secondary chicken anti-goat Alexa Fluor 488 antibodies (Molecular Probes) were employed. Cell nuclei were counterstained with 7-AAD (Sigma). FV-500 laser scanning confocal microscope images were analyzed quantitatively using the Microimage (Olympus) software on the basis of fluorescent intensity. Exogenous ghrelin led to a tendency towards reduction in body weight, and reduction of small intestine and intestinal villi length. Ghrelin administration reduced the Ki67 expression in crypts in a dose-dependent manner (linear trend, $P=0.0009$). Administration of ghrelin receptor antagonist reversed the inhibitory effect of ghrelin and caused increase of Ki67 expression over the control level ($P<0.05$). The latter indicates that the effect of endogenous ghrelin might be also abolished. Higher dose of ghrelin increased the active caspase 3 expression ($P<0.05$) in the villi and crypts, and the effect was abolished by the administration of ghrelin receptor antagonist. Mitosis/apoptosis ratio was reduced by ghrelin, whereas pretreatment with the antagonist doubled the ratio. Ghrelin (15 µg/kg b. wt.) increased the expression of MAP1LC3 as compared to control. The effect could not be blocked by antagonist pretreatment. In conclusion, our in-tissue-cytometry study suggests that in neonatal piglets ghrelin may inhibit the rebuilding of the gut mucosa by reducing cell proliferation and stimulation of apoptosis and autophagy.

P2.10

Markers of cell proliferation, apoptosis and angiogenesis in remodeling of bronchoalveolar duct junctions

Vuong Tuong, Sophia Demoura, Evgenia Kogan

Setchenov Moscow Medical Academy, Moscow, Russia

Contact: tphivuong@yahoo.com

Background. The goal of the study was to investigate the remodeling in bronchoalveolar duct junctions in patients with idiopathic pulmonary fibrosis. Design. Lung biopsies from 30 patients in early stage of IPF and 10 control patients (normal lung tissue with diagnosis of sarcoidosis) were used. Immunohistochemistry on paraffin sections had been done with anti-

bodies to Apo-protein, TGFβ, PCNA, PDGF, EGFR (Lab Vision), CD34, SMA (Novocastra). Levels of marker expression in each type of cells had been analyzed separately in the area of bronchoalveolar duct junctions (BADJ), interalveolar interstitium and in normal lung tissue. Expression levels of the investigated markers were estimated by the percentage of positive stained cells. Results were reported as mean ± SEM. The nonparametric Mann-Whitney test was used to analyze the results. Results. We found that adenomatous, myofibroblasts foci, and foci of angiogenesis were present predominantly in the area of BADJ in early stage of idiopathic pulmonary fibrosis with statistically significant higher expression of: Apo-protein by adenomatous alveolar epithelial cells (AECs) ($14.6\% \pm 8.26\%$, $P<0.05$), PCNA by adenomatous AECs ($62\% \pm 12\%$, $P<0.05$), bronchiolar epithelia ($52\% \pm 14\%$, $P<0.05$) and macrophages ($42\% \pm 13\%$, $P<0.05$), CD34 by endothelial cells ($53.44\% \pm 4.88$; $P<0.05$), and SMA by vessel smooth muscle cells and myofibroblasts, in comparison to the adjacent interalveolar interstitium and the control normal lung tissue. Conclusion. We propose that remodeling of the regions of BADJ is the most important event that contributes to the onset and progression of IPF. Better understanding of biochemical and molecular mechanisms in pathogenesis of IPF leads to more specific and targeted therapies.

P2.11

The effects on nephropathy and apoptosis of renin-angiotensin-system blockade in the STZ-diabetic rats

Matem Tunçdemir, Melek Ozturk

Istanbul University, Istanbul, Turkey

Contact: tmatem@istanbul.edu.tr

The aim of this study is to investigate effects of perindopril as an ACE inhibitor (ACEI) and irbesartan as an AT1 receptor blocker (AT1R blocker) on TGF-β1, α-SMA and apoptosis in experimental diabetic nephropathy. In this study Wistar albino rats (5 groups) were used. (1) STZ-diabetic, (2) irbesartan treated diabetics (15 mg/kg/day, 30 days), (3) perindopril treated diabetics (6 mg/kg/day, 30 days), (4) combined treated diabetic group with perindopril and irbesartan (3 mg/kg/day, 5 mg/kg/day, 30 days), (5) control group. During the experimental period blood glucose, microalbuminuria level, body weights (Bw), kidney weight (Kw) and amount of daily urine were measured. TUNEL method for apoptosis and immunohistochemical staining for TGF-β1 and α-SMA antibodies were performed. Blood glucose levels, Kw/Bw ratio and glomerular size of all STZ-diabetic groups were higher than the control group. The level of daily urine and microalbuminuria levels, Kw/Bw ratio and glomerular size were decreased in the all treated diabetic groups. Glomerular basement membrane thickening, segmental differentiation and hypertrophic glomeruli were observed in the kidney sections of untreated-diabetic rats. PAS positive glycogen accumulations were present in tubular epithelium cytoplasm in untreated-diabetic rats but they were markedly decreased in combined drug treated diabetic group. The immunostaining of α-SMA was detected within peritubular capillaries and interstitial peritubular cells of nondiabetic control group. The glomerular podocytes, some tubular cells, peritubular capillaries and interstitial area of diabetic rats showed increased immunopositivity for α-SMA. In the combined drug treated diabetic group, the α-SMA immunopositive cell number was detected significantly decreased when compared with untreated diabetic group. The expressions of TGF-β1 were significantly increased in glomeruli of untreated diabetic group when compared with the non diabetic group. In the combined treated diabetic group, a decrease of TGF-β1 expression were determined when compared with the all groups. The number of apoptotic cells increased especially in the kidney tubules of STZ-diabetic group ($p<0.001$), whereas a significant decrease was observed in the combined drug treated group ($p<0.05$). It was concluded that the increase in the expression of TGF-β1 and α-SMA in glomeruli was a marker of renal injury which is seen in the early stages of diabetes as a result of damage caused by local increased expression of RAS in the renal tissue induced by hyperglycemia. In the early stages of diabetes, the increase in the number of the apoptotic cells in the tubules was seen as one of the causes of tubular damage. Although irbesartan or perindopril treatment prevented renal injury in the diabetes, low dose application of ACEI and AT1R blocker together revealed more efficient results in preventing renal damages.

P2.12

Effect of lead acetate on the apoptosis and the expression of apoptosis-related gene XIAP and Smac in hippocampus of budding rates

Xia Huo, Xijin Xu, Dai Han

Shantou University Medical College, Shantou, China

Contact: xhuo@stu.edu.cn

Background: Lead is a ubiquitous pollutant in the environment. Brain, especially the nervous system in development phase, is more vulnerable to deleterious effects of lead than other tissues. The apoptosis have been suggested to be a causative factor in lead neurotoxicity, but little information on this process has appeared to date. Objective: To provide some scientific basis for the revelation of Lead neurotoxin mechanism, the present study was undertaken to observe the effect of lead acetate on the apoptosis and the expression of apoptosis-related gene XIAP and Smac in hippocampus of budding rates. Method: A total of 48 healthy males 30-day-old SD rats were randomly divided into 4 groups with 12 rats in each group including distilled water negative control group and 2mg/Kg, 20mg/Kg, 200mg/Kg lead poisoning groups. Lead acetate was given through gastric perfusion for 6 weeks, respectively. Lead concentration in blood of the rats was determined by atomic absorption spectrophotometry. The determination of apoptosis in hippocampus was made by TUNEL. The expression of XIAP and Smac genes in hippocampus was observed by RT-PCR. Result: TUNEL showed that there were significant differences between the four groups. There was a significant dose-response relationship between the level of lead exposure and apoptosis of cells. The expression of XIAP gene decreased in neural cells of hippocampus in high-lead treatment group compared with the control, but there were no difference in the expression of Smac gene among each group. Correlation analysis demonstrated that Lead concentration in blood correlated negatively with the expression of XIAP. Conclusion: Lead exposure results in significant increase of apoptosis of the neural cells. The high-lead exposure induced the apoptosis of neural cells in budding rats' hippocampus through depressing the expression of XIAP gene.

P2.13

Is mitosis/apoptosis index a good marker of gut mucosa rebuilding?Joanna Bierla¹, Michal M. Godlewski², Anna Kotunia³, Piotr Pietrzak¹, Romuald Zabielski¹¹Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland;²Department of Chemistry and Biomolecular Sciences, Division of Environmental and Life Sciences, Macquarie University, Sydney, Australia;³The Kielanowski Institute of Animal Physiology and Nutrition, Jablonna, PolandContact: joanna.bierla@wp.pl

First portions of colostrum trigger a period of fast development in the small intestine mucosa in mammalian neonates. Intestinal development consists of a chain of synchronized processes of cell proliferation, differentiation, maturation and death. It's manifested by a number of morphological and functional alterations which can be evaluated by microscopy and functional studies. The aim of our study was to check if the mitosis/apoptosis index is of use as a marker of intestinal mucosa rebuilding, and if it is sensitive to dietary modifications. *In-tissue* cytometry have been employed, a quantitative tool to study molecular markers important for tissue development, especially enterocytes during development of the small intestine. As a model animal the neonatal pig was proposed. The quantification of whole population of dividing cells was based on Ki-67 protein expression. It organizes chromatin structure and is expressed in every phase of cell-division cycle except G0 phase. The active caspase 3 was used as an apoptosis marker since it is a major effector caspase involved in execution of the late, irreversible phase of apoptosis. Slices of intestinal samples were labeled with the specific sets of antibodies (Ab). For mitosis analysis anti-Ki-67-FITC-conjugated Ab were used. For apoptosis assessment anti-Cpp32 fragment of active caspase 3, followed by secondary swine anti-rabbit FITC-conjugated Ab were employed. Counterstaining of cell nuclei have been done with 7AAD. At least 14 the mucosa images were collected by FV-500 laser scanning confocal microscope for each data point. Random image selection was crucial since the areas may vary significantly in the protein expression level (e.g. top of the villi compared to crypt region). Object recognition (automatically elimination under 50 pixel in size) was performed using the Microimage analysis software on the basis of fluorescent intensity. The number of objects expressing the studied protein was compared

to the overall number of cells counted on the basis of DNA-related fluorescence. Evaluated mitosis/apoptosis index in unsuckling neonates was 0.2. Within 2-3 days the index doubled due to increased mitosis in the crypts and reduced apoptosis rate on the villi. It corresponds with a rapid growth and maturation of the gut mucosa observed during the first week of life. After the phase of intensive growth the mitosis/apoptosis index returned to initial 0.2. After weaning no major change in the index was observed since increased cell proliferation in the crypts was accompanied by enhanced apoptosis on the villi. The index was found to be sensitive to modifications of sow milk and milk formula composition. Reassuring, the mitosis to apoptosis ratio is a good marker of development of intestinal mucosa in the early postnatal period. Furthermore, method of *in-tissue* cytometry proved to be a valuable asset in the analysis of molecular mechanisms of development in complex tissue of intestinal mucosa.

P2.14

Distribution of mitochondria in pEYFP-Mito *in vivo* electroporated mouse testis; its possible relation to the induction of germ cell apoptosis

Yoshitaka Hishikawa, Sucai An, Yasuaki Shibata, Yoko Sato, Takehiko Koji

Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Contact: yhish@nagasaki-u.ac.jp

During spermatogenesis, mitochondria change their structure and distribution, and have a central role in the regulation of germ cell apoptosis; the apoptosis is closely associated with redistribution of Bax and release of cytochrome c from mitochondria. It is also known that estrogen modulates various biological aspects in male reproductive organs through binding to estrogen receptor (ER). Interestingly, it has been reported that ER β is localized to mitochondria, suggesting that estrogen could directly affect mitochondrial functions through ER β . However, the precise mechanism of mitochondrial distribution and their functions related to apoptosis during spermatogenesis is largely unknown. Therefore, we investigated the change in the mitochondrial distribution and their function related to apoptosis-regulated proteins such as Bax and cytochrome c, and ER β in germ cells. We used a vector that encodes a fusion protein of enhanced yellow fluorescent protein and mitochondrial targeting sequence from subunit VIII of cytochrome c oxidase (pEYFP-Mito). pEYFP-Mito was transfected directly into mouse testis by *in vivo* electroporation, and observed by laser scanning microscopy. To clarify the localization of mitochondria, we examined the expression of SP-22 as a mitochondrial matrix marker and that of OxPhos V as a mitochondrial inner membrane one. To gain some insights into the mitochondrial pathway of germ cell apoptosis, we also examined the expression of Bax, cytochrome c and ER β . As a result, pEYFP-Mito was found in spermatogonia and spermatocytes consistently. pEYFP-Mito was localized in both a speckled cytoplasmic pattern and a whole cytoplasmic pattern in the cytoplasm of these germ cells. In the former case, SP-22, OxPhos V, and ER β were colocalized in a speckled cytoplasmic pattern, and in the latter case, OxPhos V, Bax, cytochrome c, and ER β were also distributed throughout the whole cytoplasm, which was usually associated with apoptotic germ cells. These results demonstrate that *in vivo* electroporation of pEYFP-Mito was a useful tool for examining the mitochondrial distribution during spermatogenesis, as well as the specific pattern of mitochondria to whole cells, which may be essential for the germ cell apoptosis. Furthermore, it is also possible that ER β could be potentially important role for the mitochondria related apoptotic pathway during spermatogenesis.

P2.15

The effect of bradykinin postconditioning on activities of antioxidant enzymes in postischemic rat brainViera Danielisova¹, Miroslav Gottlieb¹, Miroslava Nemethova¹, Petra Kravcukova¹, Iveta Domorakova², Jozef Burda¹¹Institute of Neurobiology, Slovak Academy of Science, Kosice, Slovakia²P.J. Šafarik University, Faculty of Medicine, Department of Histology and Embryology, Kosice, SlovakiaContact: danielis@saske.sk

Postconditioning is procedure utilizing fact that for acquirement fully-fledged ischemic tolerance repeated stress is inevitable. This stress if applied

before onset of delayed neuronal deaths in CA1 of hippocampus is able to stop this process and prevent neuronal degeneration. Moreover, these cells preserve considerable part their functions concerning learning and memory. Bradykinin is considered an important mediator of the inflammatory response in both the peripheral and the central nervous system and it has attracted recent interest as a potential mediator of brain injury following stroke. Postconditioning as well as initial lethal ischemia, increases the activity SODs and catalase. The observed increase in the activity of a mitochondrial enzyme MnSOD in the cytoplasm after lethal ischemia indicates the release of MnSOD from mitochondria during delayed neuronal death. This increase is accompanied by increase of cytoplasmic concentration of cytochrome c. Mitochondrial pathway of apoptosis supports increase of activated caspase-3 in CA1 region in beginning of delayed neuronal death, 3 days after ischemia. However, application of postconditioning 2 days after ischemia significantly prevents all these changes. The comparison of efficacy between postconditioning applied as repeated short ischemia and injection of bradykinin clearly documented higher impact of bradykinin. Importantly, bradykinin postconditioning is able to prevent cell death in most sensitive neuronal populations after cerebral ischemia. This study was supported by the VEGA 2/6211/26, VEGA 2/6210/26, VEGA 1/4237/07 and the APVV LPP 0235-06 grants.

P2.16

Expression and immune-detection of MnSOD and CuZnSOD in the posts ischemic rat hippocampus after ischemic postconditioning

Miroslava Nemethova, Viera Danielisova, Miroslav Gottlieb, Jozef Burda

Institute of Neurobiology SAS, Kosice, Slovakia

Contact: nemeth@saske.sk

Brain is highly sensitive to oxidative stress after ischemic injury and posts ischemic burst of reactive oxygen species (ROS) often causes cell death, mainly in selective vulnerable regions. ROS are known as a main cause of oxidative stress after ischemia and arise from molecular oxygen by successive single-electron reduction reactions and include not only oxygen-free radicals but also non-radical oxygen derivatives. Superoxide dismutases, (MnSOD, CuZnSOD – endogenous antioxidative enzymes) are, at least partly, capable of eliminating superoxide. It is not a particularly damaging species, but is very significant as a source of hydrogen peroxide and as a reducing agent of transition metal ions. CNS resistance to ischemic injury can be transiently augmented by exposition to sublethal repeated stress applied two days after previous ischemic attack – delayed postconditioning. The 8 min ischemic insult was evoked in male Wistar rats by standard 4-vessel-occlusion. Ischemic group of animals underwent ischemia with 3-day reperfusion. Post-conditioning group absolved two days after first 8 min ischemia next 5 min of ischemia (postconditioning) and animals survived one more day. SODs in the hippocampus were detected by immunohistochemistry and results were evaluated by microdensitometric analysis. The vulnerable CA1 region and relative resistant dentate gyrus were compared. Post-conditioning significantly increased immunoreactivity of both enzymes in the CA1 and dentate gyrus of rat hippocampus. It seems clearly documented that protein synthesis in the posts ischemic brain plays a principal role in the acquisition of ischemic tolerance. Posts ischemic pathological processes lead to the inhibition of protein synthesis machinery persisting in selectively vulnerable regions to the delayed neuronal death. We can deduce that shorter postconditioning impulse can be considered as a trigger for selective synthesis of antioxidant enzymes. This activity after ischemia seems to be predominantly focused on production of those proteins, which can provide protection against cell death. Postconditioning probably causes second remodeling of protein synthesis leading to a switch from proapoptotic to antiapoptotic pathway. The study was supported by SK-APVV 023506 and SK-VEGA 2/6211/26, 2/6210/26 grants.

P2.17

The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on viability and incidence of apoptotic granulosa cells in pigs

Olga Kraszewska¹, Anna Nynca¹, Joanna Piasecka¹, Anna Robak², Barbara Wąsowska³, Renata E. Ciereszko¹

¹Department of Animal Physiology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland;

²Department of Comparative Anatomy, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland;

³Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland;

Contact: olga.kraszewska@uwm.edu.pl

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic man-made environmental pollutant, is responsible for many endocrine-disrupting effects. TCDD was also found to directly affect ovarian steroidogenesis and it is suggested that TCDD may induce apoptosis in granulosa cells. The objectives of the study were to: 1/ examine the effect of TCDD on progesterone (P₄) and estradiol (E₂) production by granulosa cells isolated from large, preovulatory (>8 mm) porcine follicles; 2/ determine the effect of TCDD on viability of the cells; 3/ test the effect of TCDD on incidence of apoptotic granulosa cells. Granulosa cells isolated from large, preovulatory (>8 mm) porcine follicles were pre-cultured for 48 or 72 hours (medium Eagle'a; 37°C; 10% calf serum; 95% air; 5% CO₂). Then, the cells were cultured for 48 h in the presence of TCDD (10 pM, 10 nM), staurosporin (5 μM, positive control in viability test), actinomycin D (1 μM, positive control in TUNEL assay) or DMSO (TCDD solvent). Steroid hormone concentrations were determined by radioimmunoassay. Viability of the granulosa cells was tested by alamarBlue™ (BioSource International) assay. The incidence of apoptotic granulosa cells was evaluated by a fluorescent assay based on terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end-labeling (TUNEL; BD Biosciences-Clontech). Lower dose of TCDD significantly stimulated (p<0.05) P₄ and inhibited E₂ (p<0.05) production by granulosa cells isolated from large, preovulatory follicles (n=6). Higher dose of TCDD did not change steroid hormone secretion by the cells. Neither of the two TCDD doses affected cell viability (n=4) or changed the number of TUNEL-positive, apoptotic granulosa cells (n=4). In conclusion, TCDD affected follicular steroidogenesis in pigs and did not affect cell viability. This study was supported by The State Committee for Scientific Research in Poland (Grants: KBN N 301 098 31/3017, UWM 522.0206.0206) and by European Social Fund for OK.

P2.18

Quercetin synergistically co-operates with etoposide in arresting cell cycle and inducing apoptosis of rat promyelocytic leukemia LT 12 cells

Monika A. Papież¹, Małgorzata Bzowska²

¹Department of Cytobiology and Histochemistry, Collegium Medicum, Jagiellonian University, Kraków, Poland;

²Department of Immunology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Contact: mfpapiez@cyf-kr.edu.pl

Background: The flavonoid quercetin is commonly found in edible plants. This polyphenol is known to suppress proliferation and to induce apoptosis of many cancer cells by arresting their cell cycle in various phases. Etoposide is a frequently used cytostatic. Its anticarcinogenic activities consist in inducing of single and double DNA strand breaks, having an impact on cell cycle and triggering apoptosis. The aim of this study was to find out whether quercetin co-operated with etoposide in arresting cell cycle and inducing apoptosis of rat promyelocytic leukemia LT 12 cells. Material and methods: LT12 cells were treated with quercetin (0 – 20 μM) for 4, 24, 48 and 72 h or etoposide (0,5 μM) for 1 hour. Then, the cells were treated with quercetin over different time periods (4-72 h) and after those times the etoposide was applied for 1 hour. After staining with propidium iodide (PI), the DNA content was analysed by a FACScan flow cytometry (Becton-Dickinson). Apoptosis was examined using an Annexin-V-FLUOS staining kit (Roche) and CellQuest software (Becton-Dickinson). Early apoptotic cells were defined as annexin-V-positive and PI-negative, while late apoptotic and necrotic

cells were regarded as annexin-V- and PI-positive. Results: Quercetin significantly increased the sub-G1 fraction of cell cycle and the number of annexin-V-positive cells compared to the control. That polyphenol in doses as high as 10 or 20 mM arrested the cell cycle in the G2/M phase and induced apoptosis after 24 – 72-hour incubation. Etoposide exerted a similar but stronger effect compared to quercetin on cell cycle and apoptosis of LT 12 cells. Simultaneous application of quercetin and etoposide led to significant enhancement of the sub-G1 fraction and increased the number of annexin-V-positive cells compared to etoposide or quercetin applied alone. Conclusions: Quercetin enhances the impact of etoposide on cell cycle and intensifies the pro-apoptotic action of the cytostatic under study in leukemia LT 12 cells. The interaction between etoposide and quercetin points to an enhanced anticarcinogenic potential of the combination of these compounds. This study was supported by grant no. 2 P05A 162 30 from the Polish State Committee for Scientific Research.

P2.19

Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) induce apoptosis by alterations of mitochondrial respiratory chain in human colon carcinoma HCT116 cell line

Konrad Kleszczyński, Łukasz Piszczek, Andrzej C. Składanowski

Department of Molecular Enzymology, Intercollegiate Faculty of Biotechnology, Medical University of Gdańsk, Gdańsk, Poland

Contact: kleszczyński@amg.gda.pl

Perfluorinated carboxylic acids (PFAs) are widely used as industrial surfactants, in production of water- and oil-resistant food packaging and fabrication of water- and stain-resistant materials. Perfluorooctanoic acid (PFOA) is one of the most commonly used acid from this group, mainly for processing polytetrafluoroethylene, known as Teflon. Perfluorooctane sulfonate (PFOS) is a related compound, used primarily as a surfactant. Due to their chemical structure those compounds tend to be resistant to hydrolysis, microbial degradation and living cell metabolism. This feature classifies them as environmentally persistent chemicals, and is the main reason for the increase of their concentration detected in the environment, humans and wildlife. There have been numerous studies on the harmful impact of PFAs on animals, but few researches focused on the toxicity towards human cells. The aim of this study was to estimate the cytotoxic effect of PFOA and PFOS on human colon carcinoma HCT116 cells, to analyze and compare their impact on mitochondria dysfunction. Firstly, basic toxicity assessment was done for the investigated compounds using octanoic acid (OA) as a reference. Then, we focused on the mitochondrial dysfunction connected with calcium swelling induced by tested chemicals. The generation of reactive oxygen species (ROS) and alteration in mitochondria respiration were evaluated. HCT116 was used as a model cell line. To estimate toxicity of analyzed compounds, MTT and NR assays were performed. EC₅₀ values were determined by fitting viability curves using a linear logistic model. Comparative mechanistic analysis with 200 μM PFOA, PFOS and OA were carried out in a time-dependent manner with flow cytometry, using fluorescent probes for: mitochondrial calcium [Ca²⁺]_m, ROS hypergeneration, mitochondrial transmembrane potential and cell cycle analysis. The mitochondrial respiration was evaluated by Clark-type oxygen electrode. EC₅₀ values decreased in a time-dependent manner, with final values for 72 h of 3212, 312 and 204 μM for OA, PFOA and PFOS, respectively. Flow cytometry analysis showed significant difference in accumulation of [Ca²⁺]_m after 12h for PFOA and PFOS. Considerable mitochondrial potential dissipation after 4h and 12h and ROS hypergeneration after 24h and 48h were detected for PFOS and PFOA, respectively. No significant differences were observed for OA in these. Tested PFAs uncoupled oxidative phosphorylation. Obtained results indicate a role of PFAs-induced apoptosis dependent on the mitochondrial intrinsic pathway. What is more, there are indications that toxic result of a compound strongly depends on its functional group, as PFOS has generally more harmful outcome than PFOA.

P2.20

Dietary sodium butyrate stimulates rebuilding of small intestinal epithelium in neonatal calves

Piotr Pietrzak¹, Anna Kotunia², Justyna Wrzesińska¹, Paweł Górka³, Joanna Bierła¹, Zygmunt M. Kowalski³, Michał M. Godlewski¹, Romuald Zabielski¹

¹Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland;

²The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Science, Jabłonna, Poland;

³Department of Animal Nutrition, Agriculture University of Kraków, Kraków, Poland

Contact: piotr.pietrzak08@gmail.com

Direct and indirect growth-stimulating effects of sodium butyrate (Na-B) on the gastrointestinal tract mucosa were already reported, and Na-B was recently introduced to milk replacer formulas and starter feeds to stimulate the animal's performance. Na-B is an important energy source and regulator of gastrointestinal epithelial cell function, the role in rebuilding of small intestinal epithelium, however, remain obscure. Our study aimed to investigate the influence of Na-B supplementation on the jejunum epithelium proliferation and programmed cell death in neonatal calves. Four days-old calves were allocated into 4 groups (n=6): the 1st received milk formula (MF) and starter solid diet (S) without Na-B supplementation (MFC-SC); 2nd – MF without and S with Na-B supplementation (MFC-SB); 3rd – MF with and S without Na-B supplementation (MFB-SC); and 4th – MF with and S with Na-B supplementation (MFB-SB). Milk formula supplementation consisted of crystalline Na-B (0.3% dry matter). Starter diet was enriched with encapsulated Na-B (0.3% Na-B in dry matter). Calves were slaughtered at 26 d of life for gastrointestinal tissue sampling. Whole thickness samples from the mid jejunum were fixed in embedding medium, frozen and labeled with specific set of antibodies. For mitosis, anti-Ki-67-FITC-conjugated (BD Pharmingen), and for apoptosis anti-Cpp32 (DAKO) and secondary chicken anti-rabbit Alexa 488 antibodies (Molecular Probes) were used. Cell nuclei were stained with 7AAD. Confocal microscopy (FV500) and Microimage system (Olympus) were employed for in-tissue-cytometry measurement of mitotic and apoptotic indexes in the small intestinal epithelium. The mitotic index was significantly increased in MFB-SB calves which received Na-B with milk formula and starter diet (P<0.001) as compared to the non-supplemented control group (MFC-SC). Supplementation of milk formula alone (MFB-SC), but no starter diet alone (MFC-SB), also significantly enhanced the mitosis index as compared to the control. In contrast, the apoptotic index was reduced in all three supplemented groups as compared to control (P<0.001). Consequently, the supplementation caused an increase in mitosis/apoptosis ratio in all three groups as compared to the control (MFC-SC) group. In conclusion, in neonatal calves dietary Na-B enhanced the enterocyte proliferation rate and reduced the apoptosis in the jejunum, which may explain the observed growth-stimulating effects in the gut (Grant no. 1164/P01/2006/31, Ministry for Research and Higher Education, Poland).

P2.21

Effects of h[Gly²]GLP-2 on apoptosis and cell proliferation in jejunal mucosa of mice administered TNF-α/Act D

Pelin Arda-Pirinci, Sehnaz Boltekin

Department of Biology, Faculty of Science, Istanbul University, Istanbul, Turkey

Contact: parda@istanbul.edu.tr

Tumor necrosis factor-alpha (TNF-α) is a multifunctional cytokine, which activates many signaling molecules, second messengers and transcription factors. Actinomycin D (Act D) is an inhibitor of transcription that markedly increases the cytotoxic effect of TNF. h[Gly²]GLP-2 is long-acting, degradation-resistant GLP-2 analog and it has intestinotrophic effects. The aim of this study was to investigate the effects of GLP-2 on jejunal injury induced by TNF-α/Act D in mice, morphologically and immunohistochemically. BALB/c mice were divided into six groups. Group I: Control animals administered DMSO and PBS (vehicle), Group II: Animals injected 15 μg/kg TNF-α intraperitoneally, Group III: Mice injected 800 μg/kg Act D intraperitoneally, Group IV: Animals receiving Act D, 2 minutes prior to the administration of TNF-α, Group V: Animals injected subcutaneously 200 μg/kg h[Gly²]GLP-2 every 12 hr for 10 consecutive days. Group VI: Animals receiving h[Gly²]GLP-2 for 10 days, and Act D 2 minutes prior to the

administration of TNF- α at 11th day. Effects of h[Gly²]GLP-2 on jejunal injury were investigated under light microscope. In addition, its role on apoptosis and cell proliferation was detected by TUNEL assay and PCNA immunohistochemistry, respectively. Administration of TNF- α /Act D caused degenerative morphological changes and a decrease in PAS-positive reaction intensity in goblet cells and villi in the jejunal mucosa. Apoptotic index in the intestinal crypts and villi significantly increased, but index of cell proliferation in the crypts decreased in the TNF- α /Act D group. On the other hand, h[Gly²]GLP-2 pretreatment caused a significant reduction in the TNF- α /Act D-induced jejunal injury and apoptosis; an increase in cell proliferation and PAS-positive reaction intensity in the jejunal mucosa of mice. As a result, the present study indicates that h[Gly²]GLP-2 has a potent cytoprotective, proliferative and antiapoptotic effects against TNF- α /Act D-induced jejunal injury.

P2.22

The response of human colon cancer cells to C-1748 (4-methyl-1-nitroacridine derivative) treatment

Dorota Nowak-Ziatyk, Ewa Augustin, Anna Moś-Rompa, Anna Skwarska, Jerzy Konopa

Department of Pharmaceutical Technology and Biochemistry, Chemical Faculty, Gdańsk, Poland

Contact: dorota-nowak@o2.pl

Antitumor 4-methyl-1-nitroacridine derivative C-1748 has been synthesized in Gdansk University of Technology and is currently being prepared for the phase I of clinical trials. C-1748 binds to DNA non-covalently by intercalation and covalently, following metabolic reduction of the nitro group at the position 1 of acridine ring. The covalent binding is likely to underlie the demonstrated ability of C-1748 to form interstrand cross-links in cellular DNA. Previous studies have shown C-1748 to exert potent anticancer activity against several prostate and colon carcinoma xenografts in nude mice. Since C-1748 is being prepared for clinical trials, better understanding of its lethal effects is critical. Therefore, we studied the ability of this drug to induce different types of cell death, namely apoptosis, autophagy and mitotic catastrophe. Additionally, we investigated whether the process of accelerated senescence might play important role in elimination of cancer cells exposed to this drug. As experimental models, we selected human colon HCT8 and HT29 cancer cells, highly sensitive to C-1748. HCT8 and HT29 cells were treated with C-1748 at EC90 concentration for different time periods varying from 24 to 168 h to follow morphological and biochemical hallmarks typical for each type of cellular response. DAPI staining of cytospin preparations revealed that starting from 24 h of C-1748 treatment HCT8 and HT29 cells exhibited apoptosis-associated nuclear condensation. Cells with more condensed chromatin and apoptotic body-like structures were clearly visible after 72 h of drug exposure. In both cell lines, no micronucleated cells, indicative for mitotic cell death, were observed following C-1748 treatment. Starting from 96 h, HCT8 cells developed features of drug-induced senescence with flattened, enlarged morphology and increasing degree of SA- β -galactosidase staining. Such effect was not observed in HT29 cells. Exposure of HCT8 and HT29 cells to C-1748 did not result in the appearance of typical for autophagy, acidic vesicular organelles upon acridine orange staining. To conclude, in our experimental colon cancer models apoptosis seems to be the primary response to C-1748 treatment. However, the extend of apoptosis was considerably greater in case of HCT8 cell line. Those of HCT8 cells which did not die following C-1748 exposure, underwent irreversible growth arrest in process resembling accelerated senescence. Alternative types of cell death such as mitotic catastrophe and autophagy were not responsible for the elimination of both HCT8 and HT29 cells treated with C-1748.

P2.23

Effect of doxazosin treatment on rat penis

N. Dilara Zeybek¹, Ozgur Oruc², Kubilay Inci², Ali Ergen², Celik Tasar², Sevda Muftuoglu¹

¹Hacettepe University Faculty of Medicine, Department of Histology & Embryology, Ankara, Turkey;

²Hacettepe University Faculty of Medicine, Department of Urology, Ankara, Turkey

Contact: smuftuog@hacettepe.edu.tr

Benign prostatic hyperplasia (BPH) is the proliferation of the epithelial and stromal components of prostate gland. Approximately 50% of patients older than 50 years require treatment for BPH related lower urinary tract

symptoms. For the treatment, α 1 adrenoceptor antagonists such as doxazosin are used. Recently it was shown that apoptotic activity is increased in the prostate of BPH after exposure to α 1 adrenoceptor antagonists. However, effect of doxazosin on penis has not been studied. As BPH cases accompanied with erectile dysfunction, in this study we aimed to examine apoptotic effect of doxazosin on penis. Twenty-four healthy male Wistar albino rats weighing 250-300 g were obtained from the Experimental Animal Centre of Hacettepe University Medical Faculty. All rats were divided randomly into two groups with 12 rats each: the control group and the doxazosin group. For the doxazosin group, doxazosin mesylate was given in standard rat food to a final daily dose of 0.03 mg (1830 mg of drug/kg food) for 3 months. After 3 months tissue samples were taken and processed for routine light microscopy. Sections were stained by hematoxylin-eosin, Masson's trichrome. Apoptotic cells were determined by TUNEL method and apoptotic rate was evaluated. Mann-Whitney U test was used to assess statistically significant differences between the apoptotic rates of two groups. $p < 0.05$ was considered as significant. The sections were examined and photographed using Leica DMR-reflection contrast microscope-RCM with DC200. In histological sections from control group, endothelium of cavernous bodies and numerous smooth muscle cells in trabeculae were examined. Smooth muscle cells were in normal histologic appearance. Corpus spongiosum, epithelial cells of urethra and connective tissue were examined. Nerve fibers and blood vessels which were in surrounding connective tissue of corpus cavernosum and corpus spongiosum were seen in normal histologic appearance. In the doxazosin group some cavernous sinuses were congested. Endothelial cells in cavernous sinus were intact and smooth muscle cells between them showed no contraction. The collagen fibers were similar to the control group. In Tunel method, both smooth muscle cells and epithelial cells of urethra showed apoptosis both in control and doxazosin groups. The apoptotic rate of the cavernosal smooth muscle cells in the doxazosin group was higher than that in the control group and there was a statistically significant difference between the two groups. Increased apoptosis observed in smooth muscle cells of rat penis may be related with the erectile dysfunction seen in doxazosin treated BPH.

P2.24

Apoptosis and expression of relative genes in chorionic villi and decidua of spontaneous abortion in the first trimester

Melek Ozturk¹, Tugan Bese², Fatma Kaya Dagistanli¹, Fazile Akyuz Gölgeci², Matem Tuncdemir¹, Penbe Cagatay³

¹Istanbul University, Cerrahpasa Medical Faculty, Medical Biology Department, Istanbul, Turkey

²Istanbul University, Cerrahpasa Medical Faculty, Obstetrics and Gynecology Department, Istanbul, Turkey

³Istanbul University, Cerrahpasa Medical Faculty, Statistical Department, Istanbul, Turkey

Contact: mozturk@istanbul.edu.tr

Losses in the first trimester represent about 15% of all recognizable pregnancies. Apoptosis might play a crucial role in embryonic development and wastage in humans. However, the exact mechanisms regulating the survival of human fetal allograft still remain a mystery of reproductive and developmental biology. In this study, we investigated apoptosis and expression of apoptosis regulated genes (bcl-2, c-myc, p53, p21/waf-1) in the first trimester human placental tissues which were obtained following spontaneous abortion (missed abortion; n=18 and incompleting abortion; n=14), and elective terminations of apparently normal pregnancy (n=14). None of the abortions was pharmacologically induced. Patients with clinically acute infection, autoimmune, or other systemic diseases, were excluded. TUNEL method was performed for apoptosis and immunohistochemical staining was performed for p53, bcl-2, c-myc and p21/waf-1. The elective abortion group had a higher gravida and parity than those of the spontaneous abortion group. There was no statistical difference for age, gestational age and abortion rates between two groups. We observed an increase of p21/waf-1 and p53 expression in the chorionic villi and decidua of placentas of the spontaneous abortions compared to the during the voluntary termination of pregnancy. More intense immunopositivity for p53 were seen in the cells of chorionic villus and decidua of missed abortion with respect to incomplete abortion. Whereas p21/waf-1 expression was higher in incomplete abortion compared to the missed abortion. Apoptotic cells were predominant in the syncytiotrophoblast and stroma of chorionic villi and decidua in cases of spontaneous abortions compare to the elective abortion ($p < 0.001$). There was a positive correlation with apoptosis and increased expression of p53

and p21/waf-1 in the spontaneous abortion cases. There was no alteration in the levels of c-myc in both spontaneous abortion and elective termination cases, while bcl-2 immunopositivity was strong especially in chorionic villi and decidua of the elective abortion group. Our study demonstrated that p21/waf-1 and p53 may contribute to spontaneous abortion involving excessive apoptosis of the chorionic villi and decidua, bcl-2 may be one of the major preventing factors from early cell death for the maintenance of normal pregnancy.

P2.25

Effects of iloprost on ischemia/reperfusion induced apoptosis in the ipsilateral rat ovary

Sureyya Barun¹, Lale Sokmensuer², Billur Demirogullari³, Devrim Gursoy¹, Anil Dolgun⁴, Mustafa Ozat⁵, Tayfun Gungor⁵, Sevda Muftuoglu⁶

¹Gazi University Medical School Department of Pharmacology, Ankara, Turkey

²Hacettepe University Faculty of Medicine Department of Histology & Embryology, Ankara, Turkey

³Gazi University Medical School Department of Pediatric Surgery, Ankara, Turkey

⁴Hacettepe University Faculty of Medicine Department of Biostatistics, Ankara, Turkey

⁵Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey

⁶Hacettepe University Faculty of Medicine Department of Histology and Embryology, Ankara, Turkey

Contact: smuftuog@hacettepe.edu.tr

Adnexal torsion is serious cause of gynecological surgical emergency. Studies suggest detorsion rather than salpingo-oophorectomy, in order to preserve fertility. However, maintaining the circulation of ovary by detorsion leads to ischemia/reperfusion (I/R) injury in tissue including apoptosis. Iloprost (ZK 36 374) is a prostacyclin (PGI₂) analogue mainly synthesized in vascular endothelium. It has pharmacodynamic properties such as vasodilatation and inhibition of platelet aggregation. Cytoprotective and antiapoptotic properties of iloprost have also been reported previously in distinct tissues after ischemia/reperfusion injury. In this study, effects of iloprost on apoptosis seen in the granulosa cells and the stromal cells due to ischemia/reperfusion injury have been investigated. Animals were divided into four groups with 6 rats each (1. Control, 2. Ischemia, 3. Ischemia/reperfusion, 4. Iloprost + ischemia/reperfusion). Ischemia and reperfusion periods lasted 4 hours. 10µg/kg iloprost or saline were injected into the peritoneal cavity 30 min before reperfusion. At the end of the experiments, right (ipsilateral) ovaries were removed and were fixed for histological examination. Specimens were processed for TUNEL staining. After histological examination, apoptotic cells were compared statistically by Kruskal-Wallis test. During the histological examination different developmental stages of ovarian follicles and corpus luteum were observed. Stromal cells and spindle shaped theca cells were examined at the outer part of the follicles. Granulosa cells of the graffian follicles near the cavity showed more prominent degeneration than the granulosa cells of the secondary and primary follicles. Their cytoplasm have apparent cytoplasmic vacuolization, that have also prominent chromatin condensation and pyknotic chromatin mostly surrounded by a narrow rim of cytoplasm. Iloprost increased the number of apoptotic granulosa cells both in the primary and secondary follicles after ischemia/reperfusion. Moreover, it also showed the same effect in the stromal cells. These results are contradictory with most of the studies showing the antiapoptotic property of iloprost. However, in favour of the present study, it has been reported that iloprost induces apoptosis in vascular smooth muscle cells. Therefore, further studies are needed to clarify the conflicting results of iloprost on apoptosis in distinct tissues.

P2.26

The comparison of the anti-leukemic effect of selected polyphenols on rat promyelocytic leukemia LT 12 cells

Monika A. Papiez¹, Malgorzata Bzowska²

¹Department of Cytobiology and Histochemistry, Collegium Medicum, Jagiellonian University, Kraków, Poland

²Department of Immunology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Contact: mfpapiez@cyf-kr.edu.pl

Background: Phytochemicals such as: quercetin and curcumin are known to inhibit the growth of some cancer cell lines and induce apoptosis. These polyphenols differ in their anti-cancerous activity. Quercetin and curcumin

are naturally occurring topoisomerase II inhibitors and may induce double-strand DNA breaks in proliferating cancer cells, which can lead to apoptosis. Anti-cancerous activity of (-)-epicatechin is still contradictory. The aim of this study was to compare the influence of these polyphenols on DNA damage and apoptosis of rat leukemia LT 12 cells. Material and methods: LT 12 cells were incubated for 24, 48 or 72 hours with one of the polyphenols: quercetin, (-)-epicatechin or curcumin in the concentration range 0 to 20 µM. The extent of DNA damage was estimated by an alkaline single cell electrophoresis in agarose gel (a comet assay), using Comet assay 2.6 software. Apoptotic cells were detected using an Annexin V-FLUOS staining kit (Roche) and analysed by a FACScan flow cytometry (Becton-Dickinson). Results: Quercetin and curcumin caused significant DNA damage when they were used in high dose (20 mM). The extent of DNA damage induced by curcumin was larger than quercetin after 48- and 72-h incubation. In lower doses (1-10 mM), these polyphenols did not induce DNA damage even after 72-h incubation. In doses as high as 10 or 20 µM, after 24 and 72 h, the incubation with quercetin or curcumin significantly increased the number of annexin-V-positive cells. Such an effect did not appear in low doses (1 and 5 mM) of these polyphenols. Epicatechin neither induced DNA damage nor influenced the apoptotic cell death even when it was present in the culture medium in high doses (10 and 20 mM). Conclusions: Curcumin was a stronger anti-cancerous agent than quercetin in causing DNA damage and apoptosis of rat leukemia LT 12 cells. The effect exerted by both polyphenols was time- and dose dependent. Epicatechin did not exert any effect in rat leukemia cells. This study was supported by grant no. 2 P05A 162 30 from the Polish State Committee for Scientific Research.

P2.27

The effects of increased intraabdominal pressure onto the urinary system during gestation

Ibrahim Karnak¹, Pergin Atilla², Sevda Muftuoglu², Saniye Ekinci¹, F Cahit Tanyel¹

¹Hacettepe University Faculty of Medicine Department of Pediatric Surgery, Ankara, Turkey

²Hacettepe University Faculty of Medicine Department of Histology and Embryology, Ankara, Turkey

Contact: patilla@hacettepe.edu.tr

Effects of increased intraabdominal pressure occur according to diameter and length of the structures which transport urine. Renal pelvis pressure increases more than intraabdominal pressure when intraabdominal pressure is increased. This is thought to occur due to smaller diameter and longer length and as a consequence much compressibility of the ureter compared to pelvis. This mechanism can play role in development of congenital urinary system abnormalities. In this study we aimed to determine the effects of increased intraabdominal and intraamniotic pressure onto development of fetal urinary system. New Zealand pregnant rabbits were used. At 15th day of gestation intraperitoneal catheter was placed in sham and experiment groups. In experiment group, intraabdominal pressure is increased from 20th day of gestation to term. At the end of gestation fetal kidney, ureter and urinary bladder samples were taken, fixed in formaldehyde, processed according to routine tissue processing technique. Histological analysis was done on sections stained with Hematoxylin-Eosin. Apoptosis was evaluated by TUNEL method. Apoptotic index was evaluated statistically by Mann-Whitney test. Probability values p<0,05 were considered significant. Most of the glomerules in control group kidneys were developed normally with a few apoptotic cells whereas the glomerules in the experiment group were immature in structure. Bowman space and the capillary loops in the glomerule were not totally developed yet. Proximal and distal tubules both in cortex and medulla were in normal histological structure both in control and experiment group. However cells of collecting tubules had vacuoles in basal cytoplasm evaluated as degeneration of tubular cells. Apoptotic cell index was decreased in both glomerules and tubules in the experiment group (p=0,001). Control group urinary bladder had spindle shaped smooth muscle cells in normal structure, but in experiment group smooth muscle cells were arranged in short and thin fibers with many apoptotic cells when compared to control (p=0,004). Epithelial cells of the bladder were sloughed out in most parts of the bladder in experiment group. Most of the intact epithelial cells were apoptotic. Apoptotic cell index was decreased significantly (p=0,095). Ureters of control group were normal in structure with a few apoptotic cells both in the epithelium and smooth muscle layer. In the experiment group thickness of the wall of the ureter was diminished. Smooth muscle layer was very thin. Many apoptotic cells were observed in the muscle layer when compared with control group (p=0,020). Epithelium

was intact with many apoptotic cells. Apoptotic cell index was decreased significantly ($p=0,002$). The blood vessels in the adventitia of the experimental group were congested. Increase in the intraabdominal pressure during pregnancy may cause some effects in developing organs related to the urinary system of the fetus.

P2.28

The influence of (-)-epicatechin on extent of DNA damage and apoptotic cell death in the bone marrow and spleen of rats with acute myeloid leukemia

Monika A. Papież¹, Tomasz Mikolajczyk², Virginia Krzyściak³

¹Department of Cytobiology and Histochemistry, Faculty of Pharmacy, Collegium Medicum, Jagiellonian University, Kraków, Poland

²Department of Immunology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland;

³Radioligand Laboratory, Faculty of Pharmacy, Collegium Medicum, Jagiellonian University, Kraków, Poland

Contact: mfpapiez@cyf-kr.edu.pl

Background: The association between tea catechins and risk of cancer is still under investigation. Some results indicate that these polyphenols are chemopreventive agents in various *in vitro* assays and *in vivo* animal models for inhibition of carcinogen-induced tumorigenesis in digestive tract and liver. It is regarded that anti-carcinogenic activity of tea catechins may be related to their strong anti-oxidative activities. On the other hand, some studies reported no association. Nonetheless, there are some positive association between tea consumption and cancer risk. The aim of our study was to investigate the association between the effects of (-)-epicatechin administration on anti-oxidant status, DNA damage and apoptosis of the spleen and bone marrow cells of Brown Norway (BN) rats with promyelocytic leukemia (BNML). **Material and methods:** The leukemia was induced through intravenous injection (i.v.) of promyelocytic leukemia cells to the BN rats. The experimental rats were given EC in a dose of 40 mg/kg b.w. (in 0.5 ml of corn oil as a vehicle) by gavage for 23 consecutive days. The control rats were only given a vehicle. The rats were killed at 24 h after the last dose of EC. The amount of DNA damage was estimated by a single cell electrophoresis in agarose gel (a comet assay), using Comet assay 2.6 software. Apoptosis was examined using Annexin-V-FLUOS staining kit (Roche) and CellQuest software (Becton-Dickinson). Early apoptotic cells were defined as annexin-V-positive and PI-negative, while late apoptotic and necrotic cells were regarded as annexin-V- and PI-positive. The leukemic cells were identified using RM-124 antibody. The intracellular redox state was assessed in spleen and liver by measurement of the ferric ion-reducing anti-oxidant power (FRAP) level and malondialdehyde (MDA) concentration as an indicator of lipid peroxidation. **Results:** EC significantly lowered the extent of DNA damage in spleen and bone marrow cells from experimental rats in comparison with the control. The number of apoptotic cells was significantly lowered and the number of viable cells significantly increased in the spleen of EC-treated rats in comparison with the untreated ones. EC did not influence the number of apoptotic cells in the bone marrow of experimental rats as compared with control. The FRAP values decreased significantly, whereas the MDA levels increased in spleen of EC-treated rats. The opposite effect was observed in the liver of leukemic rats. **Conclusions:** EC protects the cells in the spleen in leukemic rats against DNA damage and apoptosis, whereas it does not influence the bone marrow cells. Protective effect on spleen cells is probably not associated with the influence on anti-oxidant status because EC exerted rather pro-oxidant activity in this organ. This study was supported by grant no. 2 P05A 162 30 from the Polish State Committee for Scientific Research.

P2.29

Proteins of the p53 family in psoriasis

Dana Kylarova

Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic

Contact: dana@kylar.net

Psoriasis is a chronic inflammatory disease with unclear pathogenesis and difficult to treat. The origins of psoriatic lesions include altered skin reactivity to mechanical stimuli as probably crucial, accompanied by disordered signalling between T-cells resulting in hyperproliferation and abnormal

differentiation of the epidermis. Protein p53 as a tumor suppressor plays an important role in the regulation of the cell cycle and differentiation as well as in the initiation of apoptosis. Due to splicing, its homologues p63 and p73 occur in different isoforms important mainly for cell differentiation and cell cycle control. p63 isoforms have also been found essential to ectoderm differentiation during ontogenesis. In this study we quantified the level of proteins p53, p63TA, p63deltaN, p63alpha, p73 and p73 alpha in lesional and healthy skin with the view to their possible involvement in the dysregulation of epidermal growth and differentiation in psoriatic skin. We used lesional and non-lesional skin samples of 10 patients. The samples were fixed in buffered-formol and processed by standard paraffin technique with 5mm section thickness. For the detection of p53 proteins, a three step immunohistochemical method with peroxidase visualization was performed using DO7 (to p53), p63KNApha, p63KNNTA, p63KN, p73delta1.1 and p73alpha1.1 (all specimens were from the Masaryk Oncological Institute). For each sample, 6 fields were evaluated and labeling indexes of positive cells were established. The results showed that p53, p73 and p73alpha positive cells were sparse with no significant difference between lesional and healthy biopsies. Of the p63 isoforms, p63TA showed the most prominent difference between healthy and psoriatic skin with a prevailing positivity in the latter. The findings for p63alpha and p63deltaN isoforms were similar though less pronounced. The greatest number of positive cells was observed in p63deltaN in accordance with its role in keratinocyte differentiation. These data suggest that p63 is involved in the regulation of psoriatic skin changes.

P2.30

Cell cycle analysis of apoptotic melanotic and amelanotic melanoma cells

Mirosława Cichorek, Małgorzata Wachulska, Katarzyna Zielińska-Chomej

Department of Embryology, Medical University of Gdańsk, Gdańsk, Poland

Contact: cichorek@amg.gda.pl

Introduction: The decrease ability to apoptosis and the cell cycle disturbances in different tumor cells are considered to be elements of tumor progression. Despite to popular opinion that tumor growth not only depends on the dynamics of tumor clone cells proliferation but also on the ability of cells to death – especially by apoptosis, it is still not clear which of these phenomena predominates and decides about the individual tumor growth. We also do not know at what phases of the cell cycle the tumor cells die most easily. Problems mentioned above also concern melanoma, where clear correlation between proliferation, apoptosis and this tumor progression has not been found. Our earlier comparative studies of two hamster transplantable melanoma lines: melanotic (Ma) and amelanotic (Ab) showed that the decreased ability to apoptosis could submit the higher growth rate of the amelanotic melanoma and that this line is very sensitive to camptothecin (CPT)-induced apoptosis. Therefore in this study we estimated proliferating melanoma cells (S+G2/M) and cells from other cell cycle phases (G0/G1) undergoing spontaneous and CPT-induced apoptosis. **Material and methods.** *Transplantable melanomas:* the melanotic melanoma line (Ma) derived from a spontaneous melanoma of the skin which had appeared spontaneously in a breed of golden hamsters in 1959; the amelanotic melanoma line (Ab) originated from the melanotic form by a spontaneous alteration, differing in many biological properties. **Cell cycle analysis:** Ethanol-fixed 1×10^6 melanoma cells were stained (RNaseA and PI in PBS) and analyzed on a FACS Calibur flow cytometer. Cells in S and G2/M phases of cell cycle were analyzed together as the fraction of proliferating cells. **Estimation of apoptosis:** Cytofluorimetric analysis of dead cells by the TUNEL method (APO-BRDU Kit) has been used to determine apoptotic cells and cell cycle phases they come from. **Results:** 27% of cells from the Ma line died by apoptosis, came from S+G2/M phases, while among cells of the Ab line, only 9%. After induction of apoptosis by CPT during first hours died mainly proliferating cells (S+G2/M) but later cells came also from G0/G1. **Conclusions:** In the light of our above-mentioned observations we can conclude that the higher growth rate of amelanotic melanoma arose from the decreased ability to spontaneous apoptosis of proliferating cells in comparison to the native melanotic line -less aggressive and growing slower. Under influence of CPT as the first died proliferating amelanotic melanoma cells, than cells from other cell cycle phases.

P2.31

Morphological analysis of apoptosis in hippocampus of PTSD rats and expression of apoptosis-related genes

Xiao-Ming Li, Fang Han, Yuxiu Shi

Department of Histology and Embryology, College of Basic Medical Science, Shenyang, China

Contact: shiyuxiu@163.com

To investigate the relationships between the expression of Bcl-2, Bax protein and neuronal apoptosis in the hippocampus of PTSD rats, the SPS method was used to set up rat PTSD models. There were five groups: 1d, 4d, 7d, 14d after SPS, and a control group. Apoptotic cells were detected by electron microscopy and the TUNEL method. The expression of Bcl-2 and Bax proteins was detected with immunohistochemistry, double fluorescent confocal laser scanning microscopy and Western blotting. The results showed that: 1. Apoptotic cells were present in hippocampus of PTSD rats. 2. The number of apoptotic cells increased with the development of PTSD and peaked at 7d after SPS, then decreased gradually. 3. The expression of Bcl-2 protein peaked at 4d after SPS and Bax protein peaked at 7d after SPS, then decreased gradually. 4. The ratio of Bcl-2/Bax increased at the beginning and then gradually decreased. We concluded that neuronal apoptosis in hippocampus of PTSD rats may be one of the reasons inducing hippocampus atrophy. The increase in Bcl-2 and Bax protein levels and the change of Bcl-2/Bax ratio would play an important role in regulating the hippocampal neuronal survival or death during posttraumatic stress disorder.

P2.32

Apoptosis-inducing factor reveals differentiated contribution to cell death in the cerebral cortex and basal ganglia during transient ischemia in the ratPrzemysław Kowianski¹, Grażyna Lietzau¹, Zbigniew Karwacki², Jerzy Dziewiątkowski¹, Sławomir Wójcik¹, Jan Spodnik¹, Janusz Moryś¹¹Dept. of Anatomy and Neurobiology, Medical University of Gdansk, Gdansk, Poland²Dept. of Neuroanaesthesiology, Medical University of Gdansk, Gdansk, PolandContact: kowiansk@amg.gda.pl

Cerebral ischemia evokes pathological processes, leading to necrotic or programmed (apoptotic) death pathways. In the caspase-independent pathway a flavoprotein, apoptosis-inducing factor (AIF), plays a crucial role. Under ischemic conditions AIF translocates from the mitochondrial intermembranous space to the nucleus, causing chromatin condensation and large-scale DNA fragmentation. There is some evidence that AIF and caspase-independent pathway represent one of the phylogenetically eldest signalling cascades of cellular death. However, there is no comparative study of AIF contribution to the neuronal death in evolutionary differentiated structures of the mammalian brain. The aim of our study was to address the question if there are differences in the AIF activation dynamics in the cerebral structures, characterized by the various developmental origins. In 20 adult Wistar rats of both sexes the transient focal cerebral ischemia was induced by middle cerebral artery occlusion for 1h. The reperfusion period varied from 4h to 8 days. Western blot and immunohistochemistry methods were used to study the AIF level and distribution changes in the neocortex, hippocampus and striatum. In the neocortex no significant changes in the AIF levels between the ischemic and sham-operated animals were observed during the study period. In the hippocampus the AIF level reached maximal value (6,2 fold increase in comparison to sham-operated animals) after 24h of reperfusion. The AIF-ir cells were localized predominantly in pyramidal layer of CA1-CA3 sectors and granular layer of dentate gyrus. In the striatum the AIF maximal level (9,6 fold increase) was observed after 24h of reperfusion. The AIF-ir cells were localized in the dorso-lateral part of this structure. In summary, the caspase-independent pathway reveals unequal contribution to the ischemic cellular death in evolutionary differentiated brain regions. Further comparative studies are required for establishing the significance of various death pathways in phylogenetically differentiated brain structures.

P2.33

IL-2 stimulates telomerase activity and reduces spontaneous apoptosis in human NK cellsLucyna Kaszubowska¹, Agnieszka Dettlaff-Pokora², Jolanta Myśliwska³, Andrzej Myśliwski¹¹Department of Histology, Medical University of Gdańsk, Gdańsk, Poland;²Department of Biochemistry, Medical University of Gdańsk, Gdańsk, Poland;³Department of Immunology, Medical University of Gdańsk, Gdańsk, PolandContact: kras@amg.gda.pl

Natural killer (NK) cells are a subpopulation of lymphocytes that plays a critical role in the host defense against tumor and virus-infected cells. NK cell activation process usually results in cytokine production, up-regulation of functionally relevant surface molecules and induces cytolytic activity of the cells. Our studies aimed to estimate whether *in vitro* stimulation of NK cells with IL-2 influenced a level of telomerase activity and susceptibility to spontaneous apoptosis of the activated NK cells. Isolated human NK cells were treated with different concentrations of IL-2 for increasing time periods. Then the level of telomerase activity was estimated with the use of TRAP-ELISA test both in non-stimulated and stimulated cells. Simultaneously, a cell cycle of the studied cells was analyzed by flow cytometry and fractional DNA content, typical of apoptosis and defined as "sub-G1" peak, was detected. We found that IL-2 stimulated telomerase activity in activated NK cells and reduced spontaneous apoptosis of the analyzed cells.

P2.34

The role of apoptosis in the modulation of colon carcinogenesis in rats by oligofructose and bifidobacteria

Ewa Wasilewska, Barbara Wąsowska, Maria Bielecka

Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland

Contact: barwa25@wp.pl

The study was designed to determine the effect of animal-originated *Bifidobacterium animalis* KSP4 strain and oligofructose on epithelial and luminal factors which can be relevant to the development of colorectal cancer. Four groups of male Wistar rats were injected twice with AOM (15 mg/kg body wt) and fed the standard diet (control), or the standard diet supplemented with 5% oligofructose, 2.5% freeze-dried biomass of bifidobacteria (containing $\sim 7 \times 10^9$ CFU/g), and the combination of bifidobacteria and oligofructose, at the doses as above. After 12 weeks of experimental feeding the animals were sacrificed, and carcinoembryonic antigen in blood (CEA; ELECSYS 2100, ROCHE), number of aberrant crypt foci (ACF) and apoptotic cells (TUNEL; BD ApoAlert DNA, BD Biosciences Clontech) in colonic epithelium, as well as microbiological profile (bifidobacteria, lactobacilli, enterococci, *Escherichia coli*, yeast, and total anaerobes) and enzymatic activity (a- and b-glucosidase, a- and b-galactosidase, and b-glucuronidase) in the caecal contents were examined using standard analytical, culturing and spectroscopic methods. No significant differences were found in the CEA level between the tested groups whereas the ACF number was significantly lower ($p \leq 0.05$) in the bifidobacteria receiving groups. Simultaneously however, significant reduction of the amount of apoptotic cells ($p \leq 0.05$) in the colonic mucosa in all experimental groups was observed. It probably contributed to significantly higher number of tumors ($p \leq 0.05$) in these groups. Dietary administration of lyophilized cultures of bifidobacteria resulted in significant increase of bifidobacteria population number ($p \leq 0.001$) as well as a-glucosidase and a- and b-galactosidase activity, and in decrease of b-glucuronidase activity. Supplementation of the diet with bifidobacteria in combination with oligofructose caused significant increase of bifidobacteria, *E. coli* and total anaerobes population number, and a- and b-galactosidase activity, and decreased enterococci, yeast, a- and b-glucosidase, and b-glucuronidase activity. Similarly oligofructose alone decreased number of enterococci and activity of a- and b-glucosidase, and b-glucuronidase, and increased total anaerobes, and a- and b-galactosidase activities, but unlike the others groups significantly ($p \leq 0.05$) enlarged population of lactobacilli. Supplementation of the diet with the *B. animalis* KSP4 strain or oligofructose significantly influence on composition and metabolic activity of intestinal microflora and colon carcinogenesis in rats. Significant reduction of apoptosis in the colonic mucosa may suggest the role of prebiotic and probiotic in tumor formation.

P2.35

Cell proliferation and apoptosis in normal and diabetic mice testisFulu Gao¹, Fengming Yue¹, Xin Li², Siyun Niu²¹College of Life and Sciences, Hebei Normal University, Shijiazhuang, China²Chengde Medical College, Chengde, ChinaContact: gaofulu@yahoo.com.cn

The cause of fertility problems in diabetes mellitus (DM) is poorly known. To evaluate the role of unbalance between proliferation and apoptosis of germ cells in DM, the positive rate of proliferation and apoptosis were calculated in the testes of db/db diabetic mice. C57bl/Ksj-db/db mutant diabetic mouse is a kind of good animal model as non-insulin-dependent diabetes mellitus (NIDDM). Proliferation cell nucleus antigen (PCNA) immunostaining of testicular tissue was used to quantitatively analyze DNA synthesis in germ cells. Apoptosis was signed with TUNEL and

electron microscope. The positive rate of PCNA took on a significantly decreasing tendency as the diseases progressed in DM groups. In controls, the same feature was found. However, the positive rate of DM group was significantly lower than that of controls. Positive cells in TUNEL were observed in the basal and luminal compartments of seminiferous epithelium of diabetic and non-diabetic mice testis, but it was evident that the positive tubules and apoptotic cells' rate of DM groups were significantly higher than age-matched controls. The apoptotic tubules and cells increased not only in diabetic groups with the progress of DM but also in controls with aging. Ultrastructural analysis confirmed that the cells were undergoing apoptosis. Apoptotic spermatogonium, spermatocyte and spermatozoon contained a nucleus with marginated, condensed chromatin, apoptotic body, even empty left by late apoptotic germ cell. In db/db diabetic mice, the reduced expression of PCNA and increasing TUNEL-positive apoptotic germ cells elucidate that DM induces apoptosis and inhibits proliferation, which is the reason of the infertility during diabetes mellitus, namely, degenerated spermatogenesis.

P2.36

Newly developed technique for dual localization of keratin 13 and 14 by fluorescence immunohistochemistry

Shin-ichi Iwasaki, Hidekazu Aoyagi

The Nippon Dental University School of Life Dentistry at Niigata, Niigata, Japan

Contact: iwasaki@ngt.ndu.ac.jp

The present study was designed to clarify the timing of the appearance and changes in the expression of immunoreactive K13 and K14 in the rat lingual epithelium during the morphogenesis of filiform papillae. As indicated by the previous study [1], in which we used a combination of immunofluorescence staining of semi-ultrathin sections and the corresponding differential interference contrast (DIC) images, obtained by laser-scanning microscopy, it is likely that K13 is an important marker of differentiating suprabasal cells of the interpapillary cell columns and that K14 is an important marker of mitotic activity of basal and suprabasal keratinocytes in both the papillary and interpapillary cell columns. However, to show clearly the difference, in terms of distribution, between keratins 13 and 14, we need to examine the localization of both keratins simultaneously on appropriate images of the tissue. To achieve this goal in the present study, we used confocal laser-scanning microscopy in the transmission mode to examine the same sections as those used for the detection of immunofluorescence labeling K13 and K14, in an attempt to obtain clearer images of histological and cellular structures. We also developed improvements in the retrieval [2] of the keratin antigens that allowed us to detect them with greater accuracy. Using this approach, we examined the immuno-localization of K13 and K14 on the lingual epithelium of fetal and juvenile rats by immunofluorescence immunohistochemistry while monitoring morphological changes in the filiform papillae by laser-scanning microscopy, in transmission mode, of the same sections. No K13 and K14 immunoreactivity was detected on the lingual epithelium of fetuses on day 15 after conception (E15), at which time the lingual epithelium was composed of a few layers of cuboidal cells. K14 immunoreactivity was first detected on the lingual epithelium of fetuses on E17 and K13 immunoreactivity on E19. The number of layers of cuboidal cells in the lingual epithelium also increased from E17 to E19. K13 and K14 immunoreactivity was distinct at all postnatal stages examined. Although the respective patterns of K13 and K14 immunoreactivity differed as the filiform papillae developed, K13 immunoreactivity was generally evident in the suprabasal cells of the interpapillary cell columns and K14 immunoreactivity was detected in the basal and suprabasal cells of the papillary and interpapillary cell columns. Our newly developed technique for dual localization of antigens should be useful for investigations of very small specimens, such as fetal tissues and organs. References: 1. Iwasaki S, Yoshizawa Y, Aoyagi H: *Arch Oral Biol.* 51 (2006) 416-426. 2. Shi S-R, Key ME, Karla KL: *J Histochem Cytochem.* 39 (1991) 741-748.

P2.37

Gene amplification of *Myc* and its coamplification with *ERBB2* and *EGFR*

Akishi Ooi¹, Shioto Suzuki¹, Yoh Dobashi²

¹Department of Molecular and Cellular Pathology, Kanazawa University, Kanazawa, Japan;

²Department of Pathology, Omiya Medical Center, Jichi Medical University, Omiya, Japan

Contact: aooi@med.kanazawa-u.ac.jp

This study was conducted to assess the frequencies and to identify the mechanisms of *Myc* gene amplification, especially with regards to its possible coamplification with *ERBB2* or *EGFR* in gallbladder carcinomas. By immunohistochemical analysis of a total of 97 formalin-fixed and paraffin-embedded gallbladder carcinomas, nuclear overexpression of *Myc* was found in 43 tumors. Fluorescence *in situ* hybridization (FISH) analysis revealed three tumors (3%) had *Myc* amplification. Gene amplification of *ERBB2* and *EGFR* was found in 9 tumors each including two with coamplification of *ERBB2* and *EGFR*. One of the three tumors with *Myc* amplification was a mucosal adenocarcinoma with distinct nuclear overexpres-

sion of the *Myc* protein and unequivocal overexpression of *ERBB2* and *EGFR* on the cell membrane. For this tumor, dual-color FISH clearly demonstrated the coexistence of amplified *Myc* and *ERBB2*, and *Myc* and *EGFR* as clustered signals and multiple scattered signals respectively in the individual nuclei. However, signal overlappings of amplified genes were incidental, and thus the amplified genes were thought to be located on different amplicons. The second tumor showed high level amplification of *Myc* as clustered signals without amplification of *ERBB2* or *EGFR*. The third tumor with *Myc* amplification showed less than ten non-overlapping scattered signals of *Myc* and *ERBB2*, and thus this was considered to be low level coamplification of *Myc* and *ERBB2*. Polysomy 8 was observed in 22 cases, most of which had less than five *Myc* genes, and one of which had 6-9 *Myc* genes and amplification of *ERBB2* and *EGFR*. The present study shows that FISH can detect the molecular changes of individual nuclei precisely, and that genomic instability due to preceding *Myc* amplification may cause specific amplification of *EGFR* and/or *ERBB2* to follow. This may be important for clarifying the relationship between growth factor signal transduction and *Myc* function.

P2.38

Expression of keratan sulfate on microglia in the spinal cord of amyotrophic lateral sclerosis model mouse

Iori Kojima, Kenji Kadomatsu

Department of Biochemistry, Nagoya University Graduate School of Medicine, Nagoya, Japan

Contact: ludwig2500@yahoo.co.jp

Keratan sulfate (KS) is a glycosaminoglycan composed of repeating disaccharide units with sulfate residues at the C6 positions of galactose and N-acetylglucosamine. We previously showed that N-acetylglucosamine 6-O-sulfotransferase-1 (GlcNAc6ST-1) is essential for synthesis of KS in the central nervous system, and in GlcNAc6ST-1-deficient mice, glial scar formation (gliosis) after brain injury is significantly suppressed. The activation of astrocytes and microglia occurs at lesions not only in injuries but also in neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) and Parkinson's disease. These reactive astrocytes and microglia are known to be harmful: secreting neurotoxic products. Here, we report that KS is expressed by microglia in spinal cord of SOD-G93A transgenic mouse (ALS model mouse). Double immunostaining revealed that 5D4-reactive cells were also positive for Iba1, but not for GFAP or PDGFR α . To reveal the biological significance of the association between KS expression in reactive microglia and ALS, we generated GlcNAc6ST-1-deficient SOD-G93A mouse. However, there was no significant inhibition in extent of gliosis, disturbance of motor function, or expansion of life span.

P2.39

The function of annexins in plasma membrane repair

Andrea Erlbruch¹, Alen Piljić¹, Charlotta Funaya², Claude Antony², Carsten Schultz¹

¹European Molecular Biology Laboratory (EMBL), Gene Expression Unit, Heidelberg, Germany

²European Molecular Biology Laboratory (EMBL), Electron Microscopy Core Facility, Heidelberg, Germany

Contact: erlbruch@embl.de

Plasma membrane disruptions, also termed "cell wounding", are induced by mechanical stress and are a widespread and common form of cell injury. Mammalian cell types known to be regularly subjected to plasma membrane disruptions include skeletal and cardiac muscle cells, epidermal cells as well as endothelial cells. To protect cells against the loss of cytoplasm and hence against apoptosis as a consequence of membrane lesion several signaling cascades are activated to restore the integrity of the plasma membrane. Most dangerous for the cell, but indispensable for the resealing process is the influx of extracellular Ca²⁺. The influx of Ca²⁺ through the membrane disruption site triggers protein recruitment and homotypic vesicle-vesicle fusion events spatially directed to the disruption site. Continuous

and rapid homotypic vesicle fusion leads to the formation of a patch at the impact site. Completion of resealing results finally in the fusion of the patch with the plasma membrane. Many of the proteins involved in the resealing process are Ca²⁺-binding proteins such as synaptotagmins, calpain, dysferlin, the protein kinase C (PKC) or the calmodulin dependent protein kinase (CaMK). Another group of Ca²⁺ responsive and phospholipid binding proteins are annexins, which differ in their expression pattern, in their subcellular distribution as well as in their biochemical properties. In the past we visualized annexin A4 translocation and self-association in living cells in response to calcium rise as well as the heterogeneity and timing of translocation and membrane-mediated assembly of different annexins. We find that mechanical cellular wounding by microneedle puncturing as well as laser nanosurgery induces a massive and local limited accumulation of annexin A4 at the impact site. Simultaneous live cell imaging of fluorescent fusion proteins of various annexins show differences of the annexins in their spatiotemporal translocation behaviour to a local plasma membrane disruption after microneedle puncture. Correlative Fluorescence and Immunogold Electron Microscopy reveal high resolution images of the impact site and show differences in membrane localization of annexin A2 and annexin A4 after mechanical wounding. In multiparameter live cell imaging experiments the behaviour of other signaling molecule of the membrane repair machinery e.g. calpain 2, PKC and CaMK were monitored in real time. Ca²⁺ indicators as well as a DAG-sensor were used to image the Ca²⁺ influx and the presence of diacylglycerol at the membrane disruption site. We conclude that annexin A4 is a crucial component in the repair machinery and plays a key role in this cellular process by serving as a scaffold for repair vesicles and for protein recruitment in this extensive membrane repair process.

P2.40

Fluorescent *in situ* hybridization digital image composition using computerized image analysis procedure

Marylene Lejeune, Carlos Lopez, Patricia Escriva, Ramon Bosch, Joaquin Jaen, Lluís Pons, Teresa Salvado, Tomas Alvaro

Department of Pathology, Hospital de Tortosa Verge de la Cinta, Tortosa, Spain

Contact: mlejeune.htvc.ics@gencat.cat

Background. Traditional analysis of chromosomal translocations using fluorescent *in situ* hybridization (FISH) involves the detection of co-localized fluorescent probes within the nuclei. FISH visual analysis is an intensive labor and often subjective procedure since several factors can influence in the signal probe quality. Although digital imaging is an efficient tool to reduce subjectivity and improve diagnostic efficiency, existing imaging software is not optimized yet to work with fluorescent samples. Purpose. We describe a simple FISH digital image composed analysis procedure using the software Image-pro® Plus that permits to detect all the signals observed in the captured nuclei. Material. Paraffin-embedded sections of follicular lymphoma were hybridized with split-signal *BCL2* DNA Probe (DAKO) using the manufacture procedure. Co-localization of the two probes that flank the major breakpoint region of the target gene results in a red/green (yellow) signal whereas translocation events split one signal into separate green (fluorescein) and red (Texas Red) signals. The nuclei were counterstained using DAPI. Images were acquired using a fluorescent microscope Zeiss Axioskop 2 Plus coupled to a Leica DC300 FX digital camera and controlled by the Leica LAS 2.8.1 software. The FISH signals were visualized using DAPI/fluorescein Omega filters sets and a Texas Red Zeiss filter set. The different fluorescence illumination ranges of each filter were previously determined, stored in LAS and automatically utilized during the acquisition of the images. Image capture. Representative areas involved by tumor were selected with the hematoxylin section. Using immersion oil at 100X magnification, an image of nuclei shapes were captured with the DAPI filter. Without moving the section and applying different focus settings to cover the entire depth of the section, five consecutive images were acquired with the fluorescein filter and then with the Texas Red filter. Image composition. Using the "Extended Depth of field" tool of Image-Pro® Plus 5.0 program, the 5 green images were superposed to obtain a new composite best-focus image. The same was done with the red images. Then, DAPI, green and red images were modified using the "Contrast Enhancement" tool in their respective blue, green and red channels to generate a high-contrast image with reduced background and minimization of the variations in staining intensity. Finally, the three modified images were superposed in a definitive FISH composite image. Conclusions. As compared to visual lecture, this digital image composition procedure allows the

detection of a maximum of co-localized and/or split signals in a captured nucleus and the computation of distances between signals in non-overlapping nuclei. The effectiveness of this proposed approach improves the interpretation of the FISH technique and may provide enhanced diagnostic and prognostic information.

P2.41

A natural product from marine *Agelas* sponges is a cell permeable pH-sensor

Ulf Bickmeyer¹, Achim Grube², Karl-Walter Klings¹, Matthias Köck²

¹AWI Helgoland, Helgoland, Germany

²AWI, Bremerhaven, Germany

Contact: Ulf.Bickmeyer@awi.de

Marine sponges are a well known source for interesting secondary metabolites as for example pyrrole-imidazole alkaloids, which has been shown to disturb calcium signals in neuroendocrine cells (Bickmeyer et al. 2004, 2007). One alkaloid out of this group shows a blue-green fluorescence if excited with UV-light. This fluorescence strongly depends on the pH, where the green emission is highest at pH 4 and is decreasing up to pH 9. To estimate the pH from the fluorescence we choose to measure ratiometric at wavelength of 340 and 380nm similar to the calcium dye Fura 2. Despite the fact, that in opposite to other ratiometric measurements with dyes (e.g. Fura 2), the lowering of pH increases the fluorescence at both excitation wavelengths, our approach reduces the influence of the dye concentration on measured ratio. The alkaloid ageladine is cell permeable and can be used in cultured cells as well as in transparent animals to report even small intracellular pH changes. References: 1. Bickmeyer U, Drechsler D, Köck M, Assmann M: Brominated pyrrole alkaloids from marine *Agelas* sponges reduce depolarization-induced cellular calcium elevation. *Toxicol.* 44. 45-51. 2004. 2. Bickmeyer U, Grube A, Klings KW, Köck M: Disturbance of voltage induced cellular calcium entry by marine dimeric and tetrameric pyrrole-imidazole alkaloids. *Toxicol.* 50. 490-497. 2007.

P2.42

FRET probes to monitor MMP-12 activity in lung inflammation

Amanda Cobos-Correa¹, Joanna Trojanek², Marcus Mall², Carsten Schultz¹

¹EMBL, Heidelberg, Germany

²Children's Hospital, Heidelberg, Germany

Contact: cobos@embl.de

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes whose best understood function is the degradation of matrix components in a regulated fashion. This activity is required for a correct remodeling of the extracellular matrix or in processes such as cell migration. However, MMP overexpression has been described as the major cause of some lung diseases [1]. Particularly high levels of MMP-12 have been found in diseases involving lung tissue degradation such as Chronic Obstructive Pulmonary Disease [2] and emphysema [3], as a consequence of a massive attraction of macrophages to the lung and their local stimulation by antigens. Until now, very little is known about MMP activity under disease conditions, mainly due to a lack of methods to detect it. Our approach to follow and quantify the activity of this enzyme consists on probes based on FRET. This technique allows us to elucidate spatio-temporal distribution and functional states with great sensitivity. We have designed and synthesized several probes to detect MMP-12 activity based on Foerster Resonance Energy Transfer (FRET) at different locations of potential activity. The probes contain a specific amino acid sequence for the target enzyme and two fluorophores. In the intact probe, the fluorescence emission of the donor is quenched by the acceptor. After cleavage of the reporter by the enzyme, the dyes separate accompanied by a loss of FRET that can be monitored by an increase in donor fluorescence and a decrease in acceptor fluorescence. Measuring the emission ratio values allows quantification and spatially resolved imaging, thereby providing a highly sensitive method for measuring the enzyme activity both *in vitro* and *in vivo*. Following *in vitro* experiments where the probe performance was quantified, we measured protease activity in bronchoalveolar lavage (BAL) samples of particulate matter-treated mice that show a phenotype of lung inflammation. Results demonstrating the suitability of the probes in *in vitro* and *ex vivo* experiments will be present-

ted. References: 1. Ohbayashi, H. (2002) Matrix metalloproteinases in lung diseases. *Curr Protein Pept Sci* 3, 409-421. 2. Molet, S., Belleguic, C., Lena, H., Germain, N., Bertrand, C. P., Shapiro, S. D., Planquois, J. M., Delaval, P., and Lagente, V. (2005) Increase in macrophage elastase (MMP-12) in lungs from patients with chronic obstructive pulmonary disease. *Inflamm Res* 54, 31-36. 3. Wallace A. M. (2008) Matrix metalloproteinase expression by human alveolar macrophages in relation to emphysema. *COPD* 5, 13-23.

P2.43

Age-related changes to the distribution of perisynaptic Schwann cells at the murine neuromuscular junction

Angie Rupp, Susan K Halstead, Hugh J Willison

Division of Clinical Neurosciences, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow, Scotland

Contact: a.rupp.1@research.gla.ac.uk

In recent years, research on the acute autoimmune paralytic peripheral nerve disorder, Guillain-Barré syndrome (GBS), has advanced through the development of a mouse model for the Miller-Fisher variant. In this context both the terminal axons and the perisynaptic Schwann cells (pSCs) at the neuromuscular junction (NMJ) have been demonstrated to be primary targets to circulating anti-ganglioside antibodies. Using transgenic mice expressing fluorescent proteins in all subsets of their Schwann cells and their axons, in combination with imaging technology, it has now become possible to directly watch evolving degenerative and regenerative changes to the motor nerve terminal structures affected in the acute phase of murine GBS, and during recovery. These advances, however, make it imperative to determine whether, and to what extent, the individual components of the NMJ undergo physiological changes over time. In order to address this, we quantified the average number of pSCs per NMJ in whole-mount preparations of the sternomastoid muscle of mice aged 3, 5, 7 and 9 months (each n = 3). Additionally, the distribution of NMJs in relation to their number of pSCs was calculated. In each animal 169 to 312 NMJs were assessed. The 3- and 5-month old mice exhibited a relatively stable number of 2.76 to 2.89 pSCs per NMJ (mean 2.84), whereas in the older (7- and 9-month old mice) a larger variation in the average number of pSCs per NMJ, in addition to statistically significant absolute increase of pSCs per NMJ could be observed (minimum: 2.89; maximum 3.61; mean 3.24; $p=0.016$). When compared with the younger mice, the older age groups showed a statistically significant relative increase ($p=0.031$) in the percentage of junctions with 4 pSCs or more, mirrored by a concurrent decrease ($p=0.026$) of NMJs with only one or two pSCs. The relative amount of NMJs with 3 pSCs remained stable in all animals at approximately 40% of all NMJs ($p=0.474$). The results of this preliminary study indicate, that in correspondence with other studies which have described an increased loss of NMJ stability with age, the cellular composition of the NMJ also changes over time and an increased diversity can be observed between individual animals. These observations need to be kept in mind when conducting repeated *in vivo* studies on NMJs of the sternomastoid muscle in the GBS mouse.

P2.44

Sarcoglycans and integrins in gingival epithelium during bisphosphonates treatment: immunohistochemical study

G. Cutroneo¹, E. Nastro Sinischalchi², G. Oteri², E Magaouda², A. Allegra³, C. Musolino³, D. Bruschetta¹, F. De Ponte²

¹Dipartimento di Biomorfologia e Biotecnologie, Università di Messina, Messina, Italy

²Dipartimento di Odontostomatologia, Università di Messina, Messina, Italy

³Dipartimento di Medicina Interna, Università di Messina, Messina, Italy

Contact: fatrim@unime.it

Dystrophin-glycoprotein complex (DGC) and vinculin-talin-integrin system are two system comprising cell surface receptors for extracellular matrix binding that also play a key role in cell-cell binding and in cell viability in many cellular types. Although the function of these proteins was investigated in numerous studies in many tissues, almost no data exist on their expression in gingival epithelium in normal and pathological conditions. In particular, we used immunofluorescence techniques to analyze any proteins of vinculin-talin-integrin system and of DGC in gingival epithelium

treated with bisphosphonates to evidence their role in these conditions. Bisphosphonates are an established standard of care for patients with bone metastasis. In details, first we analyzed samples of gingival epithelium treated with bisphosphonates of subjects that showed no lesions; secondly, we studied samples of gingival epithelium treated with bisphosphonates from subjects that showed a necrosis, in comparison with control samples. For the first time, our results clearly showed that all tested proteins, were almost absent in gingival epithelium of subjects without lesions, whereas in gingival epithelium with necrosis we detected a clearly visible staining, specifically in basal lamina, however, less intensive than in control samples. Moreover, in gingival epithelium with lesions there was clearly detectable new formation of cellularity and a massive neoangiogenesis in isolated zones. In our opinion the behaviour of proteins of vinculin-talin-integrin system and of DGC demonstrated a key role in cellular signaling between cell and extracellular matrix. In particular, the increase of these proteins, in basal lamina, in concomitance with the formation of lesions, could indicate a compensatory behaviour in the remodelling of the gingival epithelium in order to restore the epithelial architecture, and then to restore the signalling pathway of the cell. The occurrence of neoangiogenesis confirms the compensatory role of the two investigated protein complexes.

P2.45

Distribution of costameric proteins in normal human ventricular and atrial cardiac muscle

D. Di Mauro¹, R. Gaeta², A. Arco¹, D. Milardi¹, S. Lentini², M. Runci¹, G. Rizzo¹, L. Magaouda¹

¹Dipartimento di Biomorfologia e Biotecnologie, Università di Messina, Messina, Italy

²Dipartimento Clinico Sperimentale di Medicina e Farmacologia, Università di Messina, Messina, Italy

Contact: dedimauro@hotmail.com

Cell-cell and cell-matrix junctions in cardiac myocytes are termed intercalated discs and costameres, respectively. The intercalated discs are structures necessary for mechanical adhesion, and the costameres are sites where contractile forces generated within the cardiomyocyte are transmitted to the surrounding ECM. They also provides a site for maintenance of structure and of mechanotransduction in the cardiomyocytes. Costameres are considered as "proteic machinery" that appears to comprise two protein complexes: the dystrophin-glycoprotein complex (DGC) and the vinculin-talin-integrin system. There are structural differences between atrial and ventricular myocytes, but there have been relatively few studies that have analyzed costameres and focal adhesion function in cardiac cells. Our previous study carried out only on atrial myocytes, demonstrated that the DGC and talin-vinculin-integrin complexes had a costameric distribution that, unlike skeletal muscle, it localized only on the I band. We performed a further immunohistochemical analysis extending also the evaluation to the normal human cardiac muscle fibers obtained from ventricle, interventricular sept, and auricula, in order to define the distribution and the spatial relationship between the proteins of the two complexes also in the other heart regions. Immunofocal microscopy of cardiac tissue revealed the costameric distribution of DGC and of vinculin-talin-integrin system, the association of all tested proteins in intercalated disks, in disagreement with findings of other authors, and in T-tubule with irregular spoke-like extensions penetrating toward the center of the cell. Moreover, our data showed that tested proteins of DGC colocalize between each other such as all tested components of vinculin-talin-integrin system, and that all tested proteins of DGC colocalized with the proteins of vinculin-talin-integrin system; finally, all tested proteins were localized in the region of the sarcolemma over the I band.

P2.46

Immunohistochemical localization of DCAMLK1 on rat pancreas

Yoshiko Itoh, Johbu Itoh

Tokai University School of Medicine, Isehara, Japan

Contact: iyoshiko@is.icc.u-tokai.ac.jp

Double cortin and calcium/calmodulin-dependent protein kinase-like 1 (DCAMLK1) (Ser/Thr protein kinase family) is essential for proper neurogenesis, neuronal migration, and axonal wiring. DCAMLK1 is involved in a calcium-signaling pathway controlling neuronal migration in the deve-

loping brain, and participates in functions of the mature nervous system. DCAMLK1 protein shares high homology with doublecortin (DCX). DCAMLK1 controls mitotic division by regulating spindle formation and also determines the fate of neural progenitors during cortical neurogenesis. DCAMLK1 is known as stem cell marker on the gastric and intestinal epithelial cell in gland's isthmus. The aim of this study was to demonstrate the localization of DCAMLK1 in rat pancreas by immunocytochemistry. The pancreas of male and female Wistar rats was embedded in paraffin after formalin fixation. The paraffin sections were stained with immunofluorescence method. The primary antibodies used were anti-insulin, anti-glucagon and anti-DCAMLK1. The secondary antibodies were Alexa488 conjugated anti-rabbit IgG, Alexa 594 conjugated anti-rabbit IgG and Quantum dot 655 conjugated anti-rabbit IgG. These specimens were mounted with TOTO3/DABCO, and observed by Confocal Laser Scanning Microscope (LSM-510 META, Carl Zeiss, Germany). Immunohistochemical localization of insulin and glucagon was found in the cytoplasm of beta-cells or alpha-cells, and DCAMLK1 was observed in the cell membrane. Four patterns of DCAMLK1 distribution were found: (i) around membrane of all beta-cells, (ii) around membrane of parts of beta-cells, (iii) as scattering bubbles or (iv) negative. The immunohistochemical and immunoelectron microscopic analysis of DCAMLK1 in pancreatic islet cells is under further investigation.

P2.47

Cytogenetic characterization of brook trout (*Salvelinus fontinalis*): location of DNA sequences on chromosomes

Agnieszka Śliwińska-Jewsiewicka, Konrad Ocalewicz, Małgorzata Jankun-Woźnicka

University of Warmia and Mazury in Olsztyn, Faculty of Environmental Sciences and Fisheries, Department of Ichthyology, Olsztyn, Poland

Contact: a.sliwinska@uwm.edu.pl

Salmonid fishes are interesting objects of genetic population research. Genetic and morphologic diversity, excellent ecological adaptation and economic value put this fish in the centre of scientific attention. Comparing to other salmonid fishes brook trout (*Salvelinus fontinalis*) has not been extensively investigated, especially in Poland. Therefore we decided to study cytogenetics of brook trout from the Salmonid Laboratory Research in Rutki, Institute of Inland Fisheries in Olsztyn, Poland. The objective of this study was to define chromosomal localization of the DNA sequences after fluorescence in situ hybridisation with TTAGGG probe, after PRINS (*PRImed IN Situ*) Labelling with primers enabling 5S rDNA amplification, and to find cytogenetic markers enabling identification of sex chromosomes in brook trout. Results: Telomeric sequences (TTAGGG)_n were observed at the ends of all chromosomes. In some individuals an additional signal (ITS, interstitial telomeric sites) was found. It was present in one or two homologous chromosomes. In all cases, the ITS appeared in the pericentromeric region of subtelocentric chromosomes. PRINS with primers enabling 5S rDNA sequences amplification showed that clusters of 5S rRNA genes were placed on DAPI positive short arms of one chromosome pair. Fluorescent staining with DAPI (4',6'-diamidino-2-phenylindole) enabled identification of differences between female and male karyotypes. DAPI banding provided data about distribution of AT-rich chromatin in brook trout chromosomes: bright signals were observed in centromeres of all chromosomes. In two metacentric chromosomes of females, and in one metacentric chromosome of males, the telomeric regions of arms were sta-

ined brightly. The results enabled us to map genetic markers of X chromosome in brook trout and use such a map to identify the genetic sex, to verify gynogenesis and androgenesis efficiency and to perform studies on environmental influence on sex differentiation of this species. Localization of 5S rDNA sequences on the chromosomes could be useful as an additional cytogenetic marker in ploidy analysis. The obtained detailed cytogenetic characterization of brook trout may be useful for taking proper decisions in the management of its populations.

P2.48

A simple, rapid technique for fluorescent labeling of collagen and elastin fibers in live tissues

Natalia Celejewska¹, Krzysztof Wójcik¹, Ewa Biela-Witkowska¹, Tomasz Włodarski¹, Jerzy Galas², Gail McConnell³, Zbigniew Darzynkiewicz⁴, Jurek Dobrucki¹

¹Division of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland;

²Department of Endocrinology, Faculty of Biology and Earth Sciences, Jagiellonian University, Kraków, Poland;

³Centre for Biophotonics, Strathclyde Institute for Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom;

⁴Brander Cancer Research Institute at New York Medical College, Valhalla, United States

Contact: jurek.dobrucki@gmail.com

Collagens constitute a principal component of the extracellular matrix. Collagen molecules form a triple helix and, once outside cells, assemble into fibrils and fibers that provide mechanical strength to almost all animal tissues. Recently, we have introduced a new method of fluorescently labeling collagenous structures in live tissues for standard wide field and confocal microscopy [1]. A low molecular weight fluorescent probe Col-F exhibits affinity to polymerized collagen, readily penetrates into tissue, and binds to collagen fibers noncovalently. This simple and rapid staining technique can be used to reveal a stunning variety of intricate 3D collagenous structures in live animal tissues. Currently we are investigating a possibility of extending this technique to simultaneous two-colour staining of collagen and elastin fibers in live tissues, by using Col-F and sulphorhodamine B [2]. We demonstrate that collagen and elastin structures in walls of veins and arteries are readily stained by Col-F and sulphorhodamine B, and components of basal lamina, tunica intima, tunica media, and tunica adventitia can be clearly imaged. We also demonstrate images of collagen and elastin fibers in skeletal muscle, diaphragm and other animal tissues. Using confocal microscopy and FRAP (Fluorescence Recovery After Photobleaching) we demonstrated that Col-F binds to collagen noncovalently. In order to obtain more insight into the binding mechanism of Col-F to polymerized collagen, we performed molecular modeling of the Col-F-collagen complex. A comparison of energies of these complexes suggests a possibility of Col-F fitting into the groove on the collagen triple helix and binding via hydrophobic interactions and two hydrogen bridges. This work is supported by grants from Royal Society, London and MNiSzW, Warsaw. References: 1. Dobrucki J., Galas J., Włodarski T., Darzynkiewicz Z., Lee B., Johnson G. A fluorescent probe for confocal imaging of polymerized collagen in live tissues. *Histochemistry and Cell Biology*, submitted. 2. Ricard C., Vial J.C., Douady J, van der Sanden B. *in vivo* imaging of elastic fibers using sulforhodamine B. *J Biomed Opt.* 20.07;12(6):064017.

Molecular methods on normal and pathological specimen

P2.49

Expression profiling of MTA1 in adult mouse tissues

Yuanqiang Zhang, Wei Li, Zhen Li, Jie Zhao

Department of Histology and Embryology, the Fourth Military Medical University, Xi'an, China

Contact: zhangyq@fmmu.edu.cn

The metastasis-associated gene 1 (MTA1) was originally identified by differential cDNA screening using cell lines derived from highly metastatic mammary adenocarcinomas and the expression of its homolog, human metastasis-associated protein 1 (hMTA1) also correlated with the invasive and metastatic potential of cultured mammary tumour cells. MTA1 is thought to modulate transcription by influencing the status of chromatin remodeling. Despite its strong correlation with the metastatic potential of several cancer cell lines and tissues, MTA1 can also regulate divergent cellular pathways by modifying the acetylation status of crucial target genes. However, its fundamental physiological functions have not been characterized. To further address the possible physiological role of this protein in mammals, we examined the expression pattern of mouse Mta1 in a variety of adult mouse tissues by a combination of techniques, including semi-quantitative RT-PCR, western blotting and immunohistochemistry. All mouse tissues examined by the primary antibody showed a single band at about 80 kD, and no other bands were observed. Consistent with the immunohistochemical data, the specific 80-kD band was strong in the esophagus, lung, kidney, submandibular gland, brain, testis, epididymis, ovary and Fallopian tube, moderate in stomach, weak in heart, pancreas, WAT, thyroid gland, colon, and spleen, very weak in the trachea, and negative in skeletal muscle, adrenal gland, thymus and bladder. MTA1 was localized in both the cytoplasm and the nuclei, and was accumulated in the nuclei. In mature mice, MTA1 expression was seen in cell types that constantly undergo proliferation or self-renewal, such as testis, and cell types not constantly engaged in proliferation or self-renewal, such as brain, liver and kidney. This differential expression reveals several striking features regarding MTA1 expression: 1. MTA1 protein is expressed in numerous unexpected organs/systems; 2. its subcellular localization varies between cell karyon and cytosol in various tissues we examined; 3. the localization data suggest that MTA1 is involved in the regulation of homeostasis of multiple organ systems. The expression profile of MTA1 obtained in our systemic study suggests that this protein serves distinct functions in mammalian tissues and lays down a foundation for future studies on the roles of MTA1 under various physiological and pathological conditions such as organogenesis and tumorigenesis.

P2.50

Subcutaneous injection from birth of epigallocatechin-3-gallate, a component of green tea, limits the onset of muscular dystrophy in mdx mice: a quantitative histological, immunohistochemical and electrophysiological study

Yoshiko Nakae¹, Katsuya Hirasaka², Junpei Goto², Takeshi Nikawa², Masayuki Shono³, Mizuko Yoshida⁴, Peter J. Stoward⁵

¹Department of Oral and Maxillofacial Anatomy, The University of Tokushima Graduate School, Tokushima, Japan

²Department of Nutritional Physiology, The University of Tokushima Graduate School, Tokushima, Japan

³Support Centre for Advanced Medical Sciences, The University of Tokushima Graduate School, Tokushima, Japan

⁴Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Centre of Neurology and Psychiatry, Kodaira, Japan

⁵College of Life Sciences, University of Dundee, Dundee, United Kingdom

Contact: yoshiko.nakae@pharm.unige.ch

Previously we found that dystrophin-deficient dystrophic muscles suffer from more oxidative damage than age-matched normal controls, leading to the formation of end products of peroxidation such as lipofuscin and 8-oxoguanine in DNA. We have hypothesized that oxidative stress is a major contributing factor in the pathology of human Duchenne-type muscular dystrophy (DMD) and investigated whether administration of an antioxidant, epigallocatechin-3-gallate (EGCG), a major polyphenol of green tea, reduces their oxidative stress and pathophysiology in *mdx* mice, a mild phe-

notype model of DMD. EGCG (5mg/kg body weight in saline) was injected subcutaneously four times a week into the backs of C57BL/10 normal and dystrophin-deficient *mdx* mice for 8 weeks from either the day of birth or a day after birth. Saline was injected into normal and *mdx* controls. At the end of the treatment EGCG had almost no observable effects on normal mice or on the body weights of *mdx* mice. In contrast, it produced the following improvements in the blood chemistry, muscle histology and immunohistochemistry, and electrophysiology of the treated *mdx* mice. First, the mean activities of serum creatine kinase, an index of muscle damage, were significantly reduced by about 75 % to normal levels. Second, the mean numbers per unit volume of a marker of oxidative stress, autofluorescent lipofuscin granules, in diaphragm and soleus muscles were significantly decreased by about 50 % and 40 %, respectively, compared to the numbers in the corresponding saline-treated controls. Third, in sections of diaphragm muscles, the relative area of histologically normal muscle fibres increased significantly about 2-fold whereas the relative areas of connective tissue and necrotic muscle fibres were significantly reduced by about 40 and 90% respectively. In sections of soleus muscles, the relative area of normal muscle fibres significantly increased about 1.5-fold but that of necrotic muscle fibres decreased by 95 %. Fourth, the times for the maximum tetanic force of soleus muscles to fall by a half increased significantly 1.9-fold to almost normal values. Fifth, the amount of utrophin on the extrasynaptic sarcolemma increased qualitatively not only in immature muscle cells but also in mature muscle cells in diaphragm and lingual muscles. Quantitatively, the amount of utrophin in homogenised diaphragm muscles increased significantly by 17 %, partially compensating for the lack of dystrophin expression. Our study corroborates other recent studies that EGCG is effective for limiting the onset of muscular dystrophy in *mdx* mice, but the optimal dosage, administration route and the latest age at which treatment can be started in order to prevent muscle fibre necrosis and regeneration from occurring have still to be established.

P2.51

Mutational analysis of p53 in patients with long-lasting chronic hepatitis C

Aldona Kasprzak¹, Agnieszka Adamek², Wiesława Przybyszewska¹, Arkadiusz Czajka², Karolina Olejniczak¹, Jacek Juszczyk², Wiesława Biczysko³, Maciej Zabel¹

¹Department of Histology and Embryology, University of Medical Sciences, Poznań, Poland

²Department of Infectious Diseases, University of Medical Sciences, Poznań, Poland

³Department of Clinical Pathomorphology, University of Medical Sciences, Poznań, Poland

Contact: akasprza@ump.edu.pl

Background and aims: Chronic infection with hepatitis C virus (HCV) is regarded as a risk factor for hepatocellular carcinoma (HCC), mostly in patients with liver cirrhosis. There is also evidence for the association of HCV, mostly genotype 1b, with HCC without the intermediate step of cirrhosis. Both *in vitro* and *in vivo* different types of interactions were demonstrated between HCV proteins and p53 protein. The aim of this study was to determine whether mutations of p53 gene (*TP53*) might occur in non-neoplastic chronic HCV infected livers. Material and methods: The expression of p53 protein was analysed by immunocytochemistry in 16 liver biopsies of adult patients with long-lasting (approximately 20 years) chronic HCV (mostly genotype 1b) infection. In order to detect TP53 mutations, PCR/SSCP and gene sequencing were performed. Anti-p53 in serum were determined using enzyme immunoassay (ELISA). Results and conclusions: By immunocytochemistry we detected p53 protein in 8/16 examined liver samples. In two out of 14 examined patients TP53 point mutations were detected in the liver. In the first patient, a substitution of C to T was demonstrated in position 1 of the codon 250, resulting in the substitution of proline by serine. The other patient carried a substitution of C to G in position 13274 of intron 6. The patient carrying mutation in the codon 250 demonstrated morphological traits of liver cirrhosis and had high number of p53-immunoreactive cell nuclei in the liver tissue. None of the patients examined manifested elevated titres of serum anti-p53. In the liver, significant

positive correlations were disclosed between expression of p53 and *grading* and *staging* of the disease. A negative correlation was disclosed between cellular expression of p53 and duration of the infection. In conclusion, genetic changes in *TP53* can be detected also in non-neoplastic lesions linked to chronic HCV infection. It seems important to observe the patients with point mutations in *TP53* and with overexpression of tissue p53 with a particular care.

P2.52

Cellular expression of interleukin 2 (IL-2) and of its receptor (IL-2R, CD25) in lung tumours

Karolina Olejniczak¹, Aldona Kasprzak¹, Wiesława Przybyszewska¹, Patrycja Sujka¹, Piotr Dzięgieł², Maciej Zabel¹

¹Department of Histology and Embryology, University of Medical Sciences, Poznań, Poland

²Department of Histology and Embryology, University of Medical Sciences, Wrocław, Poland

Contact: karolinaolejniczak@op.pl

Background and aims: IL-2 represents a cytokine which is produced mainly by activated T helper (Th, CD4⁺) lymphocytes. Its critical role is to activate T cell growth *in vitro*. The role of Th cell-derived IL-2 *in vivo* is still controversial. Studies of the expression of IL-2 and its receptor (IL-2R) focused on the role of the complex in control of cell growth, IL-2 anti-tumour activity and/or appraised IL-2 value as a potential serum neoplastic marker. Roles of IL-2 and of its receptor, produced by tumour cells themselves remain poorly defined. The aim of this study was to determine whether cellular expression of IL-2 and its receptor (IL-2R α , CD25) could be detected also in lung tumours cells in histologically different types of lung cancers. Material and methods: The immunocytochemistry and hybridocytochemistry was applied to archival tissue material of typical and atypical lung carcinoids (TC, AC), small cell lung cancers (SCLC), lung adenocarcinoma, and squamous cell lung cancers (NSCLC). Results: Expression of IL-2 protein and IL-2 mRNA was detected in all types of lung tumours. IL-2 and its transcript were detected in neoplastic cells and in tumour-associated inflammatory infiltrates. The highest frequency of detection (93%) by the semi-quantitatively evaluated expression of IL-2 was found in the cells of atypical carcinoids. The expression was more pronounced as compared to SCLC (p=0.01) and NSCLC cells (p=0.005). The frequency of IL-2 receptor (IL-2R α , CD25) detection was the highest in cases of NSCLC (94%). Semi-quantitative expression of IL-2R, similarly as that of IL-2, also dominated in the group of AC but manifested a significant difference only as compared to typical carcinoids (p=0.014). Within the studied groups of tumours no correlation could be demonstrated between cellular expressions of IL-2 and IL-2R α . Our results evidence variable expression of IL-2 and its receptor in various types of lung tumours. The appraisal of diagnostic and/or prognostic significance of these results requires further studies.

P2.53

The study of the interaction between hepcidin and ferroportin

Xiao-Zhi Liu, Yan-Zhong Chang, Xiang-Lin Duan

The Institute Of Molecular Neurobiology And Neurpharmacology, Hebei Normal University, Shijiazhuang, China

Contact: xlduan0311@163.com

Ferroportin (Fpn) in the intestinal absorption cells is an important protein involved in iron absorption. Another protein recently discovered is hepcidin, a liver antimicrobial peptide involved in intestinal iron absorption thus in control of important physiological functions. Fpn is distributed in the membrane and is transferred to the cytoplasm. Is there a direct interaction between Fpn and hepcidin? What is hepcidin's role? We use the Fluorescence Resonance Energy Transfer (FRET) method, immunoelectron microscopy and immunofluorescence techniques to study the relationship between Fpn and hepcidin. In order to study the interaction between the two proteins, we have established a pRSETB-hepcidin-CFP expression vector. Hepcidin-CFP was cloned into the *E. coli* expression system for the expression of the fusion protein and then its purification. The identified fluorescent fusion protein was stable, and

with antibacterial properties - it could be used in follow-up studies. CaCo-2 or SH-SY5Y cells were transfected with the recombinant plasmid pTARGET-YFP or pTARGET-Fpn-YFP. In laser scanning confocal microscopy cells transfected with pTARGET-YFP emitted yellow-green fluorescence. Fluorescence was evenly distributed throughout the cytoplasm. Cells transfected with pTARGET-Fpn-YFP emitted weak yellow-green fluorescence, with fluorescent membranes surrounding. Fpn-YFP was expressed in CaCo-2 and SH-SY5Y cells. On the addition of hepcidin-CFP fusion protein, FRET occurred in the membrane. By adding hepcidin-CFP fusion protein to the CaCo-2 and SH-SY5Y cells in the culture medium, the Fpn hepcidin-CFP membrane protein internalization was confirmed by fluorescence microscopy. Hepcidin-CFP was scattered in the cytoplasm. The experimental results show that: there is a direct interaction between Fpn and hepcidin. Hepcidin-CFP and Fpn-YFP directly interact in the CaCo-2 or SH-SY5Y cells' membranes. Fpn protein localizes on the inside of the cell membrane, while hepcidin-CFP in the cytoplasm. In conclusion, we believe that through the hepcidin-Fpn interaction Fpn is directed into the cytoplasm. We propose the following mechanism: When hepcidin reaches a certain concentration in the blood, hepcidin and Fpn are close to the membrane. Then through direct contact Fpn-hepcidin complex is delivered into the cytoplasm where hepcidin and Fpn complex disassembles. Our study gives better insight into the mechanism of intestinal iron absorption, which may facilitate the treatment of iron metabolism disorders and provide a theoretical basis for new therapeutic strategies.

P2.54

The influence of new MDP and tuftsin analogs on cytokines levels in experimentally induced sepsis

Anna Wardowska¹, Magdalena Szaryńska¹, Piotr Trzaskowski², Krystyna Dzierzbicka³, Andrzej Myśliwski⁴

¹Department of Histology and Immunology, Medical University of Gdańsk, Gdańsk, Poland

²Laboratory of Experimental Transplantology, Medical University of Gdańsk, Gdańsk, Poland

³Department of Organic Chemistry, Technical University of Gdańsk, Gdańsk, Poland

⁴Department of Histology and Immunology, Medical University of Gdańsk, Gdańsk, Poland

Contact: anna.wardowska@amg.gda.pl

Bacterial infections still remains an important medical problem, notably in immunocompromised individuals. Pharmacological manipulation of the balance between pro- and anti-inflammatory mediators emerges as a key aspect of a successful treatment of severe sepsis. Efficient change of this balance can be achieved with adjuvants that modulate immune response and are capable of the reinforcement of standard treatment. Muramyl dipeptide (MDP) and tuftsin are two naturally occurring molecules with known immunomodulatory activity. MDP is a peptidoglycan present in bacterial wall and tuftsin is a phagocytosis-stimulating tetrapeptide (Thr-Lys-Pro-Arg) present in the blood of mammals. The compounds affect mainly innate immunity and stimulate monocytes and dendritic cells. Numerous derivatives of both immunomodulators have been designed with the aim to obtain more durable and efficient agents. In this study we assumed that targeting synthesis of pro- and anti-inflammatory agents secreted by monocytes might be a crucial point in the regulation of the severity of sepsis. *in vivo* experiments were performed on mouse strain BALB/c. Animals were infected with *Escherichia coli* (ATCC 11775) and simultaneously injected with experimentally chosen dose of conjugates of MDP and tuftsin. Real-time RT-PCR analysis was performed in order to assess transcript levels of pro- (IL6, TNF α , IFN γ) and anti-inflammatory (IL10) cytokines. A murine model of septic shock was developed in our lab to mimic severe sepsis observed in patients and then the chosen analogues were tested in this model for their usefulness as prospective antibacterial drugs or adjuvants. This study showed that three out of nine analogs of MDP and tuftsin, that is conjugates 8a, 8c and the analog T2, revealed the most promising immunomodulatory properties *in vivo*.

P2.55

Spatial identification of the proteins from the tissue of renal cell carcinoma by imaging mass spectrometrySo Dug Lim¹, Wan-seop Kim¹, Sang Hwa Lee¹, Bom Chae Chi², Chan-Hyun Na²¹Department of Pathology, Konkuk University School of Medicine, Seoul, Korea (South)²Institute of Biomedical Science and Technology, Konkuk University, Seoul, Korea (South)Contact: glsodug@yahoo.com

Renal cell carcinoma (RCC) is the most common renal epithelial neoplasm and has histologically diverse subtypes with variable clinical behavior. Gene or protein expression profiling has been a promising technique for refining the diagnosis and staging of RCC as well as for highlighting potential therapeutic targets. Recently, imaging mass spectrometry (IMS) has emerged as a new tool for the study of spatial distribution in the tissue and identification of either protein or small molecules. IMS is matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) based technology. Matrix is applied to cryosectioned-tissue and a very small area of the matrix-applied tissue is analyzed with MALDI-MS. The differences between analyzed areas are displayed by the imaging program. Based on the spatial distribution, region specific proteins can be identified by digesting the tissue with trypsin and analyzing it with MALDI-TOF/TOF. In this study, we analyzed RCC tissue with IMS, and identified some proteins that were increased or decreased in RCC. The degree of expression of protein species or members to each class was used to stain the tissue section. Thus, we successfully show the feasibility of MALDI imaging as a possible tool for the analysis of renal cell carcinomas.

P2.56

Effects of swimming on the iron absorption and utilization in ratsYu-qian Liu¹, Yan-zhong Chang², Hai-tao Wang³, Xiang-lin Duan², Jing-ze Liu²¹Department of Animal Cytobiology, College of Life Science, Hebei Normal University and College of Physical Education, Hebei Normal University, Shijiazhuang, China²Department of Animal Cytobiology, College of Life Science, Hebei Normal University, Shijiazhuang, China³College of Physical Education, Hebei Normal University, Shijiazhuang, ChinaContact: jlzliu0311@163.com

Introduction: Iron plays an essential role in the synthesis of hemoglobin and myoglobin, electron transfer, and serves as a cofactor in many enzyme systems. Iron can affect oxygen transport and the ability of aerobic oxidation and the production of ATP in mitochondria. **Materials & methods:** 20 female SD rats (90~100g) were randomly divided into two groups: control group (remained sedentary, CG), and swimming exercised group (swam 1.5h/day, SG). The rats were sampled after 10-week swimming. The serum iron (SI) and serum ferritin were mensurated using the enzyme linked immunosorbent assay (ELISA). The expression of iron transport proteins such as DMT1 (divalent metal transporter1), FP1 (ferroportin1), and TfR1 (transferrin receptor) were detected by Western Blot. **Results:** 1) The SI, serum ferritin of SG were significantly increased compared with that of CG ($P<0.05$), while the non-heam iron in bone marrow of SG was decreased ($P<0.01$). 2) Western blot analysis showed the expressions of DMT1 and TfR1 of gastrocnemius in SG were higher than that of CG ($P<0.05$). FP1 in SG was decreased compared with that of CG ($P<0.05$). 3) The expression of DMT1 (IRE) and FP1 of duodenum was significantly increased in SG compared with that of CG ($P<0.05$). The redistribution was caused by the changed expression of iron transport proteins. The increased expressions of DMT1 and TfR1 could transport more iron into gastrocnemius, while the decreased expressions of FP1 decreased iron export from gastrocnemius to circulation system in SG. **Conclusions:** The storage iron of gastrocnemius was increased and the mobilization of body iron was improved, redistribution of the stored iron was excited after exercise. The increased expressions of DMT1 and FP1 in duodenum of SG might increase iron absorption.

P2.57

Decreased TLR-5 expression in the mucosal biopsies of ulcerative colitis patientsAleksandra Gołab¹, Piotr Wierzbicki², Krystian Adrych³, Joanna Wypych⁴, Marcin Stanisławowski², Zbigniew Kmiec²¹Dept. of Biochemistry, University of Gdańsk, Gdańsk, Poland²Dept. of Histology, Medical University of Gdańsk, Gdańsk, Poland³Dept. of Gastroenterology and Hepatology, Medical University of Gdańsk, Gdańsk, Poland⁴Dept. of Gastroenterology and Hepatology, Medical University of Gdańsk, Gdańsk, PolandContact: aleksandra.golab@op.pl

Despite intensive research during the last decades, etiology and pathogenesis of inflammatory bowel disease (IBD) is still unknown. IBD encompasses two diseases, i.e. Crohn's disease (CD) and ulcerative colitis (UC). Although there is convincing evidence supporting a critical role for microorganisms in the pathogenesis of IBD, the specific mechanisms involved remain undetermined. Studies of intestinal bacterial populations in UC patients showed increased number of flagellated bacteria. The bacterial flagellum is made up of the protein flagellin. Flagellin is known to be a ligand for the Toll-like receptor 5 (TLR-5), and its ligation induces proinflammatory gene expression in epithelial cells. The family of Toll-like receptors (TLRs) is comprised of at least 11 members that recognize a variety of pathogen-associated molecular patterns, including lipopolysaccharide (LPS by TLR-4) and flagellin. The aim of this study was to characterize expression of the TLR-5 receptors in the intestinal mucosa of UC patients compared with normal controls using real-time PCR and immunohistochemistry (IHC). SYBR Green technique was used to detect the fluorescence signal. Polyclonal goat anti-TLR-5 protein antibody (AbCam, UK) was applied to detect TLR-5 protein, as a secondary antibody the biotinylated anti-goat rabbit antibody (Vector Labs, USA) was used. Mucosal biopsies were obtained from 99 patients of the mean age 47 ± 1.1 y. Control specimen were obtained from 34 irritable bowel syndrome patients (mean age 54.6 ± 2.6 y). UC patients were classified according to the disease activity criteria described by Truelove and Witts. The group I involved 41 patients with low disease activity, group II 50 patients with medium activity of UC, and group III 8 patients with severe UC. The diagnosis was confirmed by standard endoscopic and histological criteria. UC patients of group II and III had significantly lower plasma iron concentration in serum compared with normal controls. Moreover, the Erythrocyte Sedimentation Rate was highly increased proportionally to the disease activity. TLR-5 gene expression was significantly decreased in samples of group II and group III patients as compared with normal controls and group I patients. Using IHC we could observe the presence of TLR-5 receptors in the mucosal biopsies of ulcerative colitis patients and normal controls, mainly in the cytoplasm of enterocytes and at their basolateral domain, and, sporadically, in mononuclear cells of the lamina propria. However, the intensity of IHC reaction in specimens from UC patients of all groups was lower than in control samples. We suggest that the decreased expression of TLR-5 gene and protein in the mucosal biopsies of UC patients could be explained by down-regulation caused by the increased number of ligand molecules present in the intestinal lumen and at the surface of epithelial cells.

P2.58

Promoter hypermethylation and underexpression of *PLAGL1* and *LATS1* tumor suppressor genes in colorectal cancer

Piotr Wierzbicki¹, Joanna Dubowska², Krystian Adrych³,
Dorota Kartanowicz¹, Sebastian Dobrowolski⁴,
Marcin Stanislawowski¹, Janusz Chybicki⁵, Bartłomiej Korybalski⁶,
Janusz Godlewski⁷, Agnieszka Mazurek¹, Marzena Kotwas¹,
Agata Wrońska¹, Zbigniew Kmieć¹

¹Dept. of Histology, Medical University of Gdańsk, Gdansk, Poland

²Dept. of Biochemistry, University of Gdańsk, Gdansk, Poland

³Dept. of Gastroenterology and Hepatology, Medical University of Gdańsk, Gdansk, Poland;

⁴Dept. of General, Gastroenterological and Endocrinological Surgery, Medical University of Gdańsk, Gdansk, Poland

⁵Dept of General Surgery, Hospital of Internal Affairs, Gdansk, Poland

⁶Endoscopy Unit, Hospital of St. Adalbert, Gdansk, Poland

⁷Dept. of Histology and Embryology, Medical Faculty, University of Warmia and Mazury, Olsztyn, Poland

Contact: pwierzb@amg.gda.pl

Colorectal cancer (CRC) is one of the most frequent cancers and a common cause of death. Although many genes are well described in colorectal cancer, the search for new putative genes in molecular pathways of this cancer is still important. *PLAGL1* and *LATS1* are tumor suppressor genes mapped at 6q23-25. *PLAGL1* is responsible for mitotic arrest connected with TP53. *LATS1* is a serin-threonin kinase localized in mitotic apparatus dependant on CDK2/cyclin B. Those genes were found underexpressed in breast, ova-

rian, melanoma and astrocytoma cancers, however the role of *PLAGL1* and *LATS1* in the development of colorectal cancer has not been widely reported. Aim: To investigate the rate of expression of *PLAGL1* and *LATS1* in colorectal cancer (CRC) and benign adenoma at mRNA level. The second aim included hypermethylation analysis of CpG islands, which might be responsible for lower occurrence of *PLAGL1* and *LATS1* mRNA in tumor lesions. Materials and methods: We validated real-time PCR (qPCR) technique for the quantification of *PLAGL1* and *LATS1* mRNA (iQ Cycler, Bio-Rad, USA). A met-PCR analysis was applied to check the methylation status of CpG islands within *PLAGL1* and *LATS1* promoter regions on converted DNA (EZ Methylation Kit, Zymo Res., USA) from tumor and surgical margin tissue obtained from 84 CRC patients. Results: We found *PLAGL1* and *LATS1* genes underexpression in 79/84 and 66/84 cases of tumor CRC ($p < 0.001$), 64/84 and 76/84 of surgical margin tissue ($p < 0.001$), 13/14 and 7/14 of adenomas ($p < 0.05$) as compared to 37 control IBS patients. A negative correlation was found between *PLAGL1* expression and TNM stage ($R^2 = -0.42$) and G staging ($R^2 = -0.53$; $p < 0.05$ Spearman's test) as well as *LATS1*: for TNM stage ($R^2 = -0.41$) and G staging ($R^2 = -0.38$; $p < 0.05$ Spearman's test). Methylation analysis revealed hemimethylation (both hypo- and hypermethylation) status within *PLAGL1* and *LATS1* promoter regions in the majority of tumor and surgical margin CRC biopsies. Conclusion: The first study on the expression status of *PLAGL1* and *LATS1* putative suppressor genes in colorectal cancer shows strong underexpression of those genes in tumor lesion as well as in adjacent surgical margin tissue, which is also correlated with malignancy (TNM) and tumor cells' diversification (G). We suppose that hypermethylation of CpG islands within promoter regions of those genes may be partially responsible for the silencing mechanism of *PLAGL1* and *LATS1* genes.

P2.59

A novel approach to understanding neurodegeneration: an analysis of single, purified inclusions

**Federica Fulceri¹, Paola Lenzi¹, Alessandro Casini²,
Alessandra Cecchetti¹, Antonio Paparelli¹, Francesco Fornai¹**

¹Department of Human Morphology and Applied Biology, University of Pisa, Pisa, Italy

²Department of Experimental Pathology, Medical Biotechnology, Infettivology and Epidemiology, University of Pisa, Pisa, Italy

Contact: fulceri@libero.it

Neuronal inclusions characterize a variety of neurodegenerative diseases leading to movement disorders, such as Parkinson's disease (PD). Despite being well established, these intracellular bodies are poorly investigated due to technical limits to reproduce the inclusions in experimental models and difficulties to dissect this ultrastructure. In previous studies we profited from the high number of inclusions which can be reproduced *in vitro* after methamphetamine (METH) administration, in order to isolate the inclusions in specific cell fractions to study their origin, dynamic structure and molecular composition. In the present study, by using various experimental approaches we isolated pure inclusion bodies. First of all we used the transmission electron microscopy (TEM) to examine the subcellular mitochondrial fraction because it was the richest in neuronal inclusions. We found that these inclusions stain for alpha-synuclein, for proteins of the UP sys-

tem and autophagic system. In fact, these antigens, both alone and/or in combination, characterize the neuronal inclusions. Another in depth purification was obtained by using a dedicated analysis of those inclusions which retained alpha-synuclein. For this purpose we used magnetic beads conjugated with streptavidin. The Abl was first added to the mitochondrial fraction to form a complex with the specific target. After the addition of the biotinylated Abl, this complex was incubated with the beads. In this way a biotin-streptavidin bond is present and the inclusions were attached to the beads. In order to avoid non-specific reaction we further purified these bead bound inclusions by using a magnetic field, which allows to retain in the sample only those structures which were covalently bound to the magnetic beads. In this way following removal of the supernatant we got pure alpha synuclein-containing inclusions. The same procedure was carried out to isolate ubiquitin-containing inclusions. The pure inclusion-containing sample was then processed for *in situ* analysis (immunocytochemistry) and for proteomic analysis. After TEM we found that alpha-synuclein retained inclusions were much higher compared with ubiquitin. When we double stained the alpha-synuclein bound inclusions, we found that they were positive for the proteins which belong to the autophagic system (LC3, beclin, Rab 24) and to the UP system (PA700, proteasome 20S). The proteomic analysis at the level of single inclusions confirmed the presence of proteins belonging to the autophagy. This study represents a novel approach to unraveling the pure composition of neuronal inclusions and allows to disclose those steps which are involved in neurodegeneration.

Quantification in cyto- and histochemistry

P2.60

Locomotor reactivity to novelty correlates with the number of brain cells expressing tyrosine hydroxylase in a response to new environment and after electrical stimulation of the ventral tegmental area

Grażyna Jerzemowska, Karolina Plucińska, Weronika Trojnar

Department of Animal Physiology, University of Gdańsk, Gdańsk, Poland

Contact: grajer@wp.pl

The aim of the study was to test whether increased activity of the telencephalic dopaminergic systems found in rats behaviorally highly reactive to novelty (HRs) as compared to rats of low behavioral reactivity to novelty (LRs) is associated with differences in the number of cells expressing tyrosine hydroxylase (TH) in the main group of the brain dopaminergic neurons in conditions of exposure to a new environment and after mesolimbic system activation. Two groups of male Wistar rats were used: subjected to a new environment (moving from vivarium to the experimental room) (n=11) and subjected to a 14-day unilateral electrical stimulation of the ventral tegmental area (VTA) (n=9), which produces behavioral signs of psychomotor activation. After termination of the stimulation procedure all rats HRs and LR (previously designated to behavioral groups on the basis of locomotor reactivity to novelty) were subjected to immunohistochemical staining of neurons expressing TH. The number of TH+ cells was counted in the A8-A15 groups and then compared in the HR and LR rats in both experimental groups. It was found that the HRs of the novelty-exposure group had a significantly higher total number of TH+ cells in the A8-A15 groups taken as a whole. There was a strong positive correlation between the number of TH+ neurons and the locomotor activity score in 0.001). This did not concern the novelty test ($r=0.81$, $p=0.43$). In addition HR/LR differences# VTA-stimulated group ($r=0.26$, p were regionally specific: the HRs had more numerous TH+ cells in the hypothalamic arcuate nucleus and in the anterior parts of the ventral tegmental area and the substantia nigra, and the LR had a higher number of TH+ cells in the parabrachial pigmented nucleus and the posterior part of the substantia nigra, especially in the novelty-exposure group. After long-lasting unilateral electrical stimulation of the VTA this regional specifically became disordered, particularly in the A9 dopaminergic cells group. The results obtained suggest that individual behavioral and neurochemical differences which are correlated with increased susceptibility to stress and propensity to develop drug addictions, which characterize HR animals, may be determined by alterations in the morphology and activity of the dopaminergic systems.

P2.61

Quantitative colocalization analysis of RNA binding protein TLS and its target mRNA in mouse hippocampal neurons

Ritsuko Fujii¹, Olga Grossenbacher-Zinchuk², Yu Wang¹, Vadim Zinchuk³, Toru Takumi⁴

¹Waseda-Olympus Bioscience Research Institute, Biopolis, Singapore

²Institute of Anatomy, University of Berne, Berne, Switzerland

³Kochi university Faculty of Medicine, Nankoku, Japan

⁴Osaka Bioscience Institute, Suita, Japan

Contact: fujii@waseda.jp

Multi-functional RNA-binding protein TLS associates with ribosomal RNA (rRNA) and also various target mRNAs including b-actin mRNA and Nd1-L mRNA which encodes an actin-stabilizing protein. TLS transports these RNAs as a component of large mRNA-protein complex granule (mRNP) in the neuronal dendrites. In TLS-null mouse (TLS-KO) hippocampal neurons, the amount of Nd1-L mRNA in the dendrites is significantly lower than that in the wild type (WT) neurons while the amount of rRNA or b-actin mRNAs is not affected. In addition, TLS-KO neurons display abnormal spine morphology which is characterized by the reduced spine number and filopodia-like shapes as well as abnormal dendrite branching. However it has remained unclear whether the decreased amount of Nd1-L mRNA in the TLS-KO dendrites is simply attributed to the loss of RNA-transporting function of TLS and eventually leads to the abnormal

spine phenotypes. To address this question, we exogenously expressed TLS-GFP in TLS-KO hippocampal neurons and quantitatively examined the colocalization of TLS-GFP and Nd1-L mRNA in the neuronal dendrites by FISH analyses. We found that the colocalization of TLS-GFP and Nd1-L mRNA was significantly lower in both the dendrites and the spines of TLS-KO neurons than that observed in WT neurons. Consistent with these results, exogenous expression of TLS-GFP in TLS-KO neurons did not rescue the abnormal spine phenotypes. Our results suggest that critical factors for the mechanism of the dendritic mRNA transport may be impaired when TLS is not expressed throughout the neuronal development.

P2.62

Quantification of Epstein-Barr virus-infected lymphocytes by flow cytometry coupled with *in situ* hybridization

Kanae Miyake¹, Kana Nishiyama¹, Yoshinori Ito², Yohei Yamauchi¹, Yukihiro Nishiyama¹, Hiroshi Kimura¹

¹Department of Virology, Nagoya University Graduate School of Medicine, Nagoya, Japan

²Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan

Contact: kanac31415@yahoo.co.jp

Epstein-Barr virus (EBV) infects various kinds of lymphocytes and causes lymphoma or lymphoproliferative diseases. In order to diagnose EBV-related diseases, quantification and identification of EBV-infected cells are necessary. Although *in situ* hybridization is widely used to detect EBV-infected cells in tissue specimens, it is difficult to detect them in floating cells, like blood lymphocytes. We established a novel assay system to quantify and simultaneously identify EBV-infected floating cells using flow cytometry coupled with *in situ* hybridization. EBV-encoded small RNA1 (EBER1) is highly expressed in EBV-infected cells. FITC-conjugated peptide nucleic acid (PNA) probe, which specifically hybridizes EBER1, was used for *in situ* hybridization. To amplify the fluorescence signal, Alexa Fluor 488-conjugated antibodies to PNA probe were used. At the same time with the detection of EBER1, lymphocytic surface markers were stained to identify and characterize the lymphocytes. PE- or PC5-conjugated antibodies were used to label surface markers. Then, cells were analyzed by flow cytometry. The complete procedure was performed within 6-7 hours. By the flow cytometry coupled with *in situ* hybridization, EBER1 was detected in various kinds of EBV-positive cell lines including B, T, and natural killer cell lines. Each cell line was identified as B, T or natural killer cell by multiple staining with surface markers. EBER1 was not detected in EBV-negative cell lines. This assay could detect as minimum as 0.01% of EBV-positive cells among EBV-negative cells. Staining and localization of EBER1 and each surface marker were confirmed by laser scanning confocal microscopy. Finally, we quantified EBV-infected cell population in peripheral blood from a patient with EBV-related lymphoproliferative disease. Approximately, 5% of lymphocytes were positive for EBER1 and these infected cells were identified as T cells. These results indicate that this novel assay is rapid, sensitive and specific, and could be useful not only for the diagnosis but also for clarifying the pathogenesis of EBV-related diseases.

P2.63

Effects of image compression on area and roundness parameters in digital image analysis

Carlos Lopez, Marylene Lejeune, Patricia Escriva, Ramon Bosch, Salvado Teresa, Lluís Pons, Alvaro Tomas, Joaquin Jaen

Department of Pathology, Hospital de Tortosa Verge de la Cinta, Tortosa, Spain

Contact: clopezp.htvc.ics@gencat.cat

Introduction: Image compression and digital image analysis has been used in several fields of medicine. Two of the most used image formats are TIFF (uncompressed) and JPEG (compressed). In previous studies, we have observed that image compression could affect negatively the quantification of immunohistochemical stained nuclei. Automated nuclei counts on TIFF images differ from those made on the same images compressed in different JPEG formats. This variability increases with the level of compression and

was particularly elevated in images with a high number of nuclei and with high area clusters. This study evaluates the variations in the area and roundness of positive stained nuclei due to image compression and assesses their effect on automatic nuclei quantification. In addition, a mathematical algorithm is implemented in the macros to decrease these differences between TIFF and JPEG. Material and methods: The present study was performed with 47 digital images of Ki67 and FOXP3 immunohistochemical markers captured and saved in the uncompressed TIFF format. Each one of these images was converted to JPEG files with 3x, 23x and 46x compression using the ACDSee 9.0 program. Sixty-five positive objects with different shape and size were selected from TIFF images, and their area and roundness were compared with the same objects in the different compression levels. All images and positive objects were analyzed with macros previously elaborated, tested and validated with Image-Pro® Plus 5.0 program and Excel® datasheet complemented or not with a novel linear regression model. All statistical analyses were carried out using SPSS 11.0. Results: Boxplot graphics of 65 objects analyzed in TIFF and JPEG formats show practically similar area distribution whereas the roundness values decrease as the level of compression increases. Roundness variations could be corrected with a linear regression for each different level of compression calculated from these objects. When the linear regression is applied to all the positive objects analyzed, the original nuclei count differences between TIFF and the compressed images, were considerably reduced (Kaplan-Meier curves). In these conditions, the automatic nuclei counts of compressed images are more similar to the TIFF format, mainly in images with more than 100 nuclei or high area clusters. Discussion: This work shows how digital image compression could produce variations in distinct parameters (area and roundness) used to determine the positive object during an automated process of image analysis. Our new mathematical algorithms correct roundness variability measured in JPEG images, improving the accuracy of the macros developed to the automatic quantification of immunohistochemical nuclear markers. These results indicate that JPEG digital images could be an useful format for compact storage and quantitative immunohistochemical digital analysis.

P2.64

Proliferation index and percentage of positive nuclear area in follicular lymphoma evaluated with digital image analysis

Carlos Lopez¹, Ramon Bosch¹, Marylene Lejeune¹, Patricia Escriva¹, Marta Rodriguez², Maria Teresa Salvado¹, Barbara Tomas¹, Lluís E. Pons¹, Tomas Alvaro¹, Joaquin Jaen¹

¹Department of Pathology, Hospital de Tortosa Verge de la Cinta, Tortosa, Spain

²Department of Haematology, Hospital de Tortosa Verge de la Cinta, Tortosa, Spain

Contact: clopezp.htvc.ics@gencat.cat

Introduction: Histological grade is used in follicular lymphoma (FL) to choose the appropriate treatment and to predict clinical behavior in patients. Otherwise, several studies have indicated that proliferative activity is an important prognostic indicator in FL [1]. This activity was evaluated with the proliferation index (PI; % positive cells) and/or the percentage of positive nuclear area (PNA; % positive area) in Ki67 (MIB-1) immunohistochemically stained tissues. Using digital image analysis, the quantifications of these stained tissues are more reproducible, objective and faster. This study evaluates PI and PNA with digital image analysis techniques and correlates them with the histological grade of the FLs. Material and methods: Ki67 expression was assessed immunohistochemically on TMA sections from 66 samples of FL, as previously described [2]. Histological grades of all samples were reviewed twice at 40X according to the WHO criteria. Two digital images of intrafollicular areas were captured for each sample. Positive and negative cells and areas were quantified with previously elaborated, tested and validated macros with Image-Pro® Plus 5.0 program and Excel® datasheet [3]. Quantification of these macros allows calculating the PI and PNA. A comparison of the PI and PNA with the histological grade was performed with the statistical package SPSS 11.0. Results: 24 samples were classified as grade I, 25 as II and 17 as III. The Boxplot graphs showed an important dispersion of the values and revealed the existence of some low-grade patients with a high PI. Nevertheless, PI and PNA mean values appear to increase in parallel with the histological grade of the FL. The PI mean values were 12.6, 18.2 and 20.7 for grade I, II and III respectively. The PNA values were 13.1, 19.9 and 24.3 for grade I, II and III respectively. T-Student's test showed only significant differences for PI and PNA values between grade I and III ($p < 0.05$). Conclusion: FL patients with histological grade I and III present a significant dif-

ferent proliferative activity. These results, obtained with an automated process of digital image analysis, are consistent with previous studies. This method may be considered a valid alternative to manual microscopic evaluation of PI and PNA in FL patients for diagnostic, prognostic and therapeutic purposes. References: 1. Koster A, Tromp HA, Raemaekers JM, et al. The prognostic significance of the intra-follicular tumor cell proliferative rate in follicular lymphoma. *Haematologica* 2007;92:184-190. 2. Alvaro T, Lejeune M, Salvado MT et al. Immunohistochemical patterns of reactive microenvironment are associated with clinicobiologic behavior in follicular lymphoma patients. *J Clin Oncol* 2006;24:5350-5357. 3. López C, Lejeune M, Salvado MT, et al. Automated quantification of nuclear immunohistochemical markers with different complexity. *Histochem Cell Biol*. 2008 Jan 3.

P2.65

Diagnostic significance of the distribution of CD34(+) stromal cells and ASMA(+) stromal cells in the stroma of chronic pancreatitis and pancreatic cancer

Adam Nalecz, Kazimierz Jaskiewicz, Robert Rzepko

Department of Pathology, Medical University of Gdańsk, Gdańsk, Poland

Contact: adamnalecz@poczta.onet.pl

The formation of the stroma is crucial for the growth of the neoplastic tumor and in the chronic inflammatory process (CP). The pancreas has enormous ability to formation of stroma in the course of both aforementioned processes giving the unique opportunity for comparative studies. Pancreatic adenocarcinoma (PDA) is the neoplasm with a dismal prognosis, usually resistant to classic therapeutic strategies. The understanding of structural and functional differences between neoplastic and inflammatory stroma, may one day lead to discoveries of new diagnostic and therapeutic procedures of these fatal diseases. The purpose of this study was to perform a comparative morphologic analysis of the stroma in CP and PDA, considering the distribution of CD34+ and ASMA+ stromal cells. Furthermore, the diagnostic significance of above mentioned differences was assessed from the histopathologic point of view. 62 cases of PDA (38 male and 24 female) and 43 cases of CP (32 male and 11 female) were qualified. All patients underwent surgical procedures in the General Surgery Departments, Medical University of Gdańsk in the years 1998-2005. Additionally 20 samples of the pancreatic parenchyma taken during autopsies from the patient without pancreatic illness, formed the control group. Immunohistochemical staining were performed on the material from archives of the Department of Pathology, Medical University of Gdańsk. The following monoclonal antibodies were used in the study: anti-CD34 and anti-ASMA to stain stromal cells, anti-CD31 to highlight the microcirculation. There were clear differences in the distribution of CD34+ stromal cells and ASMA+ stromal cells in the CP and PDA groups: immunofenotype of 51 cases of PDA (80%) was: CD34(-), ASMA (3+ or 4+). On the other hand, the stromal cells in 41 cases of CP (95%) were: CD34(3+ or 4+) and ASMA (3+ or 4+). The revealed differences in CD34 and ASMA expression can serve as the additional tool in the histopathologic differential diagnosis between CP and PDA.

P2.66

Blood lymphocyte subpopulation after behavioral stress conditions in rats

Danuta Wrona

Department of Animal Physiology University of Gdańsk, Gdańsk, Poland

Contact: wronada@biotech.univ.gda.pl

It is known that individual sensitivity to stress conditions differently affects immune function. The present study investigated the influence of different behavioral stress conditions: handling, novelty, and social interaction stress on blood lymphocyte subpopulations in rats. Male Wistar rats ($n=20$) were divided into behaviorally different groups: high- and low- responders (HR and LR; novelty test), and dominant and submissive animals (D and S, chronic social interaction test). Blood samples were collected by heart puncture on the 1st (SI"1") or 2nd (SI"2") day following social interaction test (SI). Peripheral blood lymphocyte subsets were determined by flow cytometry using antibodies CD3-FITC/CD45RA-PC7/CD161A-APC and CD3-FITC/CD4-PC7/CD8-APC for determination of T/B/NK and T cell CD3⁺CD4⁺ and CD3⁺CD8⁺ subsets, respectively. In whole group of animals

(not divided into behavioral groups), social stress significantly suppressed ($p < 0.05$) TCD3⁺ lymphocyte ($55.13 \pm 4.13\%$ and $60.94 \pm 4.75\%$ following SI"1" and SI"2", respectively) and NK cells number (SI"1" = $3.05 \pm 0.72\%$, SI"2" = $3.63 \pm 0.98\%$). There was no significant change in B lymphocyte number following social interaction test ($25.11 \pm 2.75\%$ and $25.06 \pm 2.11\%$ for SI"1" and SI"2", respectively). In addition, stress conditions differently influenced lymphocyte subpopulations in behaviorally different animals (HR vs LR or D vs S). Significantly higher ($p < 0.05$) NK cell level was observed in D as compared to S animals ($4.11 \pm 0.68\%$ vs $2.84 \pm 0.47\%$) after SI"1". Besides, significant ($p < 0.05$) differences in the number of TCD4⁺ lymphocytes appeared between D vs S animals following SI"1" and SI"2" (SI"1"; D = $72.96 \pm 7.53\%$; S = $67.69 \pm 8.12\%$ and SI"2"; D = $66.03 \pm 5.38\%$; S = $72.09 \pm 6.23\%$) as well as between HR and LR rats after SI"2" (HR = $72.67 \pm 6.93\%$, LR = $65.75 \pm 8.13\%$; $p < 0.05$). Moreover, the lymphocyte and granulocyte number was significantly lower ($p < 0.01$) in submissive or low-reactivity rats as compared to dominant or high-reactivity animals following both novelty and social interaction tests. On the contrary, no significant differences in the number of peripheral blood TCD8⁺ and B lymphocytes between behavioral groups were observed. These results indicate that such natural stressors as novelty or social interaction suppress TCD3⁺ and NK lymphocytes which are responsible for innate and specific acquired cellular immunity. Moreover, it suggests that behaviorally and/or genetically determined individual differences in the reaction to novelty and change in social position, referred to as passive or active strategy, could be important for stress-induced immune response in rats.

P2.67

Substance P in the lymphoid intestinal tissue and intestinal lymph nodes of the growing and dysenteric pigs – quantitative studies

Waldemar Sienkiewicz, Jerzy Kaleczyc, Krzysztof Wąsowicz, Piotr Podlasz, Mirosław Łakomy

Division of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Contact: waldemar.sienkiewicz@uwm.edu.pl

Substance P (SP) is a well known neuropeptide involved in regulating lymphatic tissue function and also directly participates in the maturation of different kinds of immune cells. It also takes part in the regulation of the lymphatic tissue function in the course of inflammation. Until now studies dealing with this problem were performed mainly on laboratory animals. Considering the important role played by SP in the development and function of the lymphatic tissue, we decided to study changes in the concentration of SP in the lymphoid intestinal tissue and intestinal lymph nodes in growing and dysenteric pigs. Five groups of pigs of different age: 3 days (G1), 2 weeks (G2), 4 weeks (G3) and 4 months (G4) and 4 month old pigs infected per os with *Brachyspira hyodysenteriae* (G5) were used. Each group consisted of 5 animals. The pigs of G5 were infected per os with *Brachyspira hyodysenteriae*. Several days later, symptoms of the disease were observed in the infected animals. Few days after the first symptoms were found, at peak of their intensity, the dysenteric pigs were sacrificed. All pigs were deeply anaesthetised. Thirty minutes before the main anaesthetic,

pentobarbital (25 mg/kg b.w.) was given intravenously, all the animals were pre-treated with propionylpromazine (0,4 mg/kg b.w., i.m.). Samples of the lymphoid tissue from the ileum (Peyer's patches [PP]) and lymph nodes (LN) associated with ileum were collected and frozen. Then, the samples were homogenised and homogenates were centrifuged for 15 min. at 10000g, the clear supernatant was collected. Tissue concentrations of SP were determined by ELISA tests (Peninsula Lab., USA). Absorbance was measured with immunoplate reader Dynex MRX. Results were calculated per 1 g of fresh tissue. The data obtained were statistically analysed. The differences were considered statistically significant at $P < 0.05$. The concentration of the peptide in the LN studied increased with the animals' age and reached the highest level in pigs of G3, whereas in animals of G4 it decreased down to half of the level observed in G3. In animals suffering from dysentery, an increase in SP concentration was found. The concentration of the peptide in PP decreased with the age and reached the lowest level in animals of G4. In the dysenteric pigs SP concentration increased up to level twice as high as that found in the corresponding age group of healthy animals. Changes in SP tissue concentrations found during maturation and in the course of dysentery allow to conclude that SP is involved in the regulation of the lymphoid tissue function also in the pig.

P2.68

Human sperm chromatin evaluated by flow cytometry

Grace Chojnowski¹, Oswaldien Claassens², Bernhard Wehr², Keith Harrison², John Irving²

¹Queensland Institute of Medical Research, Brisbane, Australia

²Queensland Fertility Group, Brisbane, Australia

Contact: grace.chojnowski@qimr.edu.au

The sperm DNA-nucleoprotein complex (sperm chromatin) influences the quality and fertilisation potential of sperm. Sperm quality can be characterized using standard methods of motility and morphology but these do not always show subtle defects in sperm. The *Sperm Chromatin Structure Assay* (SCSA) is used routinely in many fertility laboratories as a diagnostic tool to determine sperm quality by using Acridine Orange (AO) staining of sperm. The aim of this study was to characterise chromatin structure of human sperm by flow cytometry after staining with three chromatin stains, Acridine Orange (AO), 4,6-Diamidino-2-phenylindole (DAPI) and Chromomycin A3 (CMA3). Twenty human semen samples were investigated by flow cytometry after AO, DAPI and CMA3 staining. Six samples were from donors of proven fertility and fourteen from patients attending the IVF unit. The percentage of sperm with fragmented DNA was expressed as the DNA Fragmentation index (%DFI). The DFI values were compared to three distinct regions obtained from the histograms after DAPI and CMA3 staining respectively. In a separate study, 64 semen samples were analysed by flow cytometry using AO and CMA3 respectively in order to establish the immature sperm populations for each stain. Flow cytometric evaluation of DAPI and CMA3 stained sperm gave DNA fragmentation results similar to AO. The immature sperm population determined by AO also correlated with CMA3. Sperm flow cytometry provides additional dimension to semen analysis not easily gained by other methods and is an objective, rapidly performed and interpreted method.

Lectures



Poster session 1



Poster session 2



Poster session 3



Poster session 4



P3.1

Simultaneous expression of Oct4 and genes of three germ layers in single cell-derived multipotent adult progenitor cells

Ji Kaihong, Liu Houqi

Second Military Medical University, Shanghai, China

Contact: jkhsmmu@yahoo.com.cn

Future application of adult stem cells in clinical therapies largely depends on the successful isolation of homogeneous stem cells with high plasticity. Multipotent adult progenitor cells (MAPCs) are thought to be a more primitive stem cell population capable of extensive *in vitro* proliferation with no senescence or loss of differentiation capability. The present study was aimed to find a less complicated and more economical protocol for obtaining single cell-derived MAPCs and understand the molecule mechanism of multi-lineage differentiation of MAPCs. We successfully obtained a comparatively homogeneous population of MAPCs and confirmed that single cell-derived MAPCs were able to transcribe Oct4 and genes of three germ layers simultaneously, and differentiate into multiple lineages. Our observations suggest that single cell-derived MAPCs under appropriate circumstances could maintain not only the characteristics of stem cells but multi-lineage differentiation potential through quantitative modulation of corresponding regulating genes expression, rather than switching on the expression of specific genes.

P3.2

Distribution of cardiac primitive cells with epithelial and mesenchymal markers expression: identification of a population of epicardially derived cells in the adult human heart

Clotilde Castaldo¹, Franca Di Meglio¹, Veronica Romano¹, Daria Nurzynska¹, Elena Piegari², Antonella De Angelis², Rita Miraglia¹, Ciro Bancone³, Gianpaolo Romano³, Stefania Montagnani¹

¹Università degli Studi di Napoli "Federico II", Naples, Italy

²Seconda Università di Napoli, Naples, Italy

³Monaldi Hospital, Naples, Italy

Contact: clotilde.castaldo@unina.it

Recently, triptase-negative, CD117(+) cells able to give rise to the cells of cardiac lineages have been observed in the adult human heart, raising questions concerning their origin and biology. Given the fact that during heart morphogenesis a subset of epicardial cells undergoes an epithelial-mesenchymal transition (EMT) generating a population of epicardially derived cells (EPDCs) that invades myocardium and contributes to the formation of myofibroblasts, vascular endothelium and smooth muscle cells, the scope of the present study was to investigate whether the EMT takes place also in the adult human heart, generating the population of cardiac resident stem cells. Fragments of adult human normal (n=11) and pathological hearts with ischemic cardiomyopathy (n=20) were analyzed by immunohistochemistry. In all regions of the pathological hearts, CD117(+) cells were significantly more numerous than in the normal hearts. These cells were localized mainly in the atria and their number was up to 38-fold higher (p<0.0001) in the epicardium/subepicardium than in the myocardium. Importantly, CD117(+) cells were identified among mesothelial cells of epicardium in the normal hearts. The presence of epicardium was confirmed by the detection of epithelial markers, E-cadherin, cytokeratin 5/6 and BVES. In the pathological conditions, however, the epithelial lining disappeared from the surface of the myocardium and, strikingly, cells with the expression of epithelial markers were distributed among loose connective tissue of subepicardium. Significant fraction of these cells expressed also CD117, vimentin, N-cadherin, Tie-2, POD-1. Real time PCR analysis of the epicardium/subepicardium layer from adult human normal and pathological hearts with ischemic cardiomyopathy confirmed the higher expression of mRNA for epithelial markers in the normal hearts and the prevalent presence of mRNA for mesenchymal markers in the cells in the

pathological subepicardium. In the latter, the synthesis of transcription repressors involved in the E-cadherin downregulation and EMT, Snail and Slug, was also detected. Our findings suggest that EMT of the epicardial cells can give rise to a population of EPDCs in the adult human heart. This process is remarkably augmented in the pathological conditions, as indicated by a nearly complete loss of mesothelial heart covering and increase in the number of CD117(+) cells with mesenchymal characteristics in the subepicardium of the hearts with ischemic cardiomyopathy. It can be concluded that the generation of EPDCs reaches its limits in the chronic pathological conditions and the regeneration of cardiac tissue is accomplished by CD117(+) cardiac primitive cells invading the myocardium. Hence, the studies of cardiac primitive cells are warranted to enhance their migration, survival and differentiation into cells of cardiac lineages in chronic pathological conditions.

P3.3

Role of laminin-1 and laminin-2 signalling mediated by $\alpha 6$ integrin in the CD117(+) cardiac primitive cells' proliferation, survival and migration

Daria Nurzynska¹, Franca Di Meglio¹, Clotilde Castaldo¹, Rita Miraglia¹, Veronica Romano¹, Ciro Bancone², Cristiano Amarelli², Valentina Mele¹, Stefania Montagnani¹

¹Università degli Studi di Napoli "Federico II", Dipartimento di Scienze Biomorfologiche e Funzionali, Naples, Italy

²Monaldi Hospital, Naples, Italy

Contact: darianurzynska@yahoo.com

It has been recently suggested that organ regeneration requires molecular reprogramming and reactivation of mechanisms operative during organogenesis. Laminin-1 ($\alpha 1\beta 1\gamma 1$) is the first extracellular matrix protein expressed during development and heart organogenesis does not proceed in its absence. The lack of laminin-2 ($\alpha 1\beta 1\gamma 1$) causes congenital muscular dystrophy involving heart. In view of the presence of cardiac stem cells in the adult heart, the scope of the study was to compare the expression of laminins in the adult human normal and pathological hearts with ischemic cardiomyopathy. Moreover, the effects of laminins on CD117(+) cardiac cells and the role of $\alpha 6$ integrin-mediated signaling were investigated *in vitro*. As revealed by immunohistochemistry, in the normal hearts (n=11) laminin-1 was restricted to epicardium/subepicardium, while $\alpha 2$ laminin bordered cardiomyocytes. In the pathological hearts (n=20), laminin-1 formed a mesh within subepicardium and filled interstitium with a granular-like pattern. Merosins lined epicardium and basement membrane of cells in myocardium. The expression of laminin-1, analyzed by immunoprecipitation and immunoblotting, in the normal atria was 4-fold higher (p<0.001) than in the normal left ventricle (LV), while in the pathological conditions it was LV that contained more laminin-1. Laminin-2 expression did not differ between the atria from normal and pathological hearts, whereas in the LV $\alpha 2\beta 1$ chains were up to 2.5-fold more abundant (p<0.001) in the pathological hearts. Specific human $\alpha 6$ integrin small interfering RNA (siRNA) was used in order to examine the role of $\alpha 6$ integrin and laminin interaction in cell biology *in vitro*. In comparison with laminin-2, the proliferation rate of CD117(+) cells on laminin-1 was 4-fold higher (p<0.001). Apoptosis in the presence of laminin-1 was 2-fold and 6-fold lower (p<0.001) than in the presence of laminin-2 and control, respectively. This effect of laminin was inhibited by $\alpha 6$ integrin siRNA, implicating $\alpha 6$ integrin and laminin interaction in the CD117(+) cells survival *in vitro*. Moreover, the speed of migration and the number of cells migrating across laminin-1 and -2 were significantly higher with respect to control. In all cases, migration on laminin-1 outweighed that on laminin-2. Transfection with $\alpha 6$ integrin siRNA reduced migration rates significantly. We conclude that chronic pathological conditions activate a regenerative response involving all, cardiac stem cells, extracellular matrix and its receptors. Laminin-1 expression in the adult human heart reconstitutes the microenvironment typical of developing heart, while $\alpha 6$ integrin on cardiac primitive cells protects them from apoptosis and stimulates their migration. All these factors should be taken into consideration when planning stem cells' transplantation or their *in situ* activation.

P3.4

Extracellular matrix composition determines proliferation and survival of cardiac primitive CD117-positive cells

Franca Di Meglio¹, Rita Miraglia¹, Daria Nurzynska¹, Clotilde Castaldo¹, Veronica Romano¹, Ciro Bancone², Gianpaolo Romano², Stefania Montagnani¹

¹Università degli Studi di Napoli "Federico II", Naples, Italy

²Monaldi Hospital, Naples, Italy

Contact: francadimeglio@hotmail.com

Stem cells and progenitor cells with the capacity to differentiate into three major cardiac cell types – cardiomyocytes, smooth muscle and endothelial cells – have been recently described in adult heart, together with numerous factors that influence their characteristics in normal and pathological conditions. It is known that the expression of extracellular matrix (ECM) proteins and their receptors changes in both qualitative and quantitative manner during adaptive and pathological heart remodeling. However, the contribution of these changes to cellular turnover and cardiac regeneration and, in particular, their influence on cardiac stem and early-committed primitive cells await investigation. Immunohistochemical analysis of sections of adult normal (n=11) and pathological hearts with ischemic cardiomyopathy (n=20) revealed different staining patterns of ECM proteins. Hence, the scope of the study was to evaluate the influence of fibronectin, laminin-1, laminin-2 and collagen IV on cardiac primitive CD117(+) cells isolated from human heart. Moreover, cardiac fibroblasts from human adult normal and pathological hearts with ischemic cardiomyopathy were isolated and cultured to obtain the natural ECM (cardiogel) *in vitro*. After non-enzymatic removal of fibroblasts, this substrate was used to compare proliferation and apoptosis of CD117(+) cells in the presence of ECM typical of normal and pathological heart. Proliferation of CD117(+) cells, evaluated by BrdU incorporation, reached maximum of 27,7±4,7% (n=4) in the presence of laminin-1 and increased 6,3-fold with respect to control albumin, while it was minimal and only 2-fold higher with respect to control, in the presence of laminin-2 (p<0,05). Apoptosis, detected in TUNEL assay, was as low as 1,8±0,4% (n=4) on laminin-1 and increased 2,8-fold, 5,4-fold and 8,5-fold during culture of cells on laminin-2, fibronectin and collagen IV (n=4, p<0,05), respectively. Components of cardiogel were characterized by immunofluorescence. Cardiogel from normal hearts consisted mainly of laminin-2, while that from hearts with ischemic cardiomyopathy contained more laminin-1, fibronectin, collagen IV and tenascin C. Interestingly, when normal CD117(+) cells were cultured on cardiogel from pathological heart, their proliferation rate was 5,7-fold higher (n=5, p<0,05) with respect to the proliferation of cardiac primitive cells on the cardiogel from normal heart. In a similar manner, although to a lesser extent, components of ECM from pathological heart diminished apoptosis rate with respect to cardiogel from normal heart. We conclude that the components of ECM, responding to the physiological changes during heart remodeling, have profound effects on cardiac primitive cells. These results should be taken into consideration when planning stem cell transplantation in diseased hearts, since the microenvironment may determine the fate of the injected cells.

P3.5

Expression of stem cell markers in human cutaneous melanoma

Franca Piras¹, Daniela Murtas¹, Luigi Minerba², Carlo Floris³, Jorge Ugalde⁴, Cristina Maxia¹, Paolo Demurtas¹, Maria Teresa Perra¹, Paola Sirigu⁴

¹Department of Cytomorphology

²Department of Public Health, University of Cagliari, Monserrato, Cagliari, Italy

³Oncologic Hospital "Businco", Cagliari, Italy

⁴Department of Pathology, Cancer Institute, Solca, Ecuador; Department of Cytomorphology, University of Cagliari, Monserrato, Cagliari, Italy

Contact: fpiras@unica.it

The potential role of stem cells in neoplasia is a subject of recent interest. The cancer stem cell hypothesis suggests that malignant tumors are comprised of both cancer stem cells, which have great proliferative potential, as well as more differentiated cancer cells, with limited proliferative potential. The involvement of cells displaying a stem cell phenotype in cancer formation and progression has been confirmed in acute myeloid leukaemia and breast cancer. More recently, a number of papers strongly support the idea that CSCs could be the basis for the origin of human brain tumours and

prostate cancer. Melanomagenesis and progression are commonly described as 'de-differentiation' processes of transformed, mature melanocytes, enabling the stepwise metamorphosis from nevus to RGP to VGP melanoma and, ultimately, to disseminated. Strikingly, the majority of melanomas emerge in normal appearing skin or unexpected sites along the neural crest migratory route, not in dysplastic nevi. Based on these, an alternative hypothesis has been put forth in light of the cancer stem cell (CSC) concept, suggesting mutated melanocyte stem cells or immature progenitor cells present in skin as precursors to melanoma. Consistent with this hypothesis is the recent observation that well established metastatic melanoma cell lines have been shown to exhibit multiple morphological, phenotypic, functional forms and retain residual characteristics of a stem cell population. Markers of melanocytic stem cells have been described recently. Among these, nestin is an intermediate filament expressed in the cytoplasm of neuroepithelial stem cells and its expression has also been found in metastatic melanomas; CD133 (human prominin-1/AC133) is a transmembrane glycoprotein that is expressed on the hematopoietic stem cells, endothelial progenitors, and dermal-derived stem cells capable of differentiating into neural cells. In this study, we evaluated the immunohistochemical expression of these stem cell markers in 170 cases of human cutaneous melanoma, including primary tumours and metastases, and their value as potential prognostic markers. In addition, we examined the association between nestin or CD133 staining and clinico-pathological variables. The results will be discussed.

P3.6

Notch signaling pathway involvement during *in vitro* differentiation of liver mesenchymal stem cells to osteoblasts

Aleksandra Bryzek¹, Ksymena Urbanek², Halina Koryciak-Komarska², Tomasz M. Czekaj², Katarzyna Bogus¹, Piotr Czekaj¹, Virginia Likus³, Aleksander L. Sieroń²

¹Department of Histology, Medical University of Silesia, Katowice, Poland

²Department of General and Molecular Biology and Genetics,

Medical University of Silesia, CoE Research and Teaching of Molecular Biology of Matrix and Nanotechnology, Network of CoE BioMedTech Silesia, Katowice, Poland

³Department of Biochemistry, Medical University of Silesia, Katowice, Poland

Contact: pcz@sum.edu.pl

It has been shown that signals generated by activation of Notch receptors play important role in both proliferation and differentiation of cells at different stages of life. Here we have investigated dynamic changes in appearance and distribution of Notch 1, 2 and 3 receptors, and Delta1 ligand in cytoplasm and nuclei of rat liver mesenchymal stem cells differentiating into osteoblasts. The cells were cultured in slide chambers for Notch signaling studies or in regular six-well plates for osteogenesis and cell number assays. Notch receptors and Delta1 ligand were identified using specific antibodies detecting domains undergoing translocation, upon activation, from cell membrane to the nucleus. The osteogenesis was determined using AlizarinS – to assay mineralization, and AlamarBlue – to assay cell number. We showed that already at 24th hour of differentiation the number of cells expressing Notch1, 2 and Delta1 as well as the number of cells with activation of these factors increased significantly in comparison to cells prior to differentiation. The number of cells expressing Notch3 remained unchanged, however, the number of cells which activated this receptor increased 7 fold. After next 24 hours the number of cells expressing Notch1 and 2 and Delta1 returned to control levels and the number of cells expressing Notch3 significantly increased, however, only small fraction of these cells did show presence of activating receptor domain in the nuclei. On day fourth, when first signs of mineralization could be detected, all analyzed receptors were in cells at control levels, however, the number of cells expressing Delta1 returned to the level observed on day one. Summing up, the three analyzed receptors and one ligand seemed to play the role at an early stage of osteogenesis, since a number of cells with activated variants were elevated 3 to 4 times. Later, during differentiation, numbers of cells analyzed for all the factors decreased below the control levels, but numbers of cells positive for Notch3 were the highest. Return to elevated numbers of cells expressing all analyzed receptors and the ligand was observed on day 8th, right before 4 fold increase in mineralization. However, on day 10th only the number of cells expressing Notch3 remained relatively high. It was concluded that Notch3 might have a role in the mineralization of the culture under osteogenic conditions.

P3.7

The phenotype of newly generated cells in the brain of adult and aged opossums

Marta Grabiec, Krzysztof Turlejski, Ruzanna Djavadian

Nencki Institute of Experimental Biology, Laboratory of Neurobiology of Development and Evolution, Warsaw, Poland

Contact: m.grabiec@nencki.gov.pl

Neurogenesis in adult mammals is active only in two brain regions: the sub-ventricular zone of the lateral ventricles (SVZ) and the dentate gyrus (DG). Here we present data concerning neurogenesis in the brain of adult and aged gray, short-tailed opossums (*Monodelphis domestica*), a marsupial species that recently became a new laboratory animal. Adult (8-10 months) and aged (26-30 months) opossums were injected with bromodeoxyuridine (BrdU). They received two intraperitoneal injections of BrdU (150 mg/kg total) in two equal doses with a 1.5 h interval. Four weeks later animals were transcardially perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). We used immunohistochemical double-labeling to characterize the phenotype of newly generated cells. Colocalization of BrdU with various markers such as NeuN (neuronal nuclear protein, marker of mature neurons), doublecortin (DCX, marker of migrating neuroblasts or young neurons), glial fibrillary acidic protein (GFAP, astrocytic marker) and 2',3' cyclic nucleotide phosphatase (CNP, oligodendrocytic marker) was examined using a confocal microscope. We found that recently generated cells (with the BrdU-labeled nuclei) were placed in the hippocampal DG, SVZ and olfactory bulb (OB), where they migrated from SVZ. The rate of neurogenesis was markedly higher in the DG and SVZ/OB of young opossums than in aged animals. Double-immunolabeling showed that in all neurogenic regions of the brain of opossums the dominating phenotype among newly generated cells was neuronal (54-74%), with a small proportion of astrocytes (10-17%). Although large numbers of CNP-positive cells were labeled in the OB, SVZ and DG, we did not find any colocalization of CNP with BrdU. Aging markedly reduced numbers of the newly generated cells and proportion of neurons among them. In the DG of old opossums the proportion of new cells expressing NeuN decreased to 44%. These results are consistent with studies in rodents and primates showing reduction in adult neurogenesis with aging. Therefore the decline in the rate of adult neurogenesis with age seems to be a common feature in both eutherians and marsupials. We suggest that old opossums may be used as a model in investigations of remedies for senile decline of the brain functions.

P3.8

Co-transplantation of bFGF-expressing amniotic epithelial cells and neural stem cells promotes functional recovery in spinal cord injured rats

Dong Chen Xiao-ting Meng, Zhi-yong Dong, Jia-mei Liu, Hui Xue, Dongguan

Changchun, China

Contact: nbumschendong@yahoo.com.cn

It has been demonstrated that amniotic epithelial cells (AECs) can enhance survival and neural differentiation of neural stem cells (NSCs) *in vitro* and enhance the function of AECs under the presence of basic fibroblast growth factor (bFGF) in our previous study. The aim of the present study was to extend those findings and investigate whether AECs modified with the bFGF gene will also enhance NSCs survival and neural differentiation in an *in vivo* environment and promote the injured spinal cord repair. Female Wistar rats were used to prepare contusive spinal cord injury (SCI) models. Contusive SCIs were induced using a weight-drop device at levels T9-T11. Seven days following contusion, rats received grafts of NSCs only, NSCs with AECs/pLEGFP-hbFGF, or NSCs with AECs/pLEGFP-C1 in the injury area. Significant locomotor improvement was observed in the NSCs/AECs co-graft group beginning at 3 weeks compared with the NSCs or NaCl only groups. These results were confirmed and extended in an electro-physiological analysis. The immunohistological analysis showed that AECs/pLEGFP-hbFGF promoted surviving (*vs* NaCl group: 194±9.17 *vs* 103.6±13.05) and neural differentiation (*vs* NaCl group: 14.24±1.11 *vs* 7±0.63) of co-transplanted NSCs. We also confirmed that AECs could promote the survival of host neurons. These results suggest that AECs/pLEGFP-hbFGF were serving as a NSCs survival and differentiated micro-environment and are useful as a source of sustained trophic supported to improve NSCs differentiation towards neurons *in vivo*. These findings sug-

gest that co-grafted AECs/pLEGFP-hbFGF and NSCs could have potential benefits for SCI. Key Words: spinal cord injury; neural stem cells; amniotic epithelial cells; microenvironment; bFGF.

P3.9

Characterization of human epidermal stem cellsMichał Pikula¹, Piotr Trzonkowski², Karolina Kondej³, Janusz Jaśkiewicz³, Andrzej Myśliwski¹¹ Department of Histology,² Department of Experimental Transplantology,³ Department of Plastic Surgery, Medical University of Gdańsk, Gdańsk, PolandContact: pikula@amg.gda.pl

Introduction: The epidermis is a constantly regenerating tissue that consists of multilayer keratinocytes. Three types of keratinocytes have been identified in the human epidermis: stem cells (ESC), transit amplifying cells (TAC) and terminally differentiated keratinocytes. ESCs are located in the bulge region of the hair follicle and in the basal layer of the epidermis. Adult human epidermal stem cells can form colonies that can be cultured and efficiently expanded *in vitro*. Cultured human keratinocytes and epidermal stem cells may be used in autografts for skin replacement in burn injuries, chronic wounds, soft tissue trauma and various skin diseases. Additionally, ESCs have become a target for gene therapy and drug testing. AIM: The main goal of our work was the isolation and characterization of human epidermal stem cells. Materials and methods: Normal epidermal cells were derived from human skin biopsies, prepared from surgery procedures. Single cell suspensions were isolated using two enzymes: dispase II and trypsin. Some samples were further sorted (FACS Aria, BD) to ESC and TAC cells thanks to the differences in the expression of beta-1 integrin, accumulation of rhodamine 123 and FSC/SSC cytometric parameters. Flow cytometry analysis of cells was performed using LSR (BD) cytometer. Keratinocytes were cultured in humidified atmosphere with 5% CO₂ at 37°C in serum-free medium (KBM) with defined keratinocytes supplements (KGM). RESULTS: In this study ESCs were identified as high beta-1 integrin expressing cells. Multicolour staining revealed that ESCs express protein 63 and possess longer telomeres than differentiating keratinocytes. When compared to TAC cells, ESCs exhibited prolonged cell cycle and morphological homogeneity. CONCLUSION: Combining flow cytometry, FACS sorting and cell culturing allows functional characterization of epidermal stem cells. The method presented is efficient enough to obtain ESCs from small human skin punch for scientific and therapeutic purposes.

P3.10

What about dairy cow stem cells? Semi-quantity and quantity evaluation of Scd1 expression in bovine mammary gland

Joanna Bierła, Tomasz Motyl

Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

Contact: joanna.bierla@wp.pl

Frequent repeating of life processes like proliferation, differentiation and involution during a life cycle make bovine mammary gland an unusual tissue. The basis for these changes seems to be stem cells but unfortunately in literature there is only little information concerning the development of this specific epithelium. Knowledge about the subject is essential, because in the future it can be used in improvement of dairy cows production capacity. Qualitative and quantitative estimation could be the basis for analysis of their expression profile. This analysis should compare stem cells presence in bovine mammary gland and visualisation of differentiation between stem cells and cells producing milk components. Therefore, maybe we can find the answer why cells in bovine mammary gland are not transformed in carcinogenesis. The frequency of tumors equals zero in this tissue. Maybe it could be the basis for understanding the fundamentals of carcinogenesis in woman's mammary gland. Our previous research allowed to determine the expression of Scd1 in bovine mammary gland which could be one of the markers of stem cells in this tissue. The aim of our new study is to determine how large cell population express Scd1. Mammary glands were obtained from 2-year-old Holstein heifers. Samples were fixed in phosphate-buffered formalin, dehydrated, and paraffin embedded. The paraffin blocks were cut into slices (5 µm) which were mounted on silanized microscope slides (Sigma-Aldrich Chemie, Germany), deparaffined, permeabilized in 70% methanol (POCH, Poland) and next rinsed with PBS

(Sigma-Aldrich Chemie, Germany). Slices were labelled with the specific set of antibodies: mouse anti-Sca1 FITC-conjugated antibodies (BD Pharmingen, USA), 1:200, 30 min in darkness, +4°C. Afterwards cell nuclei were counterstained with 7-AAD (Sigma-Aldrich Corporation, USA). For semi-quantity analysis 25 images (randomly acquired from the mammary tissue) were collected by FV-500 laser scanning confocal microscope (Olympus Polska sp. z o.o., Warsaw, Poland) 60x NA 1.4 oil immersion lens, for each data point. Object recognition was performed using the Microimage – image analysis software (Olympus Polska sp. z o.o., Warsaw, Poland) on the basis of fluorescent intensity. Objects under 50 pixels in size were automatically eliminated and the remaining cells were counted. Quantification was based on the number of objects expressing the studied protein compared to the overall number of cells counted on the basis of DNA-related fluorescence. For quantity evaluation the same procedure labelled slices were analysed in Scan*R system (Olympus Polska sp. z o.o., Warsaw, Poland). The results of both analyses show that the share of cells expressing Sca1 is from 0,47 to 3,18%. Our results confirm the theory that inter-individual differences in the presence of stem cells in bovine mammary gland exist, which can be used in animal selection.

P3.11

Transduction of stem cells from deer antler by means of pMINV-EGFP retroviral vector to confirm their contribution to auricle cartilage regeneration in rabbit

Marek Cegielski¹, Ilona Izykowska¹, Marzena Podhorska-Okolow¹, Maciej Zabel², Piotr Dziegiel¹, Dariusz Izyski³

¹ Department of Histology and Embryology, University School of Medicine, Wrocław, Poland;

² Department of Histology and Embryology, Medical University of Poznan, Poznan, Poland

³ Department of Cancer Immunology, Medical University of Poznan, Poznan, Poland

Contact: marcel@hist.am.wroc.pl

In order to check for the presence of xenogenic cells in cartilaginous tissue with regeneration defect, we mark them with the GFP protein (green fluorescent protein). More and more often, retroviral vectors are used as they allow for stable integration of the GFP gene into the cell genome. The aim of our study was the transduction of antlerogenic cells (MIC-1), isolated from the antlers of red deer (*Cervus elaphus*), by means of the pMINV-EGFP retroviral vector and evaluation of the EGFP (enhanced green fluorescent protein) expression in antlerogenic cells after they have been implanted in sites of cartilage defect in rabbit auricles. The pMINV-EGFP vector contains the EGFP reporter gene, the IRES sequence (internal ribosome entry site) as well as the neor gene determining resistance to neomycin, which remain under the control of the LTR (long terminal repeat) promoter sequence. For antlerogenic cells' transduction we used a medium with the addition of polybrene (8µg/ml) containing the pMINV-EGFP vector multiplied in TE FLY GA packaging cells. On the whole, 4-fold 24-hour-long transduction was performed. By means of a flow-through cytometer (BD FACSCanto Biosciences, Belgium) the expression of EGFP in MIC-1 cells was observed and 30% of cells showed fluorescence in the FITC range. Afterwards, the MIC-1/EGFP cells were implanted into auricle cartilage defect of a rabbit. Three weeks after the surgery samples containing the transplanted cells were taken. The analysis of the material under fluorescent microscopy (Olympus IX70, Olympus, Japan) demonstrated that the transplanted cells were present in the rabbit's auricle and showed fluorescence, which proved the EGFP expression. With regard to autofluorescence of cartilage, an additional immunohistochemical reaction with the anti-GFP antibody was performed, which confirmed the presence of antlerogenic cells in the cartilage. Material analysis under transmission electron microscopy (JEOL JEM-1011, Japan Electron Optics Laboratory Co., Japan) demonstrated that granules and greater conglomerates of the EGFP protein were present in the cytoplasm of the antlerogenic cells.

P3.12

Notch regulation of the spinal progenitor fate in the adult rodent

Masaaki Kitada, Mari Dezawa

Tohoku University Graduate School of Medicine, Sendai, Japan

Contact: Masaaki.Kitada@gmail.com

Even in the adult central nervous system, progenitor or stem cell populations have been identified. Here, we show the evidence that ependymal

cells function as the source of glial cells in the adult spinal cord and that Notch signaling regulates their proliferation and differentiation. BrdU-incorporated ependymal cells facing the central canal were gradually moved from inside to outside within the bilayered ependymal cells. Virus-mediated specific labeling of ependymal cells presented the migration to the spinal parenchyma and the differentiation into astrocytes. Forced expression of constitutively active form of Notch-1 in the ependymal cells showed three phenotypes such as migration, proliferation, and differentiation into astrocytes. These observations clearly showed that the ependymal cells in the adult spinal cord possess progenitor properties and Notch signaling is involved in the progenitor activity of the ependymal cells.

P3.13

Type I collagen inhibits differentiation of human SVF cells to cardiomyocytes

A. Balwierz¹, B. Zapala², A. Polus², A. Sliwa², B. Kiec-Wilk², L. Partyka², A. Dembinska-Kiec²

¹Dept. of Clinical Biochemistry Collegium Medicum, Jagiellonian University and Postgraduate School of Molecular Medicine, Warsaw, Poland;

²Dept. of Clinical Biochemistry Collegium Medicum, Jagiellonian University, Cracow, Poland

Contact: abalwierz@cm-uj.krakow.pl

Objectives: Progenitors have been suggested to be useful for tissue reconstructive therapy. A variety of cell types have been applied for such an approach. Since other sources of cells are limited, one of the possible alternatives became the adipose tissue. It has been recently reported that human adipose tissue contains a population of non-characterized stromal vascular fraction (SVF) cells, which are able to undergo differentiation to many lineages *in vitro* as well as *in vivo*. Besides a set of certain cytokines, SVF, similarly to other progenitor cells, need proper contact to differentiate to cardiomyocytes: cell to cell as well as with the extracellular matrix proteins. This study was aimed to assess the influence of collagen type I on the human SVF differentiation to cardiomyocytes. Methods: Differentiation of human SVF cells was induced by incubation in Methocult medium in the presence of SCF, IL-3 and IL-6. Morphological conversion was observed using optical microscope and the characteristic gene expression measured by qRT-PCR. Results: The myotubule shape changes as well as the up-regulation of the cardiac-characteristic genes: GATA, MEF2C, MYOD1, ANP were observed in the presence of myogenesis-promoting cytokines. Presence of type I collagen prevented cytokine-induced gene expression and inhibited differentiation. Conclusions: Human SVF cells differentiate to cardiomyocyte-like cells in the presence of a certain set of cytokines, whereas type I collagen attenuated this effect. Thus collagen type I cannot be recommended for the 3D scaffold used in the reconstructive therapy of the heart. Key words: SVF, adipose tissue, cardiomyocytes, differentiation, regeneration, collagen type I. This work was supported by F6 EU SC&CR (LSHB-CT-2004-502988) project.

P3.14

Lectin-binding pattern reveals involvement of glycoconjugates and carbohydrate antigens in neural stem cell niche

Masaaki Kitada, Mari Dezawa

Department of Stem Cell Biology and Histology, Tohoku University Graduate School of Medicine, Sendai, Japan

Contact: Masaaki.Kitada@gmail.com

Stem cell niche has been extensively investigated and one of the key components of it is the extracellular matrix (ECM). ECM consists of many molecules including glycoconjugates such as heparan sulfate proteoglycan, chondroitin sulfate proteoglycan, galectin-C, gp130, and SSEA-1, suggesting that neural stem cells (NSCs) are capable of binding to lectin, the protein which can bind to glycoconjugates or carbohydrate antigens. In this study, we analyzed the lectin-binding property of NSCs in the subventricular zone and the hippocampus of the adult brain. We applied 15 types of lectins for lectin-immunohistochemistry and found that 6 types of lectins accumulated in the NSC niche. Also, 3 types of lectins preferentially bound the white matter suggesting affinity to myelin. These findings suggest the potential of lectin to provide an artificial environment resembling stem cell niche.

Endothelial cell functions and angiogenesis

P3.15

Adventitial lymphatics of large arteries and atherosclerosis

Katarzyna Drożdż¹, Andrzej Szuba¹, Piotr Dziegieł², Marzena Podhorska-Okolów², Dariusz Janczak³, Dariusz Patrzalek³

¹Department of Internal Medicine, ²Department of Histology and Embryology, ³Department of Vascular, General and Transplantation Surgery, Wrocław Medical University, Wrocław, Poland

Contact: kdrozdz25@yahoo.com

Background: Adventitial lymphatics of major arteries may play an important role in development of atherosclerosis. **Aim:** The aim of our study was to evaluate the presence and number of lymphatic vessels in adventitia of aorta, iliac and internal carotid arteries in various stages of atherosclerosis. **Subjects and methods:** Fragments of arterial wall of aorta, iliac and internal carotid arteries were obtained during the surgical procedures and from cadaver organ donors. Arteries from 53 subjects were studied. Fragments of arterial wall were embedded in paraffin. Lymphatics of arterial adventitia were visualized with immunohistochemistry using LYVE-1 and anti-podoplanin antibodies. The thickness of intima was measured under magnification x100, with Olympus BX 41 light microscope using the visual mode analysis 3.2 software for computer. Intimal thickness served as a marker severity of atherosclerosis. **Results:** The lymphatic vessels were visualized in adventitia of all studied arteries. The serial sections have revealed that both LYVE-1 and podoplanin have identical specificity for lymphatic endothelium. The number of lymphatics in adventitia significantly correlated with thickness of intima ($r=0.36$; $p<0.05$). **Conclusions:** Lymphatics are present in adventitia of large arteries. The number of adventitial lymphatics correlates with intimal thickness.

P3.16

Regulation of mTOR signaling by free fatty acids in human adipose tissue progenitor cells

Urszula Czech¹, Adriana Balwierz¹, Anna Polus¹, Wojciech Dudek², Aldona Dembinska-Kiec¹

¹Dept. of Clinical Biochemistry Collegium Medicum, Jagiellonian University, Krakow, Poland

²Dept. of Gynecology and Obstetrics, District Hospital, Myslenice, Poland

Contact: uczech@cm-uj.krakow.pl

Introduction: mTOR (mammalian target of rapamycin) plays a major role in signaling caused by nutrients and other mitogens such as growth factors and the cellular energy status. mTOR has emerged as a regulator of growth related processes like development, aging and the response to hypoxia. TOR pathway up-regulation (in excess of energy) is required for fat accumulation and plays a role in development of metabolic disorders and type 2 diabetes. mTOR regulation is also involved in angiogenesis by activation of expression of HIF-1 dependent genes. **Methods:** The modified Hauner's method was used to obtain the human adipose tissue progenitor-stromal vascular fraction (SVF) cells. SVF was incubated with arachidonic acid (AA), palmitic acid (PA), eicosapentaenoic acid (EPA) and oleic acid (OA) in the concentrations 10 μ M (AA) and 30 μ M (PA, EPA, OA) for 24h. Total RNA was isolated using Trizol and (Sigma) and SV total RNA isolation system (Promega). The high grade purity RNA was used for oligonucleotide microarray (HG-U133 A, Affymetrix, 14 500 genes). The influence of FFA on gene expression was measured by real-time PCR method. To measure the activity of AKT-kinases, FAK-kinase, p38 and ERK1/2-kinases Fast Activated Cell-based ELISA (FACETM) Kits were used. **Results:** The regulation of the mTOR pathway by FFA was found: Rheb was up-regulated by unsaturated fatty acids, especially by EPA. It was paralleled by activation of AKT-kinases. The analysis of microarrays data suggests that fatty acids stimulate differentiation of SVF into adipocytes. All used fatty acids induced the activation of AKT-kinases (inhibition of apoptosis). The reduced activity of FAK-kinase was observed after stimulation with fatty acids, what may be connected with reduction of cell matrix adhesion (microarray). The p38 activity was down-regulated by unsaturated fatty acids in SVF cells. Changes in activity of ERK-kinase was not observed after stimulation of used cells with fatty acids, what is in consistence with

the lack of stimulation of cell proliferation by FAs. **Conclusions:** FFA regulated the upstream pathway of mTOR (AKT-kinases, AMPK-kinase, the TSC1-TSC2 complex and Rheb) by which TOR may influence the cell cycle apoptosis and differentiation of SVF cells. **Acknowledgements:** Supported by the EU LIPGENE FOOD-CT-2003-505944 and Polish MNiI project Nr 2 P05 132 28.

P3.17

Angiogenesis and biochemical parameters in the New Zealand Obese mice

Lukasz Wator¹, Urszula Razny¹, Anna Polus², Jerzy Stachura³, Grzegorz Dyduch³, Hans Joost⁴, Aldona Dembinska-Kiec¹

¹Dept. of Clinical Biochemistry, ²Dept. of Clinical Biochemistry,

³Dept. of Pathomorphology, Jagiellonian University Medical College, Cracow, Poland

⁴German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany

Contact: lwator@cm-uj.krakow.pl

Background: Obesity, insulin intolerance, dyslipidemia, hypertension, hyperleptinemia and hypoadiponectinemia are the main symptoms of the metabolic syndrome leading to vascular injury, atherosclerosis, diabetes and pathological angiogenesis. The aim of this study was to define the possible link between some of the above metabolic syndrome parameters and angiogenesis. **Methods:** New Zealand obese mice (NZO) were fed with standard or high fat (HF) diet for seven weeks. Body weight and biochemical parameters were monitored. Angiogenesis in subcutaneously implanted matrigel was compared by calculation of the PECAM1 positive structures, and the gene expression of the matrigel penetrating cells was analyzed by microarray (Affymetrix 430A_2 GeneChips). **Results:** Mice fed HF diet developed obesity with hyperinsulinemia and elevated glucose cholesterol and leptin concentrations. HF diet tended to increase the angiogenic response and was positively correlated with blood glucose concentration but not with serum leptin, adiponectin or insulin. Microarray results revealed changes in gene expression related to adhesion and matrix digestion, up-regulation of genes related to cytoskeleton remodeling, chemotaxis, inhibition of apoptosis, cell differentiation but not the end endothelial cell differentiation. Those changes are suggested to be mainly dependent on glucose level due to the positive correlation between blood glucose and angiogenic response. **Acknowledgements:** Project supported by Polish Committee of Science Grant No: PBZ-MIN-005/P04/2002/5.

P3.18

Hepatocyte retinoid X receptor α deficient mice respond to high fat diet with downregulation of angiogenesis

Urszula Razny¹, Lukasz Wator¹, Anna Polus¹, Yu-Jui Yvonne Wan², Grzegorz Dyduch³, Jerzy Stachura³, Aldona Dembinska-Kiec¹

¹Department of Clinical Biochemistry, Medical College, Jagiellonian University, Cracow, Poland

²Department of Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas, United States

³Department of Pathomorphology, Medical College, Jagiellonian University, Cracow, Poland

Contact: urazny@cm-uj.krakow.pl

Background. Metabolic syndrome and diabetes is characterized by gradually developing micro- and macroangiopathy, associated with pathological angiogenesis. Hepatocyte RXR α deficient mice are a unique model for investigating the effects of selected metabolic syndrome symptoms (dyslipidemia and hyperleptinemia with normal glucose level) on angiogenesis. **Methods.** Hepatocyte retinoid X receptor α deficient (hRXR α ko) mice and control mice WT were fed either standard or high fat (HF) diet for 7 weeks. Body weight, food consumption and serum biochemical parameters (glucose, triglycerides, cholesterol), adipokines (leptin, adiponectin) and insulin were monitored. At the sixth week of feeding the mice were subcutaneously injected with matrigel containing 25 nM bFGF. After 6 days matrigel plugs were removed and immunohistochemically stained against PECAM1 (CD31) antigen. The number of vessels with and without lumen and single CD31 positive cells was counted in paraffin embedded sections. The paral-

lel matrigel plug cells were harvested and used for the gene expression assessment by microarray (Mouse 430A_2, Affymetrix, 14 000 genes) and by real time PCR. Results. The feeding with HF diet of the hRXR α ko mice resulted in statistically significantly increased levels of cholesterol and leptin in serum and in a tendency to decrease angiogenesis (number of vessels with lumen). HF diet in the matrigel plug infiltrating cells down-regulated genes related to angiogenesis (*Nos3*, *Kdr*) and up-regulated genes connected with adipogenesis (*Cebpb*, *Srebf1*) (microarray analysis confirmed by real time PCR). The microarray studies also documented the up-regulation of genes related to apoptosis (activators of *caspase 3*, proapoptotic genes *Bcl2*), the activation of proinflammatory pathway (*Nfkb* pathway, *Tnf α*) and the inhibition of antioxidant pathway by HF diet. Conclusion. Our results suggest that due to impaired fatty acid metabolism in the liver, the hRXR α ko mice responded to high fat diet with impaired angiogenesis and promotion of adipogenesis. Supported by Polish Committee of Science Grant No: PBZ-MIN-005/P04/2002/5, National Institutes of Health grants CA53596, AA14147, and COBRE P20 RR021940, the Molecular Biology Core under COBRE as well as the Liver Center at KUMC.

P3.19

Immunohistochemistry and DNA profiling of VEGFs and VEGFRs during angiogenesis *in vitro*

Masumi Akita¹, Keiko Fujita², Eiko Murata³, Hiroaki Shindo⁴, Katsuji Kaneko⁴

¹Division of Morphological Science, Biomedical Research Center
²Department of Anatomy, ³Department of Health and Medical Care,
 Saitama Medical University, Saitama, Japan
⁴Nihon Institute of Medical Science, Saitama, Japan

Contact: makita@saitama-med.ac.jp

A number of genes are known to be involved in angiogenesis. Recently, we examined angiogenesis-related gene expression profiles using collagen gel culture and a DNA chip. Seventy-three out of over 35,000 transcripts were expressed after capillary tube formation in the collagen gel culture. The vascular endothelial growth factors (VEGFs) and VEGF receptors (VEGFRs) are considered as key molecules in the process of angiogenesis. In this study, we focused on the VEGF-related gene expression and immunohistochemical expression during angiogenesis. We used the collagen gel culture method with time-lapse imaging. After a 2- to 3-day culture period, light microscopic inspection revealed that fibroblastic cells were migrating in the vicinity of the aortic explants in the collagen gel. After 7 days in culture, capillary sprouts were recognized. After 10 days in culture, elongated capillary tubes with branches were observed. Micro array analysis revealed that VEGF-A and placental growth factor (PGF) genes were highly expressed. Neupilin-1 (*Nrp-1*), VEGFR-1 and -2 genes were also expressed. Immunohistochemically, these capillary tubes were strongly positive for tomato lectin, platelet/endothelial cell adhesion molecule-1 (PCAM-1) and intercellular adhesion molecule-1 (ICAM-1) antibodies. These capillary tubes were also positive for VEGFRs antibodies (especially, VEGFR-2). Pericyte-like cells resting on the outside of the capillary tubes showed immunoreactivity of actin and VEGF-A antibodies. Time-lapse imaging visualized the dynamic process in capillary tube formation from aortic explants. The present study strongly suggests that cross talk of endothelial cells and pericytes plays an important role in angiogenesis.

P3.20

Distribution of notch protein members in normal and pathological human endometrium

Maria De Falco¹, Luigi Cobellis², Gabriele Coppola³, Lucrezia Manente³, Angela Lucariello³, Ivan Cavallotti³, Nicola Colacurci², Antonio De Luca³

¹Dept. of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples "Federico II", Naples, Italy
²Dept. of Gynaecology, Obstetric and Reproductive Science and ³Dept of Medicine and Public Health, Section of Anatomy, Second University of Naples, Naples, Italy

Contact: antonio.deluca@unina2.it

Notch is a transmembrane receptor and it belongs to the growth factor family. It is structurally and functionally conserved during evolution. The mammalian family of Notch protein consists of four different isoforms (N1-N4). Notch ligands are classified into two structurally related groups:

Delta-like ligands and Serrate-like ligands. Several studies have demonstrated that Notch receptors and their ligands have an important role in cell differentiation, proliferation and apoptosis. Moreover, it has been demonstrated that the Notch pathway is involved in multiple aspects of vascular development and angiogenesis, but the mechanisms by which Notch exerts its effects on the vasculature remain to be elucidated. The purpose of this study was to investigate, by immunohistochemistry, the cellular localization of Notch-1, Notch-4 and Jagged-1 in normal endometrium and to compare these data with the distribution of these proteins in different endometrial pathologies, like hyperplasia, polyps and cancer. Moreover, we evaluated the expression levels of two major regulators of the G1 checkpoint: cyclin D1 and p21 to clarify the possible correlation between the expression pattern of Notch proteins members signalling on human endometrium. In physiologic endometrium, we observed an increase of Notch-1 and Jagged-1 from proliferative to secretory phase and an opposite trend for Notch-4 which decreased from proliferative to secretory phase. In menopause, the level of expression of all the three members of the Notch pathway decreased. Moreover, we observed a cyclin D1 increase from proliferative to secretory phase. Instead p21 showed a slight increase from proliferative to secretory phase. In pathologic endometrium, we observed an increase of Notch-1 expression from polyps to carcinoma and an opposite trend for Notch-4 and Jagged-1 which instead decreased from polyps to carcinoma. Moreover, we observed a higher expression of cyclin D1 in all the endometrial pathologies. In contrast, the expression level of p21 slightly increased from polyps to carcinoma. In human endometrium, Notch-4 seems more involved in controlling proliferation, instead Notch-1 seems more involved in differentiation program. Deregulation of these functions may induce the onset of several endometrial pathologies from polyps to cancer.

P3.21

Expression of intermediate filament nestin in vascular endothelium

Jaroslav Mokry¹, Dana Čížková¹, Jiří Ehrmann², Stanislav Filip¹, Zdeněk Kolář²

¹Charles University Medical Faculty, Hradec Králové, Czech Republic
²Palacký University Medical Faculty, Olomouc, Czech Republic

Contact: mokry@lfhk.cuni.cz

In vascular endothelial cells, expression of protein nestin has been well documented in the course of angiogenesis. Mechanism of angiogenesis is different from arteriogenesis. To find out whether nestin participates also in the formation of large blood vessels including arteries we examined developing and adult human and rat tissues and processed the samples for anti-nestin immunohistochemistry. Archival specimens were deparaffinised, processed for microwave antigen retrieval and permeabilised. Nestin was detected with monoclonal antibodies 10C2 (Chemicon) or Rat-401 (DSHB). Secondary biotinylated anti-mouse antibodies and streptavidin coupled to horseradish peroxidase or Cy3 fluorochrome were used to visualise antigen-binding sites. To achieve an intense immunostaining the specific signal was amplified with biotinylated tyramine (Catalysed Signal Amplification). Some sections were processed for double immunohistochemistry using secondary antibody conjugated to Alexa 477 fluorochrome to study co-expression of nestin with endothelial (CD31, CD34, vimentin) or proliferative (PCNA, Ki-67) markers. Nestin immunoreactive arteries were observed in most samples that contained nestin positive capillaries. The capillaries co-expressed proliferative as well as endothelial markers. The ratio between nestin⁺ capillaries and nestin⁺ arteries differed among samples, e.g. rare immunoreactive arterioles were observed in the postinfarcted human myocardium. On the other hand, large nestin⁺ arteries and veins were observed in human gliomas, human corpus luteum, uterine horn of pregnant rats and rat umbilical cord. Moreover, intensity of immunoreactivity varied between arteries and veins. In tissues that increased their volume rapidly as a result of intense proliferation, e.g. in response to hormone stimulation, nestin⁺ arteries were tortuous because they grew lengthwise. Histologically, these immunoreactive corkscrew-like blood vessels were represented by muscular arteries. Nestin expression was identified in longitudinally arranged endothelial cells and in smooth muscle cells with helical orientation. Our findings provide evidence that nestin is transiently expressed in endothelium of growing blood vessels which include angiogenic capillaries and growing arteries and veins. Nestin expression in arteriogenesis documents the important role of this intermediate filament in remodelling the cytoskeleton of vascular cells. The work was supported by a grant No. MSM0021620820.

P3.22

Role of endothelium in atherogenesis

Francesca Bonomini¹, Luigi F. Rodella¹, Tony Hayek²,
Michael Aviram², Shlomo Keidar², Raymond Coleman², Rita Rezzani¹

¹Department of Biomedical Sciences and Biotechnologies, Division of Human Anatomy, University of Brescia, Brescia, Italy

²Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Contact: bonomini.f@inwind.it

Atherosclerosis is a vascular disease with a clear focal nature, which has been shown to be correlated with shear stress levels on the endothelium, resulting from specific blood flow patterns. Changes in shear stress due to oscillatory blood flow at arterial bifurcations and curvatures leads to endothelial dysfunction as characterized by a diminished barrier function and proinflammatory gene expression. These conditions facilitate the entry of lipids and inflammatory cells in the vascular wall, ultimately leading to the formation of an atherosclerotic plaque. Under physiological conditions the vascular endothelium produces factors that inhibit blood coagulation (e.g., thrombomodulin, TM) and inhibit platelet aggregation (eg, nitric oxide, NO). Consequently, we sought to investigate the expression patterns of TM and eNOS in the endothelium of aortas of apolipoprotein E-deficient (ApoE⁰) mice aged 6, 8, 10, 12, 16 and 20 weeks. These mice are hypercholesterolemic and develop atherosclerotic lesions in the aorta. Our results demonstrated that alterations in TM and eNOS expression are coupled with atherogenesis process in ApoE⁰ mice. In conclusion, our results show that endothelial dysfunction is a systemic disorder and a key factor in the pathogenesis of atherosclerosis and its complications.

P3.23

Presence of ANP in internal carotid artery (ICA) with and without atherosclerotic lesions

G. La Marca¹, G. La Barbera¹, F. Valentino¹, B. Valentino²,
E. Farina Lipari², D. Lipari², F. Cappello², A. Gerbino³, M. Buscemi³,
G. Peri²

¹Dip. Chirurgia vascolare "G Di Cristina" Ospedale Civico, Palermo, Italy

²Dip. Medicina sperimentale Sez Anatomia Umana and

³Dip. Medicina Sperimentale, Università di Palermo, Palermo, Italy

Contact: peri@csai.unipa.it

Introduction: The cardiovascular system is regulated by haemodynamic and neurohumoral factors that play a role on both muscular tone and vasal structure. The morphological anomalies of the internal carotid artery (ICA) may determine cerebrovascular alterations. In a previous study on kinking, coiling and tortuosity of extracranial ICA, we showed a reduction of both elastic and muscular components and a compensative increase of connective fibers in presence of atherosclerotic lesions (La Barbera *et al.*, 2006). It is well known that the atrial natriuretic peptide (ANP) plays an important vasodilative and vasorelaxant action in the blood vessels, but ANP may play also an antihypertrophic action on vasal muscular tissue (Itoh *et al.*, 1990). In this work we investigated the presence of ANP in ICA with and without atherosclerotic lesions (AL). **Material and methods:** Three ICA-segments with AL and one without AL were resected during the surgical operations. Surgical specimens were fixed in Bouin's fluid and embedded in paraffin. Seven micra sections were immunostained with polyclonal ANP-antibody (Chemicon) and routine controls. **Results:** The wall of ICA with kinking and AL presented ANP-immunonegativity in endothelium; by contrast, the tunica media showed ANP-immunopositivity in muscular fibers and connective tissue. Between the connective fibers many elastic fibers were visible and they were immunonegative. In ICA with kinking but without significant AL we found ANP-immunonegative fibers and cells both in endothelium and tunica media. **Discussion:** The smooth muscular fibers of the vessels with AL may synthesize ANP. This peptide, in turn, may have inhibitory effects on the oxidation of LDL that permits the migration of the smooth muscular cells into the tunica intima (Kohno *et al.*, 1997). In conclusion, we suggest that ANP may have a role in development of AL. Further studies will follow to understand better the underlying molecular mechanisms. **References:** 1. Kohno M, Yokokawa K, Yasunari K, Kano H, Minami M, Ueda M, Yoshikawa J Effect of natriuretic peptide family on the oxidized LDL-induced migration of human coronary artery smooth muscle cells. *Circ Res* (1997) 81:585-90. 2. Itoh H, Pratt RE, Dzau VJ. Atrial natriuretic inhibits hypertrophy of vascular smooth muscle cells. *J Clin Invest* (1990) 86:1690-7. 3. La Barbera G, La Marca G, Martino A, Lo Verde R, Valentino F, Lipari D, Peri G, Cappello F, Valentino B. King-

king, coiling, and tortuosity of extracranial internal carotid artery: is it effect of a metaplasma? *Surg Radiol Anat* (2006) 28:573-80.

P3.24

AAV-mediated VEGF165 and FGF-4 gene transfer for the treatment of hind limb ischemia

Agnieszka Jazwa¹, Hevidar Taha¹, Aleksandra Sierpniowska¹,
Anna Zagorska¹, Paulina Kucharzewska¹, Justyna Leja¹,
Marco Meloni², Costanza Emanuelli², Elisa Vahakangas³,
Seppo Yla-Herttuala³, Alicja Jozkowicz¹, Jozef Dulak¹

¹Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

²Bristol Heart Institute, University of Bristol, Bristol, United Kingdom

³A.I Virtanen Institute, University of Kuopio, Kuopio, Finland

Contact: ajazwa@interia.pl

Gene transfer approach is tested for the treatment of peripheral and myocardial ischemia. Here we aimed to achieve angiogenic synergism between human vascular endothelial growth factor-165 (hVEGF165) and fibroblast growth factor-4 (hFGF-4) delivered in adeno-associated viral vectors (AAV) to protect murine limbs from the consequences of immediate ischemia. Vectors (1x10¹⁰ viral particles) carrying either B-galactosidase gene (AAV-LacZ), hVEGF165 (AAV-VEGF165), hFGF-4 (AAV-FGF4) or both angiogenic genes (AAV-FGF4-VEGF165) were injected into normo-perfused left adductor muscle of C57Bl mice (n=3/group). Some animals were subjected to hind limb ischemia by left femoral artery ligation and received either control AAV-LacZ (n=5) or bicistronic AAV-FGF4-VEGF165 vector (n=3) into ischemic left adductor. Paraffin-embedded sections were analyzed 28 days after the gene transfer. Capillary density was counted from the hematoxylin-eosin staining. Double immunofluorescent staining for proliferating cell nuclear antigen (PCNA) and Bandeiraea simplicifolia-1 (BS-1) isoelectin B4 was performed to visualize proliferating capillary endothelial cells (ECs). Pericyte coverage of capillaries was assessed by detection of chondroitin sulfate proteoglycan (NG2) on pericytes. Arterioles were quantified in skeletal muscles from the immunohistochemical staining for α -smooth muscle actin (α -SMA). Hind limb blood flow was detected using Laser Doppler Perfusion Imager. The number of necrotic toes in ischemic limbs was counted. VEGF delivered alone (AAV-VEGF165) or in combination with FGF-4 (AAV-FGF4-VEGF165) to a similar extent increased the number of capillaries in normo-perfused skeletal muscles (respectively, 1023 \pm 12,5/mm² and 1077 \pm 1,6/mm² vs 925 \pm 10/mm² in AAV-LacZ group, p<0,05). Moreover, administration of VEGF increased ECs proliferation but only in AAV-VEGF165 injected adductors (9,3 \pm 0,9/mm² vs 1,3 \pm 0,5/mm² in AAV-LacZ group, p<0,05). Both FGF-4 and VEGF augmented the integrity of capillaries by increasing the number of pericytes. Simultaneous overexpression of both angiogenic proteins (VEGF and FGF-4) from the bicistronic vector augmented the number of arterioles (6,5 \pm 0,3/mm² vs 3,4 \pm 0,3/mm² in AAV-LacZ group, p<0,05). Additionally, improved after AAV-FGF4-VEGF165 administration post-ischemic foot blood flow decreased the incidence of toe necrosis when compared to the control group injected with LacZ carrying vector. Synergy between VEGF and FGF-4 to produce stable and functional blood vessels may be a promising option for therapeutic angiogenesis in the clinical perspective. Supported by grant N302 020 31/1998 and 512/6.PR UE/2008/7 from the Ministry of Science and Higher Education. A. Jazwa is the recipient of the fellowship from the Polish Edition of the L'Oreal-UNESCO "For Women in Science" Awards.

P3.25

AAV-mediated VEGF165 and FGF-4 gene transfer for the stimulation of reparative processes in the wounded skin of genetically diabetic mice

Agnieszka Jazwa¹, Aleksandra Sierpniowska¹,
Magdalena Kozakowska¹, Anna Zagorska¹, Paulina Kucharzewska¹,
Justyna Leja¹, Elisa Vahakangas², Seppo Yla-Herttuala²,
Alicja Jozkowicz¹, Jozef Dulak¹

¹Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland;

²A.I. Virtanen Institute, University of Kuopio, Kuopio, Finland

Contact: ajazwa@interia.pl

One consequence of diabetes mellitus is the disruption of the normal process of wound healing, which is related to the decreased production of dif-

ferent growth factors involved in cell proliferation and migration. The use of those agents or their genes to stimulate healing of wounds is widely investigated. In this study we applied the combined gene therapy approach consisting of two angiogenic genes, namely human vascular endothelial growth factor-165 (hVEGF165) and fibroblast growth factor-4 (hFGF-4) in bicistronic adeno-associated viral vectors (AAV) for the stimulation of reparative processes in the wounded skin of genetically diabetic mice deficient in leptin receptor (Lepr^{-/-}). Two full-thickness longitudinal incisions (4 cm) were made on the dorsum of the mice. Then, 3×10^{10} of each AAV vector carrying either β -galactosidase gene (AAV-LacZ), hVEGF165 (AAV-VEGF165), hFGF-4 (AAV-FGF4) or both VEGF and FGF-4 genes (AAV-FGF4-VEGF165) were injected intradermally into the wound edges of Lepr^{-/-} mice (n=5/group). The rate of re-epithelialization (wound closure) was measured at day 0 (directly after wounding), 1 and then every second day till the end of the experiment (day 21), when all wounds became healed. The paraffin-embedded skin sections were stained for Bandeiraea simplicifolia-1 (BS-1) isolectin B4 binding, and capillary endothelial cells (ECs) were counted. Double immunofluorescent staining for proliferating cell nuclear antigen (PCNA) and BS-1 isolectin B4 was performed to visualize proliferating capillary ECs. Arterioles were quantified in the skin from the immunohistochemical staining for α -smooth muscle actin (α -SMA). Slightly increased total number of capillaries ($424.7 \pm 13.7/\text{mm}^2$ vs $354.1 \pm 29.08/\text{mm}^2$ in AAV-LacZ group, $p=0.09$) and significantly augmented proliferation of capillary ECs ($102 \pm 10.66/\text{mm}^2$ vs $53.13 \pm 8.08/\text{mm}^2$ in AAV-LacZ group, $p<0.05$) was observed 21 days after AAV-VEGF165 delivery into the wounded area. Moreover, the wound healing in animals injected with AAV-VEGF165 was faster than in the control mice. Interestingly, the highest rate of re-epithelialization was observed in mice injected with bicistronic vector, although there was no angiogenic effect observed at the histological level 21 days after the injury and gene transfer. Moreover, the increased re-epithelialization rate in AAV-VEGF165 and AAV-FGF4-VEGF165 group does not seem to be related to the stimulation of arteriogenesis, as no increase in the number of α -SMA-positive arterioles was observed. However, the synergy between VEGF and FGF-4 leading to the stimulation of the reparative processes in the wounded skin of diabetic animals can not be excluded. Supported by grant N302 020 31/1998 and 512/6.PR UE/2008/7 from the Ministry of Science and Higher Education. A. Jazwa is the recipient of the fellowship from the Polish Edition of the L'Oreal-UNESCO "For Women in Science" Awards.

P3.26

Cellular localization and correlation between NADPH-diaphorase activity and endothelial and inducible nitric oxide synthases immunoreactivity in periovarian vascular complex after testosterone infusion into porcine ovarian artery in the luteal phase

Barbara Wąsowska, Ewelina Bajko

Department of Local Physiological Regulations, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland

Contact: barwa25@wp.pl

The periovarian vascular complex (PVC) is composed of numerous tortuous branches of the ovarian artery, ovarian vein and lymphatic vessels. This structure participates in the local transfer of ovarian hormones, including testosterone (T), from the venous and lymphatic effluent into the arterial blood supplying the ovary. Nitric oxide (NO) is involved in vasodilatation, hormone secretion and reproduction. All known nitric oxide synthase (NOS) isoforms possess NADPH-diaphorase (NADPH-d) activity. The aim of the present study was to determine if unilateral T infusion to the ovarian artery in the luteal phase may change the activity of NADPH-d and immunoreactivity (IR) of endothelial (eNOS) and inducible (iNOS) isoforms of NO synthase in the endothelium as well as in the muscular layer of PVC arteries and veins. This study was also to examine the correlation between the histochemical activity of NADPH-d and the IR for eNOS and iNOS. T was infused (n=4) into the right ovarian artery (experimental PVC) during 3 days (10, 11, 12 day of estrous cycle) in a dose adopted on the base of the rate of the retrograde transfer of this hormone in luteal phase: 249.6 pg/min, 2×249.6 pg/min and 3×249.6 pg/min on the first, second and third day of the experiment, respectively. Saline was infused into the left ovarian artery (control PVC). Seven days following last T infusion gilts were sacrificed and both PVCs were collected. Cryostat tissue sections were stained using histo- and immunohistochemistry methods (iNOS, eNOS antibodies – BD Transduction Laboratories, USA). The intensity of the NADPH-d and eNOS, iNOS immunohistochemical reaction was estimated by measuring optical density by means of DP SOFT

(Olympus) program. The data were expressed as the mean and compared with the values obtained for control PVC. Differences were evaluated by unpaired Student's *t* test Prism 4.03 (GraphPad, USA). Infusions of T in experimental PVC, in comparison with control PVC, caused decrease of NADPH-d activity, eNOS and iNOS IR in: arterial endothelial cells ($P<0.01$ - $P<0.0001$) and veins' muscular layer ($P<0.01$ - $P<0.0001$). There was no observed correlation between NADPH-d activity and iNOS IR only in veins' endothelial cells. These results indicate that T infused during luteal phase into porcine ovarian artery may change the IR of NO synthases and in consequence – the production of nitric oxide. NO may be involved in the local control of periovarian vascular complex contractility, blood flow and in this way modulate the retrograde transfer of ovarian hormones.

P3.27

Relationships between NADPH-diaphorase staining and endothelial (eNOS) and inducible (iNOS) nitric oxide synthases in porcine uterus vessels after testosterone infusion into ovarian artery during estrous cycle

Barbara Wąsowska, Ewelina Bajko

Department of Local Physiological Regulations, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland

Contact: barwa25@wp.pl

Nitric oxide (NO) regulates vessels smooth muscle contractility and plays an important role in the control of uterine circulation during the estrous cycle. The purpose of this study was to examine the effect of unilateral testosterone (T) infusion to the ovarian artery (OA) in luteal (group I, n=4; infusion during 10, 11, 12 day of the estrous cycle), early follicular (group II, n=3; infusion: 16, 17, 18 day) and late follicular phase (group III, n=3; infusion: 19, 20 21 day) on the activity of NADPH-d (marker for eNOS) and immunoreactivity (IR) of eNOS and iNOS in the endothelium as well as in the muscular layer of veins (MLV) and arteries (MLA) in the endometrium. This study was also to determine the relationships between NADPH-d activity and IR of NO synthases. T was infused into the right ovarian artery (experimental uterine horn, Exp) at a dose: 249.6 pg/min, $2'249.6$ pg/min, and $3'249.6$ pg/min during luteal phase and at a dose: 170.4 pg/min, $2'170.4$ pg/min, and $3'170.4$ pg/min during follicular phase on the first, second and third day of experiment, respectively. Saline was infused into the left ovarian artery (control; C). Seven days following the last T infusion gilts were sacrificed, and both Exp and C uterine horn samples were collected. Cryostat tissue sections were stained using histo- and immunohistochemistry methods (iNOS, eNOS antibodies – BD Transduction Laboratories, USA). The intensity of the NADPH-d and eNOS, iNOS reactions was estimated by measuring optical density by means of DP SOFT (Olympus) program. The data were expressed as the mean and compared with the values obtained for C samples. Differences were evaluated by unpaired Student's *t* test Prism 4.03 (GraphPad, USA). The present results clearly demonstrate that infusion of T into porcine OA enhanced, in comparison with C, NADPH-d activity in the MLV in all the investigated groups ($P<0.05$ - $P<0.01$) and MLA and in the endothelium of arteries ($P<0.05$) in Group I. The correlation between NADPH-d activity and IR of eNOS and iNOS was observed only in group I in the endothelium of veins. Although the differences of NADPH-d activity and IR of iNOS were not observed, but there was significance ($P<0.01$ - 0.0001) increase of IR in the MLA and endothelium of arteries in Group III. Taken together, our histo- and immunohistochemical findings suggest that there was not clear dependences between NADPH-d activity and IR of NOS isoforms in the uterine vessels in different stages of the estrous cycle. Additionally, T besides estrogens, involving NO production, may regulate both veins and arteries tone in porcine uterine.

P3.28

The expression profiles of VEGF, its receptors and angiotensin receptors Tie-1 and Tie-2 in the early human placenta

Ramazan Demir, Yasemin Seval, Leyla Sati

Department of Histology and Embryology, Faculty of Medicine, Akdeniz University, Antalya, Turkey

Contact: akademisyen07@yahoo.com

Introduction: Vasculogenesis and angiogenesis are two consecutive processes during the development of blood vessels. While the formation of the first blood vessels in human placenta is achieved by the differentiation of

haemangiogenic stem cells from pluripotent mesenchymal cells and is referred to as vasculogenesis, the subsequent step in which new vessels form from already existing vessels is known as angiogenesis. In this study we aimed to give a full picture of vasculogenesis and angiogenesis during the first trimester in human placenta. Materials and methods: Immunohistochemistry was used to identify VEGF and its receptors VEGFR-1, VEGFR-2 and angiopoietin receptors Tie-1 and Tie-2 in very early human placenta. The staining intensities were evaluated by HSCORE analysis. Results: Our results show that while the cytotrophoblasts are the triggers of vasculogenesis and angiogenesis at the very early stages of placental development, later on as pregnancy progressed the trigger shifted from the cytotrophoblasts to Hofbauer and stromal cells. In early development of placental villi, cytotrophoblast cells show a strong immunoreactivity for VEGF, while the VEGF receptors VEGFR-1 and -2 and the angiopoietin receptors, Tie-1 and Tie-2 are expressed in the haemangiogenic stem cells, leading to the formation of the first vascular patterns in mesenchymal villi. We observed that, in addition to the expressions of VEGF and its receptor VEGFR-2, Tie-1 and Tie-2 expressions increased as pregnancy advanced while VEGFR-1 did not show any alterations. Conclusions: In conclusion, the results of this study depicts the expression profiles of angiogenic factors during vasculogenesis and angiogenesis, which can give further insights in understanding the molecular mechanisms of conditions associated with the impairment of vascular development during placentation such as intrauterine growth retardation and pre-eclampsia.

P3.29

Ultrastructural and immunohistochemical study of the inner vascular retina of the lizard *Gallotia galloti* (Lacertidae)

M.C. Alfayate¹, C. Yanes¹, E. Santos¹, M. Romero-Aleman², N. Casanas¹, R. Vinoly¹, M. Monzon Mayor²

¹Dept. of Cell Biology, F. Biology, UL Canary Island, La Laguna, Tenerife, Spain
²Dept. of Morphology, FCMS, ULPGC, Las Palmas, Spain

Contact: cyanes@ull.es

The eye of reptiles is characterized by an avascular retina and a vascular structure denominated *conus papillaris* (cp), which projects from the optic nerve head (ONH) into the vitreous, and is homologous to the *pecten oculi* of the avian eye (Wolburg 1999). The vertebrate retina and ONH have been demonstrated to possess blood-brain barrier (BBB)-like properties, which reside in the endothelial cells (ECs) of blood vessels. These ECs are induced by neighbouring astrocytes that would act as its glia limitans (Tout *et al.* 1993), and are present only in vascular retinas. We used electron microscopy to describe the cp structure, and immunohistochemical methods to detect the glucose transporter isoform 1 (*Glut1*), the transcription factor *Pax2* and the astrocyte markers *vimentin* and *GFAP* during the ontogeny for the lizard *Gallotia galloti*. Our results show that blood vessels arise at E35, and in adults they consist of a continuous endothelium with luminal and abluminal microfolds and tight junctions, besides scarce pericytes surrounded by a thick basal lamina. Closely, the pigmented glial cells (PGCs) form an incomplete sheath around the vessels and additionally, unmyelinated nerve fibers were observed. ECs expressed *Glut1* from E37 onwards, confirming that the cp represents a model of BBB. *Pax2*⁺/*Vim*⁺ cells (E34 onwards), mast cells (at hatching) and *Pax2*⁺/*GFAP*⁺ astrocytes (adult), specially in the basal and inner cp, occupied the intervacular space. These data suggest that: 1. Unmyelinated fibers in the cp might regulate the local blood support; 2. PGCs and pericytes provide vascular stability; 3. The transcription factor *Pax2* may be involved in the differentiation and maintenance of the cp. Supported by: MEC BFU2007-67139/BFI.

P3.30

Human adipose tissue stromal vascular fraction cells differentiate depending on distinct types of media

Barbara Zapala

Jagiellonian University Medical College, Cracow, Poland

Contact: b.nowak82@gmail.com

Objectives: Angiogenesis, a process involving the formation of blood vessels, is essential for many physiological as well as pathological processes. It was shown that human adipose tissue contains a population of non-characterized cells, called stromal vascular fraction (SVF), which are able to

differentiate to several lineages. The aim of the study was to find the condition promoting the differentiation of human adipose tissue progenitors toward endothelial cells as well as to show that SVF cells cooperate with differentiated endothelial cells in capillary network formation. Methods: SVF cells were isolated according to the modified Hauner's method and, after adaptation, cultured in proangiogenic or proadipogenic medium. Cells were characterized by the surface antigens (flow cytometry) and by the expression of genes characteristic for endothelial cells or for adipocytes (quantitative real-time PCR). Then tests verifying differentiation towards endothelial cells were performed. Results: The differentiation of human SVF cells towards endothelium is stimulated by the presence of serum and the absence of adipogenic factors, which was documented by the pattern of gene expression as well as different functional *in vitro* assays. Moreover SVF cells work together with HUVEC cells to perform capillary network. Conclusions: The differentiation of SVF cells to endothelial cells or adipocytes in dependence of used medium is shown. Our work provides a clear model for analyzing the differentiation capacity of SVF cells. Key words: SVF, HUVEC, adipose tissue, angiogenesis. This work was supported by F6 EU SC&CR (LSHB-CT-2004-502988), EU F6 LIPGENE (FOOD-CT-2003-505944) and Polish MNiI (2P05A 132 28) projects.

P3.31

Impaired endothelial function in subjects with multiple cardiovascular risk factors

Teresa Domagala¹, Marek Kaszuba², Katarzyna Kotula-Horowitz², Bartosz Krzyzanowski², Andrzej Szczeklik²

¹Department of Internal Medicine, Department of Biochemistry and

²Department of Internal Medicine, Jagiellonian University School of Medicine, Cracow, Poland

Contact: domagala32@yahoo.com

Background: Impaired endothelial function remains of crucial significance in atherosclerosis, being also predictive of cardiovascular events in subjects both with and without coronary artery disease. Endothelium-dependent dilation (flow-mediated dilation; FMD) of brachial artery is a validated, non-invasive physiological measure widely used to quantify endothelial function. Aim: To assess endothelium-dependent response of brachial artery and relative contributions of traditional cardiovascular (CV) risk factors in a population-based cohort of young police officers. Methods: Fifty police officers (48M, 2F; mean age 41 years) without prior myocardial infarction or stroke were enrolled into the study, with 38 healthy, normotensive, non-smoking volunteers (mean age 27 years) serving as the study controls. Physical and biochemical markers of cardiovascular risk, brachial FMD (at 7 a.m.) were measured in all subjects. After 1 min. of baseline acquisition the cuff was inflated for 5 min. and then deflated to induce reactive hyperemia. Endothelium-dependent response was construed as the dilation of the brachial artery induced by an increased flow. Results: There were: 44 (88%) overweight subjects, 22 (44%) had dyslipidemia, 8 (16%) hypertension and 17 (34%) were current smokers, 18 (36%) had positive CV disease history; CV being a risk factor throughout. Compared to the controls, the following higher mean levels were found in policemen: total cholesterol ($p < 0.0001$), LDL cholesterol ($p < 0.0001$), fibrinogen ($p < 0.002$), glucose ($p < 0.0001$) and homocysteine ($p < 0.0001$). Mean brachial FMD values were significantly lower in the study cases ($8.25 \pm 4.22\%$ vs. $13.95 \pm 4.80\%$, for cases and controls, respectively; $p < 0.0001$). Conclusions: Brachial FMD facilitate non-invasive diagnosis of atherosclerosis and may be used as a surrogate marker of early CV risk. Since in young healthy adults exposed to multiple CV risk factors endothelial dysfunction is an early feature of atherosclerosis, FMD measurement may serve as an effective clinical screening method.

P3.32

Molecular mechanisms of inner ear invagination and otocyst formation

Xiao Rei Sai

Sensory development, Riken Center for Development Biology, Kobe City Chuou-ku, Japan

Contact: saix@cdb.riken.jp

As in other developing organs, individual cells of the inner ear coordinate changes in their shapes so that the tissue can realize its final form. The chick otic placode, the primordium of the inner ear, forms from the superficial ectoderm as a result of the inductive action of mesodermal fibroblast growth factor (FGF). Soon after its induction, the epithelial placode thickens, forms a depression, invaginating and eventually vesiculating to become an otocyst, a hollow enclosed sphere within the cephalic mesenchyme. These morphogenetic events can be considered one of the first responses of the epithelium to otic inducing signals. The mechanisms by which inductive signalling causes changes in cell shape that result in tissue modelling are unclear. We find that FGF signalling promotes cytoskeletal polarization of the otic placode cells through the phosphorylation of basal non-muscle myosin II. Basal myosin II activity results in the clearance of F-actin from the basal side of the cell, establishing a polarity that causes changes in cell shape and results in the invagination of the otic placode. These results provide a mechanism by which extrinsic signals can affect and control the cytoskeletal changes necessary for cell shape and tissue morphogenesis.

P3.33

Redistribution of CacyBP/SIP from neuronal processes to somata during normal aging in rats

Gabriela Schneider, Anna Filipek, Anna Mietelska, Izabela Figiel, Grazyna Niewiadomska

Nencki Institute of Experimental Biology, Warsaw, Poland

Contact: g.schneider@nencki.gov.pl

The CacyBP/SIP protein was originally discovered in Ehrlich ascities tumor cells as a S100A6 target (Filipek and Wojda, 1996; Filipek and Kuznicki, 1998). Later, it was found that CacyBP/SIP is present in different tissues, particularly in neurons of the rat brain (Jastrzebska *et al.*, 2000). The CacyBP/SIP protein interacts not only with S100A6 but also with some other members of the S100 family (Filipek *et al.*, 2002) as well as with Skp1 and Siah-1 (Matsuzawa and Reed, 2001). Recently, we have shown that CacyBP/SIP binds to tubulin, and through this interaction might play a role in reorganization of microtubules and in differentiation of neuronal cells. In this work we examine the function of CacyBP/SIP in rearrangement of the cytoskeleton during aging. We have found that CacyBP/SIP is present in the cytoplasm and in the neuronal processes in brain neurons of young rats and only in the cytoplasm of neuronal somata of aged rats. The changes in CacyBP/SIP distribution are similar to those of a known tubulin binding partner, the tau protein. Differences in CacyBP/SIP localization between neurons of young and aged rats can not be explained by a possible loss of neuronal processes during aging because the pattern of MAP2 protein staining is similar in both aged groups. Also, the results of western blot with anti-CacyBP/SIP antibodies show no significant changes in the level of this protein in young and old rats. Thus, our present work is focused on establishing the mechanism responsible for the observed changes in CacyBP/SIP localization. In particular, we study whether there are any changes in the phosphorylation pattern of CacyBP/SIP in brains of young and aged rats. This work was supported by grants from the MNiSW (2 P04A 01030) to A. Filipek and (2 PO5A 12128) to G. Niewiadomska, and by statutory funds from the Nencki Institute of Experimental Biology. G. Schneider is a recipient of a scholarship from the President of the Polish Academy of Sciences.

P3.34

Organization of selected cytoskeletal proteins in induced senescence in non-small cell lung carcinoma A549 cell line

Anna Litwiniec¹, Alina Grzanka¹, Dariusz Grzanka², Dariusz Smoliński³

¹Department of Histology and Embryology, ²Department of Clinical Pathomorphology, ³Department of Cell Biology, Institute of General and Molecular Biology, Nicolaus Copernicus University, Toruń, Poland

Contact: annalitwiniec@wp.pl

Senescence is not only a natural barrier to tumorigenesis *in vivo*, but it also contributes substantially to the outcome of cancer therapy. In cancer cells, there are various methods of senescence induction, including: oncogene expression, chemotherapy, ionizing radiation, treatment with some cytokines or differentiating agents. Many different features, like increased cell volume, enhanced activity of SA- β -galactosidase, senescence-associated heterochromatin foci formation, are indicative of this phenomenon on the cellular level. Moreover, a possible involvement of certain cytoskeleton components in the senescence process has been suggested. Materials and Methods. We performed the analysis of vimentin and G-actin organization in A549 non-small cell lung carcinoma cell line after treatment with doxorubicin. Cancer cells were incubated in culture with seven gradually increasing doses (10nM, 25nM, 35nM, 50nM, 70nM, 100nM, 200nM) of the cytostatic, during 24 and 72 h periods of treatment. The activity of SA- β -galactosidase at pH 6 was assessed in a light microscopy examination. Organization of vimentin was analyzed using indirect labeling by classical fluorescence microscopy. G-actin organization was studied using direct and indirect labeling by classical and confocal fluorescence microscopy. Results. After treatment with doxorubicin we observed a statistically significant increase in the percentage of cells with elevated SA- β -galactosidase activity in comparison with control cells ($\alpha=0,01$), except for the lowest dose and the longest time of treatment. There were no statistically significant differences between two modes of treatment for all doses compared with one another (ANOVA). As regards vimentin, we observed the collapse on the cell nucleus, local cytoskeleton degeneration and less regular structure, especially in giant cells resulting from the highest doses of doxorubicin. We noted major nuclear G-actin localization, without significant changes in organization. Besides, fluorescence intensity of vimentin and G-actin probably change concurrently with the dose used. It is particularly visible for G-actin in the nucleus area and was confirmed by our measurements with ImageJ software. Conclusions. In our experimental conditions, the senescence program was induced more effectively by increasing the doses rather than prolonged time of treatment. Whereas changes in vimentin organization may be indicative of the senescence execution on the cellular level, in case of G-actin, differences are mainly reflected by fluctuations of its level in the nucleus. Because these processes accompany the enhanced activity of SA- β -galactosidase and broad morphological abnormalities, we suppose that they are related to cellular senescence. However, further studies are needed to understand the significance of the changes in vimentin and G-actin cytoskeleton in this complex phenomenon. This work was supported by grant UMK 33/2008.

P3.35

Application of confocal microscopy and scanning cytometry in the cell cytoskeleton imaging

Agnieszka Turowska¹, Michal M. Godlewski², Joanna Cymerys¹, Anna Chmielewska¹, Anna Tucholska¹, Marcin W. Bańbura¹

¹Department of Preclinical Sciences, Warsaw University of Life Sciences - SGGW, Warsaw, Poland

²Chemistry and Biomolecular Sciences, Division of Environmental and Life Sciences Macquarie University, Sydney, Australia; Department of Physiological Sciences, Warsaw University of Life Sciences - SGGW, Warsaw, Poland

Contact: aturowska@onet.eu

Microfilaments and microtubules play an important role in viral replication. During infection viruses take advantages of different cellular cues and signals to enlist the cytoskeleton for their mission. However, each virus

specifically affects the cytoskeleton structure. Thus, the aim of our study was to investigate the cytoskeleton changes caused by two equine herpes virus 1 (EHV-1) strains, Rac-H and Jan-E, in homologous (ED) and heterologous (Vero) cell lines. We found that the Rac-H strain disrupted actin fibers and reduced F-actin level in ED equine cells, whereas had no influence on Vero cells' cytoskeleton. On the other hand, the Jan-E strain induced F-actin polymerization in Vero cells, but not in ED cells. Noteworthy, the F-actin evaluation was based on combined methods of confocal microscopy and microimage analysis and scanning cytometry – Scan[^]R Screening System. This unique combination allowed us to interpret precisely confocal-based images showing the cellular events induced by EHV-1. Finally, we concluded that the examination of the pathogenic effects of viruses in the species specific cell lines is apparently more symptomatic than in heterologous cell lines.

P3.36

Effect of arsenic trioxide on actin reorganization in K562 erythroleukemia cells

Magdalena Izdebska¹, Alina Grzanka¹, Dariusz Grzanka², Lidia Gackowska³, Maciej Ostrowski⁴

¹Department of Histology and Embryology, ²Department of Clinical Pathomorphology, ³Department of Immunology, ⁴Department of Biochemistry, Institute of General and Molecular Biology, Nicolaus Copernicus University, Toruń, Poland

Contact: mizdebska_m@cm.umk.pl

Actin is one of the components of the cytoskeletal system in eukaryotic cells. Polymerization of this protein is involved in a large number of cellular processes (cytokinesis, endocytosis, chemotaxis). Actin is supposed to be connected with chromatin reorganization in the nucleus as well as with apoptosis. The main object of this study was to analyze the reorganization of actin in whole cells and nuclei isolated from the human leukemia cell line (K562), after treatment with arsenic trioxide. Three different doses of the anticancer drug arsenic trioxide (0.6 µg/ml, 1.2 µg/ml, 2.4 µg/ml) were applied for 24 hours in cell culture. The aim of the study was to investigate the rearrangement of actin, using classical fluorescence microscopy, transmission electron microscopy and flow cytometry. After isolation and purification by glycerol gradient, purity and integrity of nuclei were determined spectrophotometrically and by means of transmission electron microscopy. In order to evaluate F-actin involvement in apoptosis, changes in actin organization were reflected by fluorescent staining with phalloidin-BODIPY, whereas changes in the F-actin level were assessed using flow cytometry. Fluorescence intensity of F-actin rises with increasing doses of arsenic trioxide. Some symptoms of apoptotic cell death including the rate of phosphatidylserine presentation, apoptotic bodies formation and chromatin fragmentation occurred even at the lowest dose of arsenic trioxide. Moreover, at higher drug concentrations there was a tendency toward an increase in the number of cells displaying apoptotic features. The treatment with arsenic also resulted in changes of intracellular actin localization. Visible accumulation of F-actin appeared in the site of apoptotic blebbing. Our fluorescence and electron microscopy observations revealed characteristic apoptotic morphology. The work was supported by the grant UMK 37/2008. Acknowledgements: Trisenox (arsenic trioxide) was kindly provided by Cephalon France.

P3.37

Effects of Taxol treatment on morphology and cell death in CHO AA8 cells

Aleksandra Stepień¹, Alina Grzanka¹, Dariusz Grzanka², Mariusz Szczepański¹, Anna Helmin-Basa³, Lidia Gackowska³

¹Department of Histology and Embryology, ²Department of Clinical Pathomorphology, ³Department of Immunology, Collegium Medicum, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland

Contact: agrzanka@cm.umk.pl

Purpose: The aim of this study was to investigate if Taxol-induced microtubular reorganization in CHO AA8 cells is accompanied by apoptosis. Materials and methods: CHO AA8 cells were incubated with the indicated Taxol doses: 0.25 µM; 0.5 µM; 1 µM for 24h. On the next day, the drug-containing medium was replaced with drug-free medium. The cells were incubated in fresh medium for up to 3 days, in the 37°C incubator. The analyses were performed immediately (day 1), 24h (day 2) and 48h (day 3)

after treatment, using fluorescence microscopy, electron microscopy and flow cytometry (TUNEL assays). Results: The effects of Taxol treatment were time- and dose-dependent. Exposure to 0.25 µM Taxol resulted in the appearance of few giant, multinucleated cells with extensive arrays of fine filaments. There was a slight increase in the level of polyploidy that reached to approx. 8% on day 3 as well as the percentage of TUNEL positive cells (approx. 6% on day 3). At 0.5 µM and 1 µM Taxol, a significant number of multinucleated giant cells with loosely/tightly packed bundles of microtubules or extended microtubular network was observed. The cells showing shrinkage of the cytoplasm and condensation of their nuclei were also seen. These cells formed ring-like microtubular structures surrounding the nuclei. At both 0.5 µM and 1 µM Taxol, the appearance of multiple spindle poles was evident. Disruption of microtubular network was most noticeable in cells incubated with 1 µM Taxol, observed 48h after treatment. It was accompanied by strong multispotted nuclear DAPI labeling. Cell cycle analysis revealed a marked increase in the percentage of polyploid cells (DNA content greater than 4n), which was the highest after incubation with 1 µM Taxol, 48h following treatment (approx. 56%). The results of TUNEL showed the occurrence of DNA fragmentation in a population of cells treated with 0.5 µM and 1 µM Taxol (approx. 90% on day 3). Bivariate analysis of the percentage of TUNEL-positive cells vs. DNA content revealed that dUTP was incorporated into DNA breaks of cells positioned in all phases of the cell cycle. Conclusion: Following Taxol treatment, CHO AA8 cells undergo changes different from apoptosis or necrosis. We suggest that, in the proportion of cells, Taxol may induce cell death through mitotic catastrophe.

P3.38

Immunolocalization of dynamin-2 around discoid vesicles in umbrella cells of mouse urinary bladder

Nobuo Terada, Nobuhiko Ohno, Sei Saitoh, Chao Dong, Yasuhisa Fujii, Shinichi Ohno

Department of Anatomy and Molecular Histology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo-city, Japan

Contact: nobuot@yamanashi.ac.jp

Objectives: Umbrella cells of transitional epithelium in urinary bladders usually expand and contract according to volume changes of urine. It has been thought that the hydrostatic pressure controls exocytosis/endocytosis of discoid vesicles (DVs) in the umbrella cells, but little is known about the way how the special membranes are controlled to form the DVs. Dynamin is a GTPase that has been reported to have a function in endocytotic processes by mediating scission of cell membranes. In this study, we have focused on dynamin expression and immunolocalization in the mouse urinary bladder epithelium. Methods: Immunoelectron microscopy with a pre-embedding method was performed as reported before. Cryostat sections of paraformaldehyde perfusion-fixed urinary bladder tissues of mice were incubated with a primary antibody against dynamin-2, followed with a horseradish peroxidase-avidin-biotin-conjugated secondary antibody, and finally visualized with diaminobenzidine-hydrogen peroxide incubation and osmium tetroxide. Some cryostat sections were incubated with the primary antibody and visualized with 10 nm gold-conjugated secondary antibody. To examine three-dimensional ultrastructures around the discoid vesicles of umbrella cells, the quick-freezing and deep-etching (QF-DE) method was also performed for the paraformaldehyde perfusion-fixed urinary bladder tissues, which were quickly frozen by the metal-contact method. Results: At the light microscopic level, dynamin-2 was more strongly immunolocalized in the umbrella cells than intermediate and basal cells of the transitional epithelium, especially near the apical cytoplasmic regions. By the immunoelectron microscopy, dynamin-2 was localized on and around membranes of the DVs in the umbrella cells. By the QF-DE method, branched filaments were found to be surrounding the DV-membranes. The immunoreactive products for dynamin-2 were detected along the filamentous networks around the DVs, indicating the dynamin-2 interaction with the DV-membranes and such filaments. Conclusions: Our results demonstrate the presence of dynamin-2 in the umbrella cells, closely related to DVs and the surrounding filaments. Considering the previous report that little clathrin or caveolin is immunolocalized around the membranes of DVs, it brings the suggestion of some functional roles of dynamin in the clathrin/caveolin-independent system, to form DVs from the cytoplasmic side of their membranes.

P3.39

The changes of actin cytoskeleton in CHO AA8 cell line after apoptosis and mitotic catastrophe induction by hyperthermia**Dariusz Grzanka¹, Alina Grzanka², Magdalena Izdebska², Anna Helmin-Basa³, Lidia Gackowska³, Andrzej Marszałek⁴**¹Department of Clinical Pathomorphology, ²Department of Histology and Embryology,³Department of Immunology, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland;⁴Department of Clinical Pathomorphology, Collegium Medicum, Nicolaus Copernicus University and Department of Clinical Pathomorphology, Poznan University of Medical Sciences, Bydgoszcz, PolandContact: d_grzanka@poczta.onet.pl

Here, we present studies on the reorganization of the actin cytoskeleton and ultrastructural changes in CHO AA8 cell line after induction of cell death by apoptosis and mitotic catastrophe. For achievement of the aim we used Chinese hamster ovary cells (CHO AA8) cultured in minimum essential medium eagle (MEM). Cell culture were exposed to hyperthermia (45°C) for two hours. To estimate the morphology and changes in actin cytoskeleton fluorescent microscopy and transmission electron microscopy (including immuno-gold technique) were used. Flow cytometry (TUNEL and Annexin V-FITC/7-AAD) was used for estimation of level cells' death. In the present study we showed changes in actin cytoskeleton and in cell morphology. Moreover, expose of cell culture to hyperthermia caused inhibition of cell proliferation. Cell cycle analysis showed increase cell polyploidy. Paralelly, TUNEL assay revealed significant increase of cells with DNA fragmentation and Annexin V-FITC/7-AAD flow cytometry analysis revealed dose dependent increase in the percentage of cells with phosphotyrosine externalisation. Two populations of the cells were observed: rounding, shrinked and detached from the substratum also with bubbles on their surface as well as flattened and giant mono- or multinucleated. In giant and flattened cells F-actin was demonstrated as extensive arrays network and stress fibers. There were also observed cells with strong labeling of F-actin in enlarged nuclei. In the rounded and shrinking ones strong F-actin labeling was observed in the centre of the cells as well as in the buds on their surface. On the ultrastructural level two types of cells were seen. Apoptotic cells with margination and condensation of chromatin in nucleus and cells which were multinucleated or with multisegmented nuclei and distinct chromatin pattern. Giant cells with intracellular small and large vacuoles and containing electron-dense material were also seen. Positivity immunogold labelling for actin was localized in the nuclei, predominantly in the areas of chromatin condensation and also in cytoplasm. Our results suggest that CHO AA8 cells following elevated temperature exposure underwent two different mode of cell death: apoptosis and mitotic catastrophe. Analysis of our results might indicate that reorganization of actin system is involved in both processes.

P3.40

Gelsolin in human melanoma A375 cells grown on lumican**Agata Radwańska¹, Monika Mach¹, Dagmara Baczyńska¹, Yanusz Wegrowski², Maria Malicka-Błaszkiwicz¹**¹Faculty of Biotechnology, University of Wrocław, Wrocław, Poland²Faculty of Medicine, University of Reims-Champagne-Ardenne, Reims, FranceContact: mmb@ibmb.uni.wroc.pl

The recognition of the role of actin binding proteins (ABPs), as wells as extracellular matrix proteins (ECM), involved in cancer cells motility and metastasis formation is required for the development of new therapeutic approaches. Gelsolin (the one of ABPs) is known to control the dynamic rearrangement of actin cytoskeleton in the response to the external stimuli. The level of gelsolin in highly invasive human melanoma A375 cells was found to be elevated to compare with other cancer cell lines. Gelsolin has multiple effects on actin filaments organization i.e. regulation of the length of actin filaments by severing and capping activity. Lumican, the member of small leucine rich proteoglycans (SLRPs) family of ECM, contributes to the collagen network organization. Its role in tumor growth control has been suggested. The suppression of melanoma cells migration in the presence of lumican core protein was already reported. The goal of these studies was to characterize the gelsolin level and its distribution in A375 cells grown on lumican core protein. Moreover these observations were correlated with the changes of invasive factor, induced by lumican in A375 cells. Confocal microscopy was applied for the observations of gelsolin and filamentous actin distribution in A375 cells. Submembrane actin condensation and the decrease in the number of stress fibers in A375 cells induced by lumican were followed by the changes in the gelsolin distribution. Gelsolin was localized only in the center of the A375 cells grown on lumican. On the contrary, in cells grown on glass and collagen gelsolin was condensed also at the cell periphery and colocalized with actin stress fibers. The quantitative densitometric analyses of immunoblots were done to estimate the level of gelsolin in lysates of A375 cells grown at the different concentrations of lumican. The results were compared with those obtained for cells grown on collagen and glass. Statistically significant, decreased concentration of gelsolin was observed in A375 cells grown at the highest concentration of lumican. It was accompanied by the decreased level of the invasive factor calculated by the assay done on Matrigel-coated Transwell filters. The distinct correlation between the decreased gelsolin level and the reduced migration ability of A375 melanoma cells grown on lumican was confirmed.

P3.41

Expression of decorin in esophageal cancer in relation to expression of three isoforms of transforming growth factor-beta (TGF- β 1, - β 2 and - β 3) and matrix metalloproteinase-2 activity

Katarzyna Augoff¹, Krzysztof Grabowski¹, Jerzy Rabczynski², Adam Kolondra³, Renata Tabola¹, Aleksander F. Sikorski³

¹Department of Gastrointestinal and General Surgery, ²Department of Pathological Anatomy and ³Institute of Biochemistry and Molecular Biology, University of Wrocław, Wrocław, Poland

Contact: augoffka@chir.am.wroc.pl

Background and aims: Generation of a reactive stroma environment occurs in many human cancers and is likely to actively contribute and promote tumorigenic processes. Decorin (DCN), an ubiquitous matrix macromolecule belonging to the family of small leucine-rich proteoglycans, is involved in the regulation of collagen fibrillogenesis, the control of cellular growth and, along with metalloproteinases, serving as a modulator of the availability of active transforming growth factors-beta (TGF- β s) in extracellular milieu. In order to define the reactive stroma environment and to determine its role in esophageal cancer progression we investigated the distribution and expression of decorin in relation to the expression of three basic TGF- β isoforms and an activation level of matrix metalloproteinase-2 (MMP-2). **Methods:** Normal esophageal mucosa and cancer tissues taken from 9 patients during esophagectomy for esophageal cancer were examined by Western-blot and RT-PCR methods, immunohistochemistry and zymography to determine the expression of decorin, TGF- β 1, -2, -3 and MMP-2 activity. **Results:** Immunohistochemical studies performed in tumor and normal tissues revealed generally high levels of DCN in the extracellular matrix surrounding clusters of cancer cells as well as in stroma adjacent to normal epithelium of unaffected esophageal tissues. Tumor and epithelial cells did not show any decorin staining. DCN expression on mRNA level was decreased in neoplastic relative to normal stroma, but the differences were not statistically significant. Despite general trend towards increasing levels of TGF- β s expression, observed in pathological tissues, no statistically significant differences were found in cancers as compared to controls. There were no correlations between the levels of DCN and TGF- β mRNAs expression. Activation ratio of MMP-2 was found significantly higher in cancers as compared to normal tissues and inversely correlated with TGF- β 1 mRNA expression in both investigated groups. However, we did not observe elevated levels of TGF- β dimeric forms in cancers in relation to controls. **Conclusions:** The expression and accessibility of factors such as DCN and TGF- β 1, - β 2 and - β 3 isoforms are not significantly altered during the esophageal cancer growth. It may indicate that DCN is not included in the process of stroma activation during the tumorigenesis in the esophagus. Moreover, it seems that the system of TGF- β liberation from the complex with DCN by activated MMP-2 does not play an important role in the ECM reorganization resulting in cancer progression.

P3.42

Immunohistochemical studies of decorin expression in polyps and carcinomas of the colon

Katarzyna Augoff¹, Jerzy Rabczynski², Renata Tabola¹, Leszek Czapl¹, Katarzyna Ratajczak³, Krzysztof Grabowski¹

¹Dept. of Gastrointestinal and General Surgery, ²Dept. of Pathological Anatomy, ³Dept. of Traumatic and Hand Surgery, Wrocław Medical University, Wrocław, Poland
Contact: augoffka@chir.am.wroc.pl

Background: Recent studies have shown that small leucine-rich proteoglycan, decorin (DCN) may suppress tumor progression as a natural anticancer agent negatively controlling cellular growth. We hypothesized that the physiological expression of decorin may be associated with cellular senescence of colorectal mucosa and its down-regulation promoting an increase in cellular proliferation could participate in the progression of adenoma to adenocarcinoma. Therefore, the expression of decorin in hyperplastic polyps and neoplastic polyps of the colorectum was examined and compared

to normal colon mucosa and colon cancer tissues. **Material and Methods:** Tissue samples were obtained from 41 patients with different types of colon polyps (6 hyperplastic adenomas, 34 neoplastic adenomas and 1 adenomatous polyp with focal carcinoma) and from 12 patients with colon cancer. Seven samples of normal colon tissue were used as controls. Paraffin-embedded samples were used for an immunohistochemistry study. **Results:** Normal and hyperplastic tissues as well as the majority of tubular adenomas showed strong expression of decorin in the stroma. Adenomas with the villous component were characterized by moderate and very low decorin immunoreactivity. The decrease of decorin reactivity in tubulovillous adenomas was significant as compared with other polyps and with controls. Weak decorin immunoreactivity in stroma adjacent to clusters of cancerous cells was also found in most cases of the common type of adenocarcinoma but not adenocarcinoma mucinosum. **Conclusion:** These findings suggest that: (i) expression of decorin may be involved in the differentiation of colon polyps; (ii) reduced expression of decorin may abrogate the defensive potential of stromal tissue and promote the development of the common type of colon carcinoma.

P3.43

Evaluating the effects of intermittent hypoxia on collagen

Mine Gultomruk, Tugba Kotil, Fadime Aktar, Ebru Karabulut, Seyhun Solakoglu, Yurdagul Canberk

Dept. of Histology and Embryology, Istanbul Faculty of Medicine, University of Istanbul, Capa, Istanbul, Turkey

Contact: ycanberk@istanbul.edu.tr

Repeated episodes of hypoxia interspersed with episodes of normoxia, namely intermittent hypoxia has long been evaluated as a stimulant for adaptive mechanisms of tissues and cells. Although much is known about the acute and chronic effects of sustained hypoxia, less is known about the effects of intermittent hypoxia. Research data suggest that there are distinct protective effects of hypoxia-reoxygenation episodes against oxidant stress, both in animal experiments and in human studies [1-3]. It has been shown that brief periods of hypoxemia (the same as during the usual interval hypoxia training [IHT] session) result in adaptation to hypoxia without destructive effects. In this study we aimed to evaluate the effects of intermittent hypoxia on dermal tissue components using quantitative and qualitative methods regarding collagen synthesis. Besides several histochemical staining methods, spectrophotometric analysis for collagen precursor hydroxyproline and immunohistochemical staining for procollagen were applied. The change of collagen content and other parameters of the skin induced by hypoxia were investigated. 40 wistar male rats weighing 300-350g were used and divided into 2 groups: air-breathing group (A group, 20 rats) and intermittent hypoxia group, (IH group, 20 rats exposed to a gas mixture containing 10% of O₂ for 60 min daily for 23 days). Collagen amount was analysed by spectrophotometric hydroxyproline assay and in skin specimens. These values were used to estimate glucosaminoglycan content. Histological analyses were performed on skin samples taken at 1st, 15th and 30th days which were fixed in 10% buffered formalin and embedded in paraffin. Sections were examined under bright field and polarized light. Dermal thickness was measured using a micrometer eyepiece with a 40x objective. Spectrophotometric analyses showed that collagen content was increased to 154% (P<0.05). Histochemical and immunohistochemical evaluation revealed accelerated deposition of collagen molecules in the hypoxia treated group. These results suggest that hypoxic enhancement of the posttranscriptional step of collagen synthesis contributed to the accelerated deposition of collagen fibrils and elastotic masses in the papillary dermis of treatment group when compared with the control group. **References:** 1. Manukhina *et al.* Role of Nitric Oxide in Cardiovascular Adaptation to Intermittent Hypoxia. *Experimental Biology and Medicine* 231:343-365 (2006). 2. Robert M. Douglas, *et al.* Chronic intermittent but not constant hypoxia decreases AA/Cr ratios in neonatal mouse hippocampus and thalamus. *Am J Physiol Regul Integr Comp Physiol* 292: R1254-R1259 (2007). 3. Acute intermittent hypoxia activates myocardial cell survival signaling Ah-Mee Park, *Am J Physiol Heart Circ Physiol* 292: H751-H757 (2007).

P3.44

Matrix metalloproteinases and their tissue inhibitors in remodeling of bronchoalveolar duct junctions

Evguenia Kogan, Vuong Tuong, Sophia Demoura

Setchenov Moscow Medical Academy, Moscow, Russia

Contact: tphivuong@yahoo.com

Background. The goal of the study was to investigate the relationships between the expression of matrix metalloproteinases as well as their inhibitors and processes of apoptosis, cell proliferation and angiogenesis in patients with idiopathic pulmonary fibrosis (IPF). Design. Lung biopsies from 30 patients with early stages of IPF and 10 control patients (normal lung tissue with diagnosis of sarcoidosis) were used. Immunohistochemistry on paraffin sections had been done with antibodies to MMP 1, 2, 7 and TIMP 4, Apo-protein, TGF β , PCNA, PDGF, EGFR (Lab Vision), CD34, SMA (Novocastra). Levels of marker expression in each type of cells had been analyzed separately in the area of bronchoalveolar duct junctions (BADJ), interalveolar interstium and in normal lung tissue. Expression levels of the investigated markers were estimated by the percentage of positively stained cells. The results were reported as mean \pm SEM. The nonparametric Mann-Whitney test and Spearman correlation test were used to analyze the results. Results. We found that adenomatous, myofibroblasts' foci, and foci of angiogenesis were present predominantly in the area of BADJ in an early stage of idiopathic pulmonary fibrosis. Strong feed-forward correlation was found between the expression level of MMP-7 by proliferating alveolar epithelial cells (AECs) and macrophages with the level of AEC apoptosis ($r_s = 0,97$; $P < 0,001$) in the regions of BADJ. Statistically significant correlation exists (strong feed-forward) between the level of Apo-protein expression in AECs in the regions of BADJ and the expression level of PCNA by myofibroblasts in adjacent myofibroblast foci ($r = 0,856$, $P < 0,001$). Moreover, strong feed-forward correlation was observed between the expression level of MMP-2 by proliferating AECs and macrophages in the regions of BADJ with the intensity of angiogenesis in adjacent angiogenesis foci ($r_s = 0,82$; $P < 0,05$). There was no correlation between the investigated markers at the sites of interalveolar interstium in IPF and in normal lung. Conclusion. We propose that remodeling of the regions of BADJ together with imbalance of MMPs and their inhibitors are the key events that contribute to the onset and progression of IPF. Better understanding of biochemical and molecular mechanism in the pathogenesis of IPF will lead to more specific and targeted therapies.

P3.45

Stromal reaction at the invasive margin of pT3 colorectal adenocarcinomaRobert Rzepko¹, Rafał Pęksa¹, Adam Nałęcz¹, Wiesław Kruszewski², Kazimierz Jaśkiewicz¹¹Medical University of Gdańsk, Department of Pathomorphology, Gdańsk, Poland²Medical University of Gdańsk, Gdańsk, PolandContact: czita123@poczta.onet.pl

The colorectal cancer tumours are composed of neoplastic cells and fibrovascular connective tissue stroma. The type and intensity of stromal reaction most likely play a role in tumor development and progression. We examined the presence of different types of stroma and occurrence of different patterns of neoplastic growth at the invasive margin of 113 colorectal adenocarcinomas. Patients were operated between 2001 and 2004 in the Departments of Surgery and Surgical Oncology, Medical University of Gdańsk. All the investigated neoplasms were pT3 tumours (according to TNM classification) located in sigmoid colon and rectum. The examined parameters were evaluated in tumour fragments (with most advanced invasion) stained HE, Masson trichrome and immunohistochemically using anti-ASMA antibody. Based on the results published by Ueno *et al.* (Gut 2004;53:581), we distinguished two different types of cancer stroma – mature (composed of mature collagen fibres) and immature (with keloid like broad bands of collagen and/or myxoid stroma). We classified the pattern of infiltrative growth with regard to the presence of cancer foci with "direct adipose tissue invasion" (in these fragments of tumour myofibroblastic stromal reaction was not observed). In the second group of tumours without "direct adipose tissue invasion" on the edge of cancer intensive myofibroblastic proliferation was found. Results: We found the mature type of stroma in 72 (64%) investigated tumours and the immature in 41 (36%) cases. Infiltrative growth "with" and "without direct invasion of adipose tis-

sue" were observed in 49 (43%) and 64 (57%) cancers respectively. Mature type of stroma and cases "without direct invasion" correlated with better survival time (Kaplan-Meier method and log-rank test; $p = 0,29$ and $p = 0,004$ respectively). We have found a strong correlation between the mature type stroma and tumours without "direct invasion", while the immature stroma was correlated with "direct invasion of adipose tissue". (R Spearman correlation coefficient 0,66; $p < 0,001$). Conclusions: There are correlations between the type of stroma and the pattern of invasion in colorectal cancer tumors. The examined parameters are related to the clinical course of neoplastic disease.

P3.46

Effects of iloprost on calvarial suturesGökhan Tunçbilek¹, Petek Korkusuz², Figen Özgür³¹Hacettepe University Faculty of Medicine Dept. of Plastic & Reconstructive Surgery²Hacettepe University Faculty of Medicine Dept. of Histology & Embryology and³Hacettepe University Faculty of Medicine Dept. of Dept Plastic & Reconstructive Surgery, Ankara, TurkeyContact: petek@hacettepe.edu.tr

Objectives: Premature fusion of calvarial sutures is the result of a long and complex reaction and several growth factors including transforming growth factor-B and basic fibroblast growth factor have important roles in this event. Several prostaglandins have important functions in local bone modeling and remodeling by autocrine and paracrine mechanisms. Although the effects of prostaglandins on long bones were studied both experimentally and clinically, there is limited data about cranial bones and sutures. In this study we investigated the effect of iloprost – a stable prostacyclin analogue, which is widely used for the treatment of pulmonary arterial hypertension (PAH) even in early pregnancy, on rat calvarial sutures. Methods: In two study groups, iloprost was injected intraperitoneally 10 and 15 Mg/kg/day, respectively. In the third group dexamethasone 2 mg/kg/day + iloprost 15 Mg/kg/day was injected intraperitoneally to antagonize the effects of iloprost. In every group, four rats were sacrificed at the postoperative 15, 30 and 45 days and specimens including the sagittal and frontal sutures were excised immediately. Routine histological and immunohistological FGF and TGF staining were performed on the specimens. Morphological measurements were performed on the skulls. Results: In histological evaluation, bone formation in both the frontal and sagittal suture areas was increased and accelerated in iloprost groups. Dexamethasone inhibited the effects of iloprost on the third group. Expression of TGF-b and bFGF was also increased in immunohistological staining. In morphological measurements statistically significant differences were found between the control and study groups. Iloprost did not fuse the rat calvarial sutures prematurely but it narrowed the sagittal and frontal sutures especially after the second week of the study. This situation might affect the sutures of the babies of the pregnant patients with PAH treated with iloprost. Conclusion: Cranial sutures, calvarial bones, and cranial shape of the babies of the pregnant patients who were treated with iloprost should be monitored to clarify the topic. Key words: cranial suture, prostacyclin, TGF, FGF.

P3.47

Expression of MMP9 in guinea pig skeletal muscles after administration of selected neuropeptidesHanna Gendek-Kubiak¹, Ewa Gendek²¹Department of Cytophysiology, Histology and Embryology and²Department of Chemistry and Clinical Biochemistry,

Medical University of Łódź, Łódź, Poland

Contact: h_kubiak@hotmail.com

In our earlier papers we have described the appearance of some muscle fiber damage and cellular infiltrations after intramuscular injections of substance P, guinea pig VIP and neuropeptide Y. Among the known factors which might influence inflammation spreading in tissues, there are matrix metalloproteinases (MMP) secreted into extracellular matrix by different cells, including inflammatory infiltration cells. An idiopathic inflammatory myopathy (IIM) group of diseases includes the ones in which inflammatory infiltrations, comprising mononuclear cells, and muscle fiber damage/regeneration signs are present. From among the metalloproteinases studied by different researchers, in muscle specimens from IIM patients, the most characteristic feature was a positive reaction for matrix metalloproteinase 9 (MMP9) in the extracellular matrix, infiltrating cells and in

some changed and regenerating myofibers, while it was absent in muscle specimens from healthy persons. In this study, we have aimed to elucidate changes of immunodetectable MMP9 presence in guinea pig muscle specimens during up to 72 hours after a single intramuscular (into a quadriceps muscle) injection of SP, guinea pig VIP or NPY solutions at concentrations reported by us earlier. Specimens taken from the quadriceps muscle at the opposite side of the body, on 0.9% NaCl solution injection or on no injection at all, served as control muscle specimens. In the connective tissue of control muscle specimens, only single cells tested positive for MMP9. After a single injection of any from among the neuropeptides used, vascular alterations, inflammatory infiltrations and myofiber damage signs, similar to the ones described by us earlier, were observed. MMP9 immunostaining was found in scattered infiltrating cells, some injured muscle fibers and in regenerating myofibers, as well as in endothelial cells and smooth muscles of the blood vessels. Overexpression of MMP9 may facilitate increased penetration of not only different molecules, but also of infiltrating cells, to endomysium and thus their immediate contact with the muscle fibers. The neuropeptides studied by us, on their intramuscular injection, induce a cascade of reactions leading to MMP9 activation. The expression of MMP9 in the course of IIM in people and after intramuscular neuropeptides administration seem to be worthwhile further comparative studies. This work has been supported by the Medical University of Łódź (grant numbers: 502-18-558 and 502-17-555).

P3.48

Left ventricular fibrillar collagenous network remodeling in ischemic heart disease

Jolita Palubinskiene, Dalia Pangonyte, Elena Stalioraityte, Danute Kazlauskaitė, Reda Zhiuraitiene

Institute of Cardiology, Kaunas University of Medicine, Kaunas, Lithuania

Contact: jolipalu@itc.kmu.lt

The aim of the study was to evaluate peculiarities of human myocardial interstitial collagen network remodeling in the left ventricular and interventricular septum myocardium, distant from acute injury or healed infarct

site. Object and methods. Hearts of suddenly deceased 86 males (aged 48.6 ± 8.2 years) who died suddenly out-of-hospital (within 6 hours of the onset of the terminal event) due to the first acute ($n=51$, preinfarction ischemic heart disease (IHD) group) or repeated ($n=35$, postinfarction IHD group) event of IHD, and had no other cause of increased load on the heart, except ischemia, were studied morphologically, macro- and histomorphometrically. The control group consisted of males ($n=26$) of similar age (46.7 ± 7.3 years), who succumbed to external causes, not associated with cardiac pathology. As Sirius-red in saturated picric acid solution is specifically binding to interstitial collagen fibres, we employed this quality in quantification of myocardial interstitial collagen. Computerized image analysis system Quantimet 520 (UK) was applied for histomorphometry. The volume percent, perimeter, numbers of fiber foci of interstitial collagen per field were assessed from left ventricular myocardium intramural part area, distant from acute ischemic injury or healed infarct. Approximately 100 fields were examined in each slice, magnification 700 times, area of one field 35578 m^2 . Multifactorial analysis of variance (ANOVA) was applied to compare histomorphometric parameters between groups. Results. Interstitial collagen percentage volume, perimeter and the number of separate fiber foci per field of left ventricular myocardium in the preinfarction IHD group was greater than in the controls (by 31, 26 and 19 percent respectively, $p < 0.05$). Pearl-Reed's limited augmentation curve of regression was determined between percentage volume of collagenous fibers and stenotic index of coronary arteries (scored points of total segmentary stenosis) ($R=0.77$, $p < 0.0001$). The induced collagen network remodeling process progresses during the postinfarction phase: collagen percentage volume of left ventricular myocardium was higher by 40 percent, perimeter by 39 percent, and number of fiber foci per field by 32 percent in the postinfarction IHD group as compared with the same indices of preinfarction IHD group ($p < 0.05$). The largest parameters of left ventricular collagen percentage volume were determined in the tercile of the largest healed infarct. Conclusions. Remodeling of the left ventricular myocardium interstitial collagen network – interstitial fibrosis – is induced by chronic myocardial ischemia (prior to the first myocardial infarction) and progresses after the myocardial infarction.

P3.49

Alterations of testicular morphology and blood-testis barrier in irradiation and hyperthermia exposed rats: evaluation for infertility

Gozde Erkanli¹, Feriha Ercan¹, Cenk Umay², O. Uzel²

¹Marmara Univ. School of Medicine, Dept. of Histology-Embryology, Istanbul, Turkey

²Istanbul Univ. Cerrahpasa Medical Faculty, Dept. Radiation Oncology, Istanbul, Turkey

Contact: eferiha@hotmail.com

Administration of irradiation (IR) and hyperthermia (HT) causes infertility by decreasing the spermatogenic colony growth and the number of sperms in rats. The aim of this study was to investigate the alterations of testicular morphology and blood-testis barrier in rats exposed to in utero irradiation and postnatal hyperthermia. Four groups of rats were used in this study: 1) Control group, 2) HT group (rats exposed to hyperthermia in post-natal 10th day), 3) IR group (rats exposed to IR on the 17th gestational day), 4) IR+HT group (rats exposed to IR on the 17th gestational day and then to HT in post-natal 10th day). Three and six months after the experimental procedures rats were perfused with 4% paraformaldehyde and testis tissue samples were prepared for ZO-1 and occludin immunohistochemistry, and electron microscopy. Regular seminiferous tubules with spermatogenic cells and tight junctions, ZO-1 and occludin immunoreactivity were observed in control rats. Some degenerated seminiferous tubular cells with vacuoles and quite regular tight junctions in HT group; decreased number of spermatogenic cells and degenerated seminiferous tubular cells with many vacuoles in the cytoplasm and beneath the dilated tight junctions in the IR group; atrophic tubules with only Sertoli cell line and severe degeneration in tight junctions in IR+HT group were observed after three months. ZO-1 and occludin immunoreactive cells were decreased in HT and IR groups but were absent in IR+HT group after three months. These ultrastructural and immunohistochemical results were more severe after six months in all experimental groups. In conclusion, irradiation and hyperthermia administration caused a decrease in the blood-testis barrier proteins ZO-1 and occludin, and a severe degeneration of seminiferous tubules with increased spermatogenic cell death. Administration of irradiation and hyperthermia in the developing rats may cause infertility due to a decreased number of spermatogenic colony cells and an altered blood-testis barrier proteins ZO-1 and occludin in testis.

P3.50

Ultrastructural and histological alterations of rat testis after bilateral vasectomy

Gazi Contuk, Feriha Ercan

Marmara University, School of Medicine, Dept. of Histology-Embryology, Haydarpasa, Istanbul, Turkey

Contact: eferiha@hotmail.com

Vasectomy is a simple, effective and one of the most widely used methods of male contraception. The operation causes an interruption in the luminal continuity of the vas deferens and, thus, interferes with the transport of spermatozoa from the testis and epididymis into the ejaculatory duct. The aim of this study was to investigate the effect of vasectomy on histological appearance of the testis. In this study, 18 young adult Wistar Albino male rats underwent bilateral vasectomy operation and 18 rats were sham-operated. Then, 6 animals per group were sacrificed 1 month, 3 months and 6 months after the operations. The changes in tubular structure, germinal epithelium, interstitium and connective tissue were examined at light and electron microscopic (TEM) level. Regular seminiferous tubules with spermatogenic cells were observed in sham-operated rats. One and 3 months after vasectomy, degenerated seminiferous tubules with decreased number of spermatogenic cells, vacuolated appearance of the epithelium, accumulation of spermatozoa with immature germ cells in the lumen of the seminiferous tubules were observed. Atrophic seminiferous tubules and increased connective tissue in peritubular spaces were also observed 3 months after

vasectomy. Six months after vasectomy, increased number of atrophic seminiferous tubules, diffuse cellular damage and severe increase in connective tissue in peritubular spaces were observed. In conclusion, bilateral vasectomy caused increased degeneration of seminiferous tubules and spermatogenic cell loss. In the present study, degenerative changes were shown to be increased with time after vasectomy.

P3.51

Effects of in utero ethanol administration on rat testicular morphology and cell death: evaluation for infertility

Yasemin Ersoy, Feriha Ercan

Marmara University, School of Medicine, Department of Histology-Embryology, Haydarpasa, Istanbul, Turkey

Contact: eferiha@hotmail.com

Ethanol, a reproductive toxin, has inhibitory effects on testicular functions in human and animals. Chronic ethanol use causes testicular atrophy, decrease in sperm production and testosterone levels. Its use in pregnancy also causes teratogenic effects. The aim of the present study was to investigate testicular morphology and cell death in the offspring of rats which were given ethanol on the last week of their pregnancy. Pregnant rats were given either water (control group) or 30% aqueous ethanol (ethanol group) beginning from the 14th gestational day until birth. Testicular samples were then obtained from newborns, and from 15-day-, 1-month- and 3-month-old offspring after the administrations. Testis samples were prepared for light microscopy and transmission electron microscopy. Paraffin sections were stained with the TUNEL method to observe cell death. Ultrathin sections were stained with uranyl acetate and lead citrate for ultrastructural investigation. Control rats showed regular testis morphology. The ethanol administration caused disarrangement of cells in seminiferous tubules in newborns and 15-day-old rats; disarrangement of spermatogenic cells and abundance of cell debris in seminiferous tubule lumen in 1-month-old rats; and decreased cell numbers in spermatogenic cells and interstitial fibrosis in 3-month-old rats. The number of TUNEL-positive cells were more increased in ethanol administrated 15-day-old rats compared to all other groups. TUNEL-positive cells were also increased in 1-month- and 3-month-old ethanol administrated rats when compared to the control rats. In conclusion, in the present study, in utero ethanol administration was shown to cause severe degeneration of seminiferous tubules with increased spermatogenic cell apoptosis, and these findings may suggest to result in infertility due to decreased spermatogenic cells in testis.

P3.52

Adenosine improves burn-induced dermal and gastric injury: a histopathological study

Pinar Turan, Yasemin Kocabas, Rumeysa Durak, Berrak Yegen B, Serap Arbak

Marmara Un. Medical Faculty, Istanbul, Turkey

Contact: arbaks@yahoo.com

Introduction: Numerous studies have highlighted the anti-inflammatory role of adenosine. Owing to its immunomodulatory and anti-inflammatory properties, we investigated the potential therapeutic effect of adenosine against burn-induced skin and gastric tissue injuries using a histopathological approach. Material and methods: Under ketamine anesthesia, dorsum of the Wistar albino rats was exposed to 90°C water bath for 10 s, while the dorsum was dipped in 25°C water in the control group. Adenosine (5 mg/kg) or saline was administered intraperitoneally immediately after the burn and the injections were repeated twice a day. In both saline- and adenosine-treated burn groups, rats were decapitated at 6 h and 48 h following burn injury. Skin and gastric tissue samples were processed for light and scanning electron microscopy. Paraffin sections were stained with Masson's trichrome and hematoxylin & eosin for skin and stomach. Results: 1. Stomach. In saline-treated burn groups (6 h and 48 h), stomach mucosa was significantly disrupted with severe mucosal hemorrhage and epithelial desquamation. In adenosine-treated burn groups (6 h and 48 h) a nearly

regular surface epithelium and slightly degenerated glandular cells indicated a tissue recovery. Scanning electron microscopical investigations of the saline-treated burn groups (6 h and 48 h) revealed extensive epithelial loss with totally exposed basal lamina, hemorrhage and widened gastric pits. In the adenosine-treated 6-h burn group gastric mucosa represented a regular epithelial arrangement with few widened gastric pits, while in the adenosine-treated 48-h burn group stomach tissue depicted gastric mucosal integrity implying a high degree of tissue improvement. 2. Skin. In both burn groups prominent skin tissue degeneration was reflected by totally desquamated epidermis and excessively damaged hair follicles. Adenosine-treated 6-h group represented an increased epithelial regeneration with mostly intact dermal structures, while the 48-h adenosine group demonstrated a prominent increase in epidermal height and minimal damage in dermal structures, reflecting good skin histology. Conclusion: Findings of the present study suggest that adenosine possesses an anti-inflammatory effect on burn-induced dermal and gastric damage and protects the tissues against burn-induced degeneration.

P3.53

***In vitro* effect of chitosan on fibroblastic cell activity: immunocytochemical and scanning electron microscopical study**

Bahar Uslu¹, Serap Arbak¹, Ayhan Bilir², Julide Tanbuga³, Suna Ozbas³, Burcu Biletkin⁴, Seçkin Denir⁴

¹Dept. of Histology and Embryology, Medical Faculty, Marmara University, Marmara, Turkey

²Dept. of Histology and Embryology, Medical Faculty, Istanbul University, Istanbul, Turkey

³Faculty of Pharmacy, Marmara University, Marmara, Turkey

⁴Dept. of Histology and Embryology, Medical Faculty, Istanbul University, Istanbul, Turkey

Contact: arbaks@yahoo.com

Chitosan, a linear polysaccharide, has been recently used in biomedical applications. *in vitro* studies demonstrated the effect of on cellular growth and its stimulatory action on cellular layer formation. Our present study aims to compare the proliferative effects of chitosan in membranous and solution forms on Swiss 3T3 mouse embryonic fibroblasts. Methods. Cells were cultured in a normal medium without chitosan (Control Group); cells were cultured either in a medium containing 2.0% chitosan in membranous form (Membrane Group) or 100µl of chitosan solution in concentration 2.0% (Solution Group). Two different methods have been used in the experiments: cells cultured in the medium containing chitosan in solution or membranous form (Method 1); containing previously cultured cells chitosan solution or membranous forms were added into the medium (Method 2). On the 5th and 10th day of the experiment for both methods, cells were treated with 5-bromo-2-deoxyuridine (BrdU) and then incubated with anti-BrdU primary antibody to assess cellular proliferation. MTT {3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrasolium bromide}-test was performed in order to assess cellular viability. Mouse monoclonal anti-collagen 1 primer antibody was used to indicate collagen synthesis. For each group, spheroid cell cultures grown in the above-mentioned media were investigated at transmission (TEM) and scanning electron microscopical (SEM) levels. Results. BrdU labeling test indicated a higher proliferation index in membrane group of method 1 on the 5th and 10th day. For second method, membranous form on the 10th day, and solution form on the 5th day were the most effective groups by means of cellular proliferation. MTT results reflected a high cellular viability in the method 1 on the 5th day of membranous form, whereas cellular viability was highest in the solution form of method 2 on the 5th day. SEM investigations on the experimental groups showed well-defined cellular projections, intact cellular membranes and tight intercellular junctions. They were prominent especially in the membrane group of Method 1 and in the membrane and solution groups of Method 2. Prominent collagen synthesis by the cells was demonstrated in the membrane groups on the 10th day for both methods. In conclusion we found that chitosan in membranous form on the 10th day of the experiment exhibited a significant proliferative effect and increased the number of cell-to-cell junctions in the cultures of Swiss 3T3 mouse embryonic fibroblasts.

P3.54

Effects of zinc deficiency on the epithelial and Paneth cells in the rat small intestine

Eiko Murata¹, Masumi Akita², Kumiko Yamada¹, Yuka Eiki¹, Makoto Nodera¹, Kunio Kitamura¹, Hiroaki Shindo³, Katsuji Kaneko³

¹Faculty of Health and Medical Care, Saitama Medical University, Hidaka, Japan

²Division of Morphological Science, Biomedical Research Center, Saitama Medical University, Moroyama, Japan;

³Nihon Institute of Medical Sciences, Moroyama, Japan

Contact: emurata@saitama-med.ac.jp

Zinc deficiency is associated with a variety of clinical features such as hypogeusia, hyposmia, growth retardation, dermatitis, alopecia, gonadal hypofunction, abnormal pregnancy, susceptibility to infections, delayed wound healing, impaired glucose tolerance, and increased carcinogenesis. In an inflammatory bowel disease such as Crohn's disease, deficiencies of trace elements including zinc are observed, and Paneth cells and their intracellular granules are decreased. We investigated the effects of zinc deficiency on the epithelial and Paneth cells. Materials and methods: Five weeks old male SD rats were fed for 16 weeks with a zinc deficiency diet (0% Zn diet: Zn deficiency group) as well as with a control diet (10mg ZnCO₃ added to 100g Zn deficiency diet: control group). Zinc concentration in serum and a tissue fragment of the small intestine were measured by atomic absorption spectrometry. Histochemical, immuno-histochemical and electron microscopical studies were also performed. Results and discussion: In the Zn deficiency group, 15-30% decrease of zinc concentration was observed in the serum and the tissue fragment. Electron microscopical observation revealed some morphological changes of microvilli accompanied with different histochemical reactivity of the intracellular granules in the Paneth cell. Intracellular granules contain an antibacterial peptide α -defensin, which is activated by processing of a zinc related enzyme (matrilysin; matrix metalloproteinase-7; MMP-7). Localization of positive granules in the apical part of Paneth cells for α -defensin 5 suggests the reflection of this processing. We will conduct further investigation on the involvement of matrilysin on the activation of α -defensin.

P3.55

***In vitro* changes of cell proliferation and *in vivo* lung injury after exogenous surfactant treatment**

Andrzej Marszałek¹, Lidia Gackowska², Monika Seget³, Elżbieta Kaczmarek³, Ewa Florek⁴, Wiesława Biczysko³

¹Department of Clinical Pathomorphology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Torun, Poland and Department of Clinical Pathomorphology, Poznan University of Medical Sciences, Poznań, Poland

²Department of Immunology, Collegium Medicum in Bydgoszcz,

Nicolaus Copernicus University, Bydgoszcz, Poland

³Department of Clinical Pathomorphology and ⁴Department of Toxicology, Poznan University of Medical Sciences, Poznań, Poland

Contact: amars@cm.umk.pl

Semi-natural and artificial pulmonary surfactant preparations have been used for decades with very good results in treatment of respiratory distress syndrome in newborns. Treatment of acute respiratory insufficiencies in adults was less spectacular and provides still unsatisfactory results. The aim of the present study was the evaluation of effects of therapeutic dose of semi-natural surfactant on cell properties in *in vitro* and *in vivo* experimental models. We studied the effect of a single dose of exogenous surfactant on activation of peripheral blood mononuclear cells (PBMC) as well as the lung parenchyma morphological changes related to exogenous surfactant treatment. In this project we used pathogen-free rats. Young adult animals (210-230 g of b.w.) were given single dose of semi-natural surfactant (Curosurf; 150 mg of lipids/kg of b.w.). Lungs (at least 5 animals in one time group) were collected at specific time intervals after 1, 6, and 24 hours and 5, 10, 21 and 42 days. Tissue samples were processed and finally examined by light microscopy and transmission electron microscopy. In a second (parallel) experiment, we used PBMC in cell cultures which were treated with different concentrations of aforementioned preparation of surfactant. In the cell culture studies we found that high surfactant concentrations caused inhibition of cell proliferation. Using lower concentrations of surfactant in the next experiment we found that brief pre-stimulation of cells in culture by surfactant caused much higher proliferative response in both cultures of peripheral blood lymphocytes (PBL) and monocytes. The

response was found exponentially high in monocytes and PBMC cultures. In the material from *in vivo* studies we found different morphological effects. After surfactant treatment we observed different levels of atelectasis in different lung areas (at 1h observations in some samples only about 10% of lung was aerated) which were statistically significant as compared with controls. Moreover, at first time intervals in experimental lungs there were collapsed alveoli with parenchymal and intraalveolar edema with ultrastructural features of cell membrane destruction (in epithelial and endothelial cells, and erythrocytes). After 24h of observations enormous inflow of pulmonary macrophages into areas with abundance of exogenous surfactant was found. Moreover in such areas soon after acute injury we observed proliferation of type II pneumocytes. At 21 and 42 days of observations alveolar septa were widened by the proliferation of fibroblasts as well as accumulations of extracellular components (mainly collagen). In conclusion, our results suggest that surfactant treatment activates macrophages and to some degree lymphocytes-macrophage axis in the *in vitro* studies. Macrophage activation could partially explain some features of acute (partially reversible) and especially prolonged injury (fibrosis) to normal lung tissue after surfactant treatment.

P3.56

Immunohistochemical and ultrastructural findings on human placental stem villi

Leyla Sati, Yasemin Seval, Ramazan Demir

University, Antalya, Turkey

Contact: akademisyen07@yahoo.com

Introduction: During pregnancy, the placenta undergoes growth and remodeling that require degradation and synthesis of extracellular matrix components. Differentiation of mesenchymal cells, fibroblasts, myofibroblasts and muscle cells occur during stromal villi maturation. In this study we aimed to investigate the differentiation of cellular elements in the stroma of the human placental stem villi. **Methods:** A total of 14 human placental tissues (36-38 weeks) were studied. Double immuno-labeling was performed for desmin and vimentin or α -smooth actin and desmin or α -smooth actin and vimentin on formalin-fixed, paraffin embedded tissue sections. Parallel tissue samples were taken for electron microscopy. Distribution of cells in stem villi stroma was evaluated semiquantitatively according to ultrastructural parameters. Group comparisons were drawn using ANOVA. **Results:** Desmin-vimentin double immunostaining was mainly localized in the triangular shaped stroma surrounded by vessels and to a lesser amount in the perivascular area and vessel walls ($P = <0.001$). However, desmin- α smooth actin staining was observed predominantly in the vessel wall and perivascular area ($P = 0.063$). Vimentin- α smooth actin immunoreactivity was significantly stronger in the triangular and perivascular areas compared to the vessel walls ($P = 0.003$). Ultrastructurally, cells in stem villi stroma were reticulum, Hofbauer, mesenchymal, filamented, and vacuolated and smooth muscle cells, fibroblasts and myofibroblasts, each showing varying degrees of maturation and polymorphism. The mesenchymal stem cell was rarely observed. Transitional myofibroblastic forms with characteristics of both fibroblast and smooth muscle cells were observed having invaginated nuclei, well-developed rough endoplasmic reticulum, mitochondria. **Conclusions:** Cellular differentiation between fibroblastic cells and smooth muscle cells may play a role in villous contractility and modulation of the intervillous space with effects on materno-fetal placental circulation. The cross-talk between stromal cells under severe hemodynamic stresses is crucial and may lead to either normal pregnancy or spontaneous abortion. These studies will also lead to practical applications in the control of clinical situations such as unexplained infertility.

P3.57

Changes of intercalated disk in case of congenital heart malformations in humans – immunohistochemical, ultrastructural and morphometrical studies

Dariusz Tosik¹, Jacek Moll², Jadwiga Moll³, Andrzej Kulig⁴, Hieronim Bartel¹

¹Department of Histology and Tissues Ultrastructure, Medical University, Łódź, Poland

²Department of Cardiosurgery, ³Department of Cardiology and

⁴Department of Clinical Patomorphology, Polish Mother's Memorial Hospital Research Institute, Łódź, Poland

Contact: tosik@achilles.wam.lodz.pl

Intercalated disk is treated now as a single functional unit of the cardiac muscle. Its function is connected with mechanical junction of cardiomyocytes and regulation of signal conduction. The influence of the adherens junction-associated molecules (N-cadherin and desmoplakin) on heart development was also investigated. The aim of the study was to investigate potential changes in the intercalated disk ultrastructure in case of three common types of congenital heart malformations: hypoplastic left heart syndrome (HLHS), tetralogy of Fallot (TOF) and ventricular septal defect (VSD). The specimens were obtained during the surgical correction of these malformations. The N-cadherin expression, qualitative and quantitative changes of intercalated disk components were examined. We have found that expression of N-cadherin is significantly lower in case of TOF and HLHS in comparison to VSD. Ultrastructural investigations showed widening of adherens junctions and desmosomes areas, disarrangement of filaments and lower number of gap junctions per intercalated disk in TOF and HLHS cases. The obtained results suggest that N-cadherin deficiency influences the structure of the adherens junctions. In relation to this, the cell-to-cell contact, one of the most important factors of correct organogenesis, is broken down. We suggest that this could be one of the factors which induce heart malformations. The study was supported by the grant 0714/P05/2005/28 from the State Committee for Scientific Research.

New light microscopic techniques

P3.58

Immunohistochemical study of functional proteins in cells and tissues of living animal organs by the *in vivo* cryotechnique

Shinichi Ohno, Nobuhiko Ohno, Nobuo Terada, Sei Saitoh

Department of Anatomy and Molecular Histology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

Contact: sohno@yamanashi.ac.jp

Various analyses of molecular distribution in animal organs by light microscopy have been necessary in biological and medical fields. However, the conventional chemical fixation of resected tissues always yields technical problems, such as molecular movement, structural alteration and anoxic modification of cells and tissues. Our *in vivo* cryotechnique (IVCT) has been used to demonstrate their functional morphology in living animal organs. It is an original method of cryofixation, in which target organs of anesthetized mice are frozen *in vivo*, indicating that they are directly embedded in ice crystals without perfusion-fixation or tissue-resection. In our presentation, some features of IVCT will be described on the basis of immunohistochemistry. (I) Detection of soluble components: As the first application of IVCT to immunohistochemistry, serum albumin and immunoglobulin G (IgG) were detected exclusively in blood vessels of living mouse cerebellum, but quickly leaked out across blood-brain barriers owing to anoxia with resected fresh tissues. Subsequently, the extracellular distribution of serum proteins was well retained in the kidney or liver tissues of living mice with IVCT, but significantly modified with the conventional fixation or the quick-freezing method. These findings indicated that IVCT would have some beneficial effects on immunohistochemical detection of soluble molecules in cells and tissues of living animals. (II) High time-resolution of molecular localization: Some rapid morphological changes occurring within seconds are difficult to capture with conventional chemical fixation methods. All the fixatives used for biology and medicine take some time to penetrate deeper areas of tissue blocks. In contrast, another IVCT instantly immobilizes molecular conformation of functional components in cells and tissues at the time of cryofixation. Such molecular analyses were made in an experiment on rapid phosphorylation of rhodopsin, in which immunoreactivity of rhodopsin-phosphorylated 334Ser was detected after 30 sec of exposure to light, but not after 10 sec in dark-adapted mouse retina. The IVCT would be the most useful approach for immunohistochemical analyses of rapid molecular changes in functioning cells. As described above, blood circulation is inevitably stopped in resected tissues. In contrast, IVCT directly cryofixes functioning organs of anesthetized animals without perfusion-fixation or tissue-resection. The preservation of both morphology and molecular conformation is a technical advantage of IVCT, different from the conventional methods. The IVCT has been already used for various organs of living animals. Considering its advantages over the conventional methods, the application of IVCT will be expanded from standard microscopic analyses to other new analytical fields.

P3.59

High resolution microscopy gives insight into DNA repair

Grace Chojnowski, Derek J Richard

Queensland Institute of Medical Research, Brisbane, Australia

Contact: grace.chojnowski@qimr.edu.au

Cells have developed a vast array of surveillance, repair and signalling proteins in order to prevent the loss of valuable genetic information that results from DNA damage. It has been estimated that each cell can suffer up to 30000 DNA damaging insults in a 24 hour period, each damage event, if not repaired correctly, could consequently result in a loss of cellular programming which can lead to cancer development. After the induction of DNA breaks, repair centres, which are marked by specific repair proteins are seen to develop at the break sites. These repair centres are dynamic and their composition alters in a time dependent manner. Repair centres however are large and must represent hundreds of molecules of each repair protein. Due to the lack of resolution it is difficult to utilise microscopy to see in more detail the composition of the repair centres. In this study we examine the localisation of a newly described DNA repair protein, hSSB1, with known DNA repair proteins, after inducing double strand DNA breakers with ionizing radiation. We initially compare two microscope systems, the Olympus IX81 microscope with a resolution of approximately 250nm and the Deltavision Personal DV with a 100nm resolution. We show in this study that the resolution capabilities of a microscope can greatly effect data interpretation especially in colocalisation studies. This enables us to further examine in detail hSSB1s placement in the repair centres.

P3.60

The influence of bacteria on sperm viability and mitochondrial activity

Monika Fraczek¹, Małgorzata Piasecka², Dariusz Gączarzewicz³, Maria Laszczyńska², Maciej Kurpisz¹

¹Institute of Human Genetics Polish Academy of Sciences, Poznań, Poland

²Laboratory of Histology and Developmental Biology Pomeranian Medical University, Szczecin, Poland;

³Department of Animal Reproduction University of Agriculture, Szczecin, Poland

Contact: framon@man.poznan.pl

The relationship between the quality of standard semen parameters and male genital tract infections is still actively studied due to the influence of the latter ones on the fertilizing potential of spermatozoa. The aim of the study was to establish the effect of bacteria on the motile and immotile sperm fractions in *in vitro* conditions. Study was performed on ejaculated spermatozoa from 12 volunteers with normal sperm parameters (sperm concentration $>20 \times 10^6$ /mL, motility $>50\%$ cells with progressive motility and morphology $>14\%$ normal sperm forms) evaluated according to WHO criteria. Motile and immotile spermatozoa were obtained using the swim-up technique (F10 medium, 1h, 37°C). Different spermatozoa fractions were incubated with following bacterial strains: *Escherichia coli*, *Staphylococcus haemolyticus*, *Bacteroides ureolyticus* at a concentration of 1×10^5 cells/mL. Sperm viability was checked by the LIVE/DEAD Sperm Viability Kit (SYBR14 and propidium iodide – PI) and assessed in fluorescent microscope. Mitochondrial activity was evaluated using JC-1 test as well as NADH-dependent NBT assay (DT-diaphorase). The number of dead spermatozoa (PI-positive) and cells which absorbed simultaneously SYBR-green and PI increased after incubation of male gametes with bacterial strains, especially with *B. ureolyticus* and *S. haemolyticus*. Moreover, energetic disorders of sperm mitochondria were found. The percentages of spermatozoa with decreased mitochondrial transmembrane potential ($\Delta\Psi_m$) and spermatozoa with decreased formazane deposits were elevated compared to untreated cells. Our results indicate that the presence of bacteria or their toxins in semen may have harmful effects on sperm viability and mitochondria function.

P3.61

Fluorescence correlation spectroscopy of vesicle movements in the hemocyte cytoplasm

Grzegorz Tylko

Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, Krakow, Poland

Contact: grzegorz.tylko@uj.edu.pl

Fluorescence correlation spectroscopy (FCS) combined with scanning laser microscopy (LSM) is a powerful tool for investigations of physical and chemical processes between fluorescent compounds or fluorescently labeled molecules. It also opened new possibilities for cell biologists involved in protein or vesicle movement analyses in the cell cytoplasm. This technique includes investigation of one or two molecular complexes. The advantage of FCS is that it requires low concentrations of molecules (10^{-9} - 10^{-11} M) and the analytical volume does not exceed 1 fl. The scope of the work was to investigate the applicability of FCS to the assessment of endocytotic dynamics of hemocytes of the housefly's larvae (*Musca domestica*) – a model of phagocytotic cells. The autocorrelation method of analysis was exploited. The FCS system was calibrated before the measurements using green rhodamine (RH6G) and cyanine (CY5) dye were performed. The cells obtained from the third instar larvae were cultured on coverslips in Hank's buffer and fed with 40 nm fluorescent beads (540/560 and 660/680) for 30 min. Excess of the beads was removed by cell washing with fresh buffer and the samples were observed in bright field and fluorescence mode. FCS measurements were performed in the cytoplasm of cell processes, the endocytoplasm and the perinuclear region. The samples were exposed to Ar-Ion (488 nm) and HeNe Laser (633 nm) light and focused with water immersion 40x/1.2 C-Apochromat objective. Out-of-plane fluores-

cent light was reduced with pinholes set to the value of 1 Airy unit and registered by avalanche photodiodes. The radii of the illuminated volume were calculated at 0.18 mm and 0.32 mm for 488 and 633 nm lines, respectively. Thus, structural parameter (S) reached 3.55. The validity of this calculation was proved by the autocorrelation measurement of the fluorescent bead of a known radius and the diffusion coefficient. The experimental function was fitted to model one with the value of the structural parameter fixed. All autocorrelation functions were obtained using the Confocor3 software and diffusion times were determined after their fitting to non-linear models with least-squares fitting method. The FCS measurements of the hemocytes revealed different mobilities of internalized particles in three investigated cell regions. Movements of the particles in the cytoplasm of cell processes were the fastest. In the endocytoplasm particle transport decreased significantly leading to immobilization of endocytosed particles. Lack of movements of the particles characterized perinuclear region. The study has showed that FCS enables tracing of endocytotic processes in different parts of cell, determining the speed of the cargo movement and its fraction.

P3.62

Quantitative fluorescence recovery after photobleaching (FRAP): the role of photon flux and object translocation

Mateusz Kuzak, Dominika Trembecka, Jurek Dobrucki

Division of Cell Biophysics, Faculty of Biochemistry, Jagiellonian University, Kraków, Poland

Contact: mateusz.kuzak@gmail.com

Background: Fluorescence recovery after photobleaching (FRAP) is a method widely used for measuring parameters characterizing the dynamic behaviour of proteins *in situ*, in live cells [1]. Unfortunately FRAP protocols and methods of data analysis, which are used in various laboratories, vary considerably. It is unclear if fluorescence recovery parameters obtained using different protocols can be directly compared. Goal: The goal of this work was to define FRAP conditions, which will enable inter-experiment data analysis. Results: In order to identify critical parameters, which influence estimates of fluorescence recovery, we investigated dynamics of eGFP-tagged linker histone H1 in stably transfected HeLa cells. We demonstrated that photon flux of the bleaching beam strongly influenced recovery times, while the total dose of light and bleaching time had only minor effect. Slight movements of chromatin, nucleus and the whole cell introduced unexpectedly large systematic errors in recovery determinations. Exciting laser light of a high intensity, which is used to avoid redistribution of the fusion protein during the bleach insult, can also be a source of artifacts. Intense illumination can lead to undesirable photoactivated reactions; we show that illumination of some fluorophores promotes covalent binding (photoaddition) to cellular components or causes structural changes in the bleached area. Photoaddition of the fluorescent probe to cell components leads to overestimation of the immobile fraction of the investigated molecule and adversely influences cell physiology. Preliminary data indicate that such effects can occur not only when a low molecular weight fluorescent dye like acridine orange is subjected to exciting light, but also when the enhanced green fluorescent protein is photobleached in live HeLa cells. Reactivity and cell damage may in part be explained by generation of singlet oxygen, what occurs as a result of exciting fluorescent heterocyclic probes and proteins [2,3]. Conclusion: Probe photoaddition and structural damage in the bleached region influences cell physiology and bound/free ratio of the tagged protein. Using the same photon flux of the bleaching beam, and due controls for probe photoaddition and cell photodamage in the bleached region are preconditions for inter-experiment comparisons of protein dynamic behaviour. References: 1. Lippincott-Schwartz, J. Snapp, E., Kenworthy, A. (2001). Studying protein dynamics in living cells. *Nat Rev Mol Cell Biol* 2, 444-456. 2. Greenbaum, L. Rothmann, C., Lavie, R., & Malik, Z. (2000). Green fluorescent protein photobleaching: a model for protein damage by endogenous and exogenous singlet oxygen. *J Biol Chem* 381, 1251-1258. 3. Jiménez-Banzo, A., Nonell, S., Hofkens, J., Flors, C. (2008). Singlet oxygen photosensitization by EGFP and its chromophore HBDI. *Biophys J*, 94, 168-172.

Sub-cellular molecular tracing in living cells

P3.63

Detection of the effects of cholesterol level alterations in plasma membrane on the TRH receptor mobility with fluorescence recovery after photobleaching

Pavel Ostasov, Zuzana Burdikova, Lucie Kubinova, Petr Svoboda

Institute of Physiology Academy of Sciences of the Czech Republic v.v.i., Prague, Czech Republic

Contact: ostasov@biomed.cas.cz

Plasma membrane integrity plays an important role in GPCR signalling. Here we investigated the effect of disruption of membrane integrity by cholesterol depletion on the thyrotropin releasing hormone receptor mobility in the plasma membrane. For our experiments, we used cells derived from HEK 293 cells, expressing a fusion protein between thyrotropin releasing hormone receptor and an enhanced form of GFP (TRH-R-eGFP). The control cells were compared to cells treated with β -cyclodextrin (β -CD), which causes depletion of cholesterol from the plasma membrane and alters its integrity. To determine the possible changes in receptor apparent diffusion coefficients and mobile fraction after β -CD treatment we used fluorescence recovery after photobleaching (FRAP). We used FRAP at different bleach spot sizes (2, 3 and 6 μm) to determine if the receptor apparent diffusion coefficient is independent of bleach spot size (suggesting that the receptor is diffusing in homogenous environment) or the coefficient changes with bleach spot size which would occur if its diffusion is restricted into some sort of membrane domains. For imaging, the Leica SP2 AOBIS microscope was used, with 488 nm laser line for excitation and 458 nm, 476 nm and 488 nm lines for photo bleaching. One group of the cells was treated for 30 minutes with 10 mM β -CD dissolved in serum free medium, which was enough to decrease cholesterol content in the plasma membrane by about 50%. The second group was treated for 30 minutes with serum free medium without β -CD. Then the cells were immediately transferred onto microscope stage and FRAP was performed. In the control cells we did not detect any significant change in the apparent diffusion coefficient, determined at bleach spots of different sizes, which was about $0,24 \pm 0,05 \mu\text{m}\cdot\text{s}^{-1}$. However, after β -CD treatment the coefficient was changed from $0,14 \pm 0,02 \mu\text{m}\cdot\text{s}^{-1}$ at 2 μm size of bleach spot to $0,27 \pm 0,01 \mu\text{m}\cdot\text{s}^{-1}$ at 6 μm spot. At the largest bleach spot the recovery of TRH-R-eGFP was apparently faster in cholesterol depleted cells than the recovery in the control cells. These results indicate that cholesterol depletion induces changes in receptor distribution in the plasma membrane and also suggest that β -CD treatment causes receptor confinement into some sort of domains. This study was supported by AV0Z50110509, LC06063, GACR 102/08/0691 and GACR 305/08/H037.

P3.64

Solid Lipid Nanoparticles loaded with Doxorubicin and 6-Coumarin (Doxo-CoumSLNs): uptake, distribution and drug release in human U373 astrocytoma cell cultures

Calderoni Sara¹, Strada Alberto¹, Brioschi Andrea², Colonna Alessandra¹, Gasco Maria Rosa³, Mauro Alessandro⁴

¹Istituto Auxologico Italiano, Laboratory of Neurobiology and ²Istituto Auxologico Italiano, Department of Neurology, Piacavallo di Oggebbio, Italy

³Nanovector S.r.l., Turin (TO) and ⁴Istituto Auxologico Italiano, Dept. of Neurology and Turin University, Dept. of Neuroscience, Piacavallo di Oggebbio and Turin, Italy

Contact: s.calderoni@auxologico.it

Introduction: Solid Lipid Nanoparticles (SLNs) are colloidal carriers used to enhance the pharmacokinetic profile of drugs with low blood half-life, poor body distribution and high systemic toxicity. Doxorubicin is an anthracycline antibiotic of highly effective in the treatment of haematopoietic and solid tumors. Due to its high systemic toxicity and its poor ability to pass through the Blood Brain Barrier many colloidal carriers for this drug were successfully tested *in vitro* and *in vivo*. Nevertheless, the exact mechanism by which SLNs transport and release vehiculated drugs into cells is still poorly understood. The aim of this study was to test SLNs temporarily loaded with Doxorubicin and 6-Coumarin (Doxo-CoumSLNs) in U373 human astrocytoma cells to evaluate their cellular uptake, distribution and drug release. Methods: Doxo-CoumSLNs (2.64 mg/ml Doxo+27.5 $\mu\text{g}/\text{ml}$ Coum) and CoumSLNs (20.5 $\mu\text{g}/\text{ml}$) were obtained from Nanovector; their mean diameter was $114.4 \pm 1.1 \text{ nm}$ (Doxo-CoumSLNs) and $187 \pm 2.1 \text{ nm}$ (CoumSLNs). U373 cells seeded on confocal dishes after 24h were incubated with Doxo-CoumSLNs and observed at different times (0 to 24h). The cellular content of doxorubicin was evaluated by LineProfile analysis (LaserSharp 5.1 software, Bio-Rad). Moreover, cell cultures were treated with CoumSLNs (4 and 24h) and then processed for subcellular organelles stainings. Results: Doxo-CoumSLNs were visible in the cytoplasm within 5 minutes and well retained till the last observation; Doxorubicin red staining became detectable in the cell nucleus after 30 minutes while the green Coumarin signal remained confined to the cytoplasm. Moreover, a great amount of red emitting intracellular clumps was observed after 24h. The presence of small and spread green spots (mainly located in the cytoplasm near the nucleus) was observed after administration of large amounts of SLNs (10 μl instead of 2.5 μl) and these dots tended to disappear after 24h. Doxorubicin LineProfile showed that the nuclear concentration increases from 4h and remains stable till 24h. CoumSLNs signal was present in the cytoplasm and partially co-localized with lysosomes and peroxisomes. Nanospheres were not detectable in Golgi and mitochondria. Conclusions: Doxo-CoumSLNs rapidly entered the cells (5 min.) and were well retained for almost 24h. They tended to release Doxorubicin after 30 minutes and continued till 24h acting as "drug reservoir". SLNs aggregated after 24h forming red-emitting clumps, while very few green-emitting dots were observed. These results suggest that Doxorubicin is better retained than Coumarin in SLNs. Moreover, the partial co-localization with catabolic organelles implies that SLNs can be metabolized by their acid lipases. Taken together these results showed that these Doxo-CoumSLNs work well in both releasing the vehiculated drug and tracing the SLNs intracellular fate but only at short times (5 min. to 4h).

Lectures



Poster session 1



Poster session 2



Poster session 3



Poster session 4



Imaging of nerve cell structure and function

P4.1

Immunohistochemical changes of tyrosine hydroxylase expression in substantia nigra and VTA rat brain nuclei under dopaminergic hyperfunction

Rudolf Khudoerkov, Dmitry Voronkov

Neurology Research Center RAMS, Lab. of Functional Morphochemistry, Moscow, Russia

Contact: voronkovdm@gmail.com

An immunohistochemical method was used to study the localization of tyrosine hydroxylase in substantia nigra (SN) and ventral tegmental area (VTA) under experimentally induced hyperfunction of the dopaminergic system in Wistar rats. Wistar male rats (180±20 g) were injected intraperitoneally everyday for 4 weeks with levodopa (l-dopa 50 mg/kg and benserazide 12.5 mg/kg) and control rats at that time were injected with saline. After 4 weeks of injections the control and experimental animals were tested in open field trials by automatic movement registration, and then sacrificed. The brains of the studied rats were fixed in Carnoy's, embedded in paraffin and cut into 7 µm sections. Neurons expressing tyrosine hydroxylase (TH⁺) were labelled with monoclonal antibodies, using avidin-peroxidase detection with DAB. For morphological staining the Nissl method was used. The intensity of cell immunoreactivity of TH-positive neurons was assessed by the Leica Qwin semi-automatic digital imaging system. The intensity of neuron staining and background values were measured on separated blue channel of 24bit RGB image, and the registered values were evaluated in optical density units (OD). The intensity of TH expression in neurons was also scored visually using a 3-point scale (high, medium and low intensity). The Leica Qwin was also used to measure the neuron body area and to calculate the number of TH⁺ neurons per field of view. Mann-Whitney and Kolmogorov-Smirnov non-parametric tests were used for statistical analysis. The cell body area of TH⁺ neurons after 4 weeks of l-dopa injections was significantly decreased on 31% and 20% of neurons in SN and VTA respectively. The overall number of TH⁺ neurons (per field of view) did not differ from that of the control animals, but both in SN and VTA the average OD was significantly lower than in the control group. The TH expression level in various populations of TH⁺ neurons was changed differently under l-dopa treatment. In comparison to the control group, the number of high-expressing TH⁺ cells was significantly decreased by 50% in SN and 40% in VTA. The number of moderately expressing TH⁺ neurons was decreased by 60% in SN and did not change in VTA. In contrast, the quantity of low-expressing TH⁺ neurons was increased by 38% in SN and 25% in VTA. It was concluded that long treatment with exogenous dopamine precursor l-dopa results in different changes of dopamine synthesis in various neuronal populations of SN and VTA. Neurons with high level of TH expression displayed more involvement in dopamine synthesis regulation than the low-expressing TH⁺ neurons.

P4.2

Role of TLS in MeCP2-dependent NR1 alternative splicing in neurons

Widyawilis Binte Selamat¹, Loo Chin Wong¹, Toru Takumi², Ritsuko Fujii¹

¹Waseda-Olympus Bioscience Research Institute, Biopolis, Singapore

²Osaka Bioscience Institute, Osaka, Japan

Contact: fujii@waseda.jp

Translocated in liposarcoma (TLS) is a multifunctional protein component of hnRNP and may play an important role in biological processes that link the splicing of pre-mRNA and somatodendritic transport of mRNA in neurons. In efforts to better understand the functions of TLS in neurons, we identified interacting protein partners for TLS by the TAP method from PC12 cells. The purified TLS-protein complexes contained methyl-CpG binding protein 2 (MeCP2) and RNA binding protein YB-1 as well as ribosomal proteins, poly(A)-binding proteins and cytoskeleton

control proteins. MeCP2 has been reported to interact with YB-1 and is also known to regulate the alternative splicing of N-methyl-D-aspartate receptor (NMDAR) 1 (NR1) pre-mRNA in neuronal activity-dependent manner which generates two major functional NR1 mRNA splice variants, C2 type (NR1-1 and 1-2) and C2' type (NR1-3 and 1-4). In addition, mutations in MeCP2 have been implicated in a variety of neurodevelopmental disorders including X-linked mental retardation and some cases of autism. In this study, we investigated a possible involvement of TLS in the NR1 splicing control by MeCP2 and YB-1 in mouse cortex neurons. In mouse cortex neurons, TLS was co-immunoprecipitated with all NR1 splice variant mRNAs (NR1-1, NR1-2, and NR1-4). However, after treatment of cortex neurons for 48 hr with type I mGluR agonist (DHPG), NMDAR antagonist (D-AP5) or GABA receptor antagonist (bicuculline), TLS was found to be predominantly associated with NR1-4 in bicuculline-treated neurons, and with NR1-2 in D-AP5 and DHPG-treated neurons. Another set of experiments showed that selective inhibition of NMDAR by D-AP5 increased the level of NR1-4 splice variant and decreased the levels of NR1-1 and NR1-2 in cortex neurons whereas the increasing neuronal activity by bicuculline resulted in predominating levels of NR1-1 and NR1-2 over NR1-4. These results suggest that TLS may reciprocally bind or sequester the NR1 splice variants that are not produced under the control of MeCP2 and YB-1 in neuronal activity-dependent manner. In fact, the TLS bound to the NR1 splice variants were largely free from MeCP2 and YB-1 proteins. Moreover, protein interaction between TLS and MeCP2/YB-1 was enhanced in the presence of RNase. When the neurons were treated with D-AP5, MeCP2 displayed a diffuse localization within the nucleus which was accompanied by increased nuclear localization of TLS. Our live cell monitoring of TLS-GFP also revealed that dynamic nuclear-cytoplasmic shuttling of TLS could be dependent on neuronal activity and neuronal maturation. Our data suggests that TLS may play a supportive role in selective expression of NR1 splice variants by MeCP2 and YB-1.

P4.3

αB-crystallin distribution in the dog central nervous system

Siyami Karahan¹, Tolga Güvenç²

¹Dept. of Histology and Embryology, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Turkey

²Dept. of Pathology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey

Contact: siyamikarahan@yahoo.com

αB-crystallin is one of the two subunits of the multimeric protein α-crystallin (αA and αB), which is a major component of the lens. As a molecular chaperon and heat shock protein, αB-crystallin prevents false protein folding and has anti-apoptotic properties. In this study, αB-crystallin expression was investigated in the central nervous system (CNS) using tissues collected from the cerebrum, cerebellum, olfactory bulb, pituitary gland, medulla oblongata, cornu ammonis, hypothalamus and cervical spinal cord. Except in the pituitary gland, αB-crystallin immunolocalized in glial cells in all the nervous tissues investigated. No immunoreactivity was observed in neurons. In double staining of αB-crystallin and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase), the immunoreactive glial cells were determined to be CNPase positive as oligodendrocytes. Immunoreactive oligodendrocytes located mainly in the white matter and the layers of the gray matter adjacent to the white matter. However, the spinal cord had numerous αB-crystallin-positive oligodendrocytes both in the white and gray matter. In the white matter of the spinal cord, a limited number of glial cells were positive for αB-crystallin, but negative for CNPase. αB-crystallin immunoreactivity was weak in the cytoplasm but very light in the nucleus. As observed in oligodendrocytes of normal dog CNS tissues, αB-crystallin may have a role in the anti-apoptotic process of oligodendrocytes and in the prevention of false folding of proteins such as myelin basic protein, which is synthesized intensively by oligodendrocytes.

P4.4

Predisposition of distal nodes of Ranvier to injury in a mouse model of Guillain Barre syndrome

Rhona McGonigal, Sue Halstead, Hugh Willison

University of Glasgow, Glasgow, Scotland

Contact: 0105442m@student.gla.ac.uk

Guillain-Barré syndrome (GBS) is a peripheral neuropathy that causes acute flaccid paralysis. Pathology of the axonal variant of this syndrome has been associated with antibody-dependent complement-mediated injury to the peripheral axon. *In-vivo* modelling of this syndrome through fluorescent imaging in the mouse is a major step towards identifying pathogenic pathways. In particular, the blood-nerve barrier (BNB) is a major protectant for axons against factors in the external environment. The BNB does not extend completely to the neuromuscular junction, leaving this terminal portion of the axon unprotected. It is here that susceptibility to antibody attack has been demonstrated in our mouse model of GBS. We are now determining to what extent the rest of the distal axon is at risk from circulating antibodies. The ganglioside GD1a, which is a target for pathological antibodies in GBS, is expressed at the nodes of Ranvier (NoR) of intramuscular axons. To investigate the injury caused by antibodies raised against this ganglioside and the permeability of the BNB, we used mice genetically engineered both to over-express GD1a and express fluorescent proteins in the cytoplasm of axons and Schwann cells (SC). Fluorescence allowed identification of axons, particularly NoR, where IgG could be localized in a gradient dependent manner. Furthermore, MAC deposition at NoR and loss of axonal cytoplasmic fluorescence was indicative of injury to the distal axon. Our studies are suggestive of a resilient proximal barrier that becomes more permeable towards the nerve terminal. Therefore, it is not only the axon at the terminal that can be a target of injury, but also the distal axons at their nodes of Ranvier.

P4.5

The effects of NOS inhibitor 7-nitroindazole and NO precursor L-arginin on immunoreactive neuronal nitric oxide synthase (nNOS) in rat brains and its relation to memorySüheyla Gonca¹, Füzuzan Yıldız², Cannur Dalçık³, Pelin Coştur Biyiksiz¹, Hakki Dalçık¹¹University of Kocaeli Medical School Department of Histology and Embryology, Kocaeli, Turkey²University of Kocaeli Medical School Department of Pharmacology, Kocaeli, Turkey³University of Kocaeli Medical School Department of Anatomy, Kocaeli, Turkey

Contact: suhgonca@gmail.com

In adult mammals, learning, memory and restoration of sensorimotor lost functions imply synaptic reorganization that requires diffusible messenger-mediated communication between presynaptic and postsynaptic structures. A candidate molecule to accomplish this function is the gaseous intercellular messenger nitric oxide (NO), which is involved in synaptogenesis and projection refinement during development. However, the role of NO in synaptic reorganization processes in adulthood remains to be established. Therefore, the aim of the present study was to investigate the possible role of NO particularly in learning and memory. The role of NO on reference and working memory performance was investigated by using the NO synthase (NOS) inhibitor 7-nitroindazole (NI) (10mg/kg), and NO precursor L-arginine (200mg/kg) by a three-panel runway task in rats. The number of animals was sixteen in a group in reference memory and seventeen in a group in working memory studies. Deeply anesthetized rats were perfused transcardially with 0.9% NaCl followed by 4% buffered paraformaldehyde fixative. After perfusion, brains were removed and postfixed in 4% paraformaldehyde containing 30% sucrose. Each brain was sectioned in the coronal plane at 30 µm using a vibratome, into twelve sets of serial sections, thus separated by a rostral-to-caudal distance of 360 µm. Sections from brains prepared at different times were preserved for later simultaneous processing in cryoprotectant. Brain sections were processed "free-floating" for nNOS immunocytochemistry exposing the sections to rabbit anti-nNOS (at a dilution of 1:25) antibody for 24 h. Stained sections were mounted in rostral-to-caudal order and examined under a microscope. nNOS immunoreactive cells were highly distinct in various brain areas including cortex, hippocampus, paraventricular nucleus, periventricular nucleus, supraoptic nucleus, ventromedial and anterior hypothalamic nucleus, medial preoptic nucleus, bed nucleus of stria terminalis and thalamus. nNOS immunoreactivity was demonstrated to be decreased in many brain

areas such as: paraventricular nucleus, cortex and hippocampus of the 7-NI treated animals. However, immunoreactivity appeared to be the same in the brain areas of the L-arginine treated brains compared to the controls. There was a distinct decrease in nNOS immunoreactivity in the hippocampus in the 7-NI treated animals, however no change was observed in the hippocampus of the L-arginine group. In the memory test, 7-NI induced impairment in reference memory and was reversed by L-arginine. The present results may demonstrate a possible involvement of nNOS in memory formation.

P4.6

Galanin system in the brain of zebrafish (*Danio rerio*)Piotr Podlasz¹, Ville Salinen², Yu-Chia Chen², Hisaaki Kudo², Natalia Fedorowska¹, Pertti Panula²¹Division of Animal Anatomy, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland²Neuroscience Center, Institute of Biomedicine/Anatomy, University of Helsinki, Helsinki, Finland

Galanin is a 29-30 amino acids long neuropeptide widely expressed in the central and peripheral nervous system. Galanin has been implicated in several higher order physiological functions including cognition, feeding, nociception, mood regulation, and neuroendocrine modulation, which may be relevant to disease states and clinical therapeutics. Galanin is a highly inducible neuropeptide, showing distinct up-regulation after pathological disturbance within the nervous system. Significant increase in galanin expression is observed after peripheral nerve injury, inflammation, in the basal forebrain in Alzheimer's disease, during neuronal development, and after stimulation with estrogen, while seizure activity depletes galanin in the hippocampus. These early studies suggested that increased galanin level might have a trophic influence on nerve repair. Later studies on galanin gene knockout and galanin over-expressing mice supported such role of galanin in nerve survival, regeneration and functional recovery. The zebrafish is a potential model for human disease and drug screening. Lack of detailed reports on the galanin system in zebrafish motivated us to perform this study. In zebrafish, galanin mRNAs were detected with RT-PCR 20 min. after fertilization, which implies that they are maternal transcripts. *in situ* hybridization studies with a probe against mRNA encoding galanin detected cells in the ventrocaudal forebrain, and two groups of neurons in the midbrain 24 h after fertilization (hpf). Similarly, immunocytochemistry showed galanin-ir nerve structures in 24 hpf embryos. A dense network of galanin-ir fibers was located mainly in the ventral part of brain. Nerve fibers were organized into longitudinally and vertically extending fascicles connecting galanin-ir groups of neurons in the preoptic area, hypothalamus and tegmentum. Immunocytochemistry also showed separate galanin-ir nerve fibers in the hindbrain and descending fibers to the spinal cord. In the adult zebrafish brain *in situ* hybridization showed that preprogalanin mRNA-expressing neurons were located in four regions: the preoptic nucleus, the lateral recess nucleus, the midbrain tegmentum, and the commissural nucleus of Cajal. Organization of galanin gene in zebrafish was also identified. It resembled the mammalian galanin gene. We found 2 different isoforms of preprogalanin in zebrafish. We hypothesized that the 2 forms of preprogalanin cDNA are splice variants, and the observed 72 nucleotide-long insert is a part of the intron 3. The results suggest that the well-preserved galanin gene in zebrafish gives rise to active peptides similar to mammalian ones. Galanin gene expression starts early in zebrafish, and the peptide-containing fibers innervates important regions of the CNS. Zebrafish may become a useful model to study the role of galanin in brain development and regeneration, provided that suitable mutants become available.

P4.7

Detection of protein gene product (PGP) 9.5 as a marker of epidermal nerve fibre depletion in simultaneous pancreas and kidney transplant patientsAlena Lodererova¹, Ludek Voska¹, Petr Boucek¹, Terezia Havrdova¹, Frantisek Saudek¹, Milos Adamec¹, Claudia Sommer²¹Institute for Clinical and Experimental Medicine, Prague, Czech Republic²Neurologische Klinik der Universitaet, Wurzburg, Germany

Contact: allo@ikem.cz

The aim of the study was to assess the effect of normoglycemia following simultaneous pancreas/kidney transplantation (SPK) on neurological function and epidermal nerve fiber density in patients with type 1 diabetes (DM). The panaxonal marker protein gene product 9.5 (PGP 9.5) was used

for epidermal nerve fibre detection. Methods: Twenty two patients with DM undergoing a simultaneous pancreas/kidney transplantation and fourteen healthy controls participated in the study. Skin biopsies were performed using a 3-mm punch from the distal thigh and the proximal calf at the time of transplantation and at 30±5 (mean±SD) months post transplant. After fixation (4% paraformaldehyde for 3 hours at 4°C, then cryoprotection with 10% sucrose in 0.1M phosphate buffered saline) and freezing (in isopentane cooled by liquid nitrogen), 40-µm sections were immunoreacted with a rabbit polyclonal antibody to PGP 9.5 (Dako, Glostrup, Denmark), followed by mouse anti-rabbit IgG conjugated with rhodamine (Jackson Immuno Research, West Grove, PA). Samples were analyzed with an Olympus microscope BX 51(Olympus Optical, Hamburg, Germany). The mean number of intraepidermal nerve fibers (IENF) per mm epidermis was derived using the software Olympus DP-SOFT (Software Imaging Systems, Münster, Germany). Results: Normoglycemia with insulin independence and satisfactory renal graft function was achieved in 18 patients. At follow-up 21- 40 (median 29) months after SPK, increases in intraepidermal nerve fiber density of the thigh samples were seen in 3 patients (median: 4.1; range 1.9-10.2 IENF/mm). No significant changes occurred in neurological function or intraepidermal nerve fiber density (Table 1.) of the transplanted group as a whole. Conclusion: We observed some improvement of nerve fiber counts in the biopsies from the more proximal lower thigh area in 3 patients. However prolonged follow-up is clearly needed, as these results confirm the presence of structural, poorly reversible changes in advanced diabetic neuropathy. Supported by the NR 7929-4/2004 grant, Internal Grant Agency, Czech Ministry of Health.

Table 1.

	SPK patients baseline	SPK patients post-Tx	Controls
IENF/mm - thigh	0.8 ± 1.3***	1.6 ± 2.5***	11.4 ± 4.2***

*** p<0.001 SPK patients vs. Controls

P4.8

Inflammatory pain induces eNOS and endothelial cell marker alterations in dorsal root ganglia

Elisa Borsani, Roberta Albertini, Rita Rezzani, Luigi F. Rodella

Department of Biomedical Sciences and Biotechnologies, Unit of Human Anatomy, University of Brescia, Brescia, Italy

Contact: eborsani@med.unibs.it

The involvement of nitroxidergic system in dorsal root ganglia (DRG) in acute and chronic inflammatory conditions is an important biological and clinical problem not yet clearly defined. Moreover, also the relationship between circulatory and nervous system during pain state is an open question. We investigated, in a mouse model of inflammatory pain, the neurochemical alterations of the active form of endothelial nitric oxide, phosphorylated on ser-1177 (p-eNOS), the expression of vascular endothelial growth factor (VEGF) and CD31, a marker for endothelial cells also called PECAM-1 (platelet-endothelial cell adhesion molecule-1), using immunohistochemical technique and confocal microscopy. Experiments were carried out on 20 C57BL/6 male mice subdivided into three experimental groups. The first group was the saline control, the second group was the CFA (complete Freund's adjuvant) treated for 24 h and the third group was CFA treated for 14 days. The mice received a 50-µL subcutaneous injection of CFA or saline into the plantar surface of the right hindpaw. The animals were behavioural tested before and 14 days after the CFA injection. At the end of the experiment the mice were sacrificed, perfused with 4% paraformaldehyde and DRG processed for p-eNOS, VEGF and CD31 immunohistochemistry. The behavioural test showed a thermal hyperalgesia persisting for 14 days. The immunohistochemistry showed a co-localization of CD31 and p-eNOS staining in vessels and a VEGF expression in neurons. In saline injected mice, p-eNOS expression was found also in vessels, CD31 well described the vessel morphology and VEGF expression was found in the cytoplasm of the neurons. In 24 h CFA treated mice, p-eNOS and CD31 expression was increased in vessels in respect to the control; VEGF expression decreased in the neuronal cytoplasm in respect to the control. In 14 days CFA treated mice, p-eNOS and CD31 expression decreased in vessels while VEGF expression increased in neuronal cytoplasm in respect to the 24 h CFA treated mice. These data suggest that pain alters the neurochemistry of nitric oxide system, associated with vessels, and the CD31 expression, implicated as a critical mediator of transendothelial

migration; moreover it induces a lacking of the neurotrophic and neuroprotective effects of VEGF. The close relationship between pain and vessels could have important clinical implications.

P4.9

Co-localization of IL-6 and BDNF immunostaining in the dorsal root ganglia of rat neuropathic pain model

Petr Dubovy, Ilona Klusakova, Ivana Svizenska, Radim Jancalek

Department of Anatomy, Medical Faculty, Masaryk University, Brno, Czech Republic

Contact: pdubovy@med.muni.cz

BDNF contributes to the survival-promoting activity of IL-6 on embryonic DRG neurons. On the other hand, IL-6 is involved in the injury-induced synthesis of BDNF in adult DRG neurons [1]. Although detection of BDNF-mRNA is reported in the satellite glial cells (SGC) of DRG [2], there is no direct evidence on BDNF protein localization in these cells. A nerve injury results in an elevation of both BDNF and IL-6 proteins in the DRG neurons to be involved in the neuropathic pain induction [3]. In the present study, we sought to determine if changes of BDNF and IL-6 immunoreactivity in DRG display a similar pattern following rat sciatic or spinal nerve ligatures, the most frequently used experimental models of neuropathic pain. A double immunofluorescence (IF) staining for BDNF and IL-6 was compared in DRG (L4-L5) of naive rats and those operated for unilateral sciatic (ScNL) and spinal L4-5 nerve ligature (SNL) for 1, 3, 7, and 14 days. To test neuropathic pain induction, withdrawal threshold of mechanoallodynia and thermal hyperalgesia was measured by Dynamic plantar aesthesiometer (UGO BASILE) and Plantar test (UGO BASILE), respectively. A weak IL-6 and BDNF IF was observed in the small- and medium-sized DRG neurons as well as SGC surrounding large-sized neurons of naive rats. Both IF was elevated by nerve injury in many large- and medium-sized neurons. Increased IL-6 IF was located mainly at the surface of large-sized neurons and their SGC. The same pattern of increased IF was observed for BDNF in DRG associated with nerve ligatures. Double immunostaining for IL-6 and BDNF proteins revealed a simultaneous increased IFs and their same distribution in the ipsilateral DRG following unilateral ScNL and SNL. In addition, we have unequivocally evidenced that SGC surrounding large-sized DRG neurons display IF for BDNF protein. The results confirmed close relation of IL-6 and BDNF proteins in the injured DRG in respect of neuropathic pain induction. References: 1. Murphy et al. (2000) Eur. J. Neurosci. 12, 1891-1899. 2. Zhou et al. (1999) Neuroscience 92, 841-853. 3. Hoheise et al. (2005) Pain 114, 168-176. Supported by GACR 309/07/0121 and MSM0021622404.

P4.10

The neurosecretion of oxytocin in the hypothalamic magnocellular nuclei of the training rats

E. Farina Lipari¹, A. Lipari¹, M. Bellafiore¹, G. Peri¹, A. Gerbino², M. Buscemi², B. Valentino³

¹Dip. Medicina sperimentale Sez. Anatomia Umana

²Dip. Medicina sperimentale

³Dip. Medicina sperimentale Sez. Anatomia Umana, Università di Palermo, Palermo, Italy

Contact: peri@csai.unipa.it

Introduction. Studies on hypothalamic vasopressin-oxytocin secreting nuclei showed that vasopressin (VP) and oxytocin (OT) play a role in regulating cardiovascular responses induced by contraction of skeletal muscle. In the hypothalamic paraventricular (hPVN) and supraoptic (hSON) nuclei VP/OT neurons showed a cFOS-like immunoreactivity indicating that they were activated during static muscle contraction. As OT peptide plays an important role in cardiac metabolism by regulating fluid homeostasis by hormones, the aim of our work was to investigate the OT expression in hPVN and hSON of trained rats by resistance exercise. Material and Methods. Sixty-four male Wistar rats were divided into a sedentary (C) and a resistance-trained (R) group. Every two weeks of training (15, 30, 45 days) eight rats from each group were weighed and then sacrificed. After 45 days the trained rats were obliged to rest for two weeks and afterwards they were sacrificed (60 day). The training protocol consisted in climbing a 1 m ladder (2 cm grid ladder) with an initial weight attached to the tail equal to 50% of rat body weight and gradually this weight increased in the following 6 weeks of training. Sections were immunostained for OT-expression analy-

sis. Results. hPVN and hSON resulted to be specifically positive to OT-ab. In both nuclei the number of OT-positive neurons significantly increased after 45 days of training, while it was lower in 15 day-trained rats than in the corresponding control animals. Both nuclei of 30 day-trained rats showed a little difference in OT-positive neurons compared with the controls. In both nuclei of 60 day-trained rats the number of OT-positive neurons was significantly greater than in the trained and control animals. Discussion. Exercise training determinates tachycardia and hypoxia in the skeletal muscle. It is known that the hOT-ergic fibers go to the solitary tract and motor nucleus of the vagus nerve and some studies showed that exercise increases OT-content in dorsal and ventral brainstem. Thus the hOT-neurosecretory nuclei induce a reduction in tachycardia by stimulation of the ventral area of the brainstem. We investigated the modifications of the activity of the hPVN and hSON during 15, 30, 45 days of training and also 15 days after finishing exercise. The reduction in OT-positive neuron number after 15 days of training might be due to higher degranulation that might be related to tachycardia and hypoxia of the skeletal muscles by exercise. The increase in the positive neuron number observed from 15^o to 45^o days of training results to be associated with lower OT degranulation in hPVN and hSON. This phenomenon might be due to adaptation of the heart in response to exercise training.

P4.11

Immunolocalization of alpha-synuclein in the rat spinal cord by two novel monoclonal antibodies

Giorgio Vivacqua Loredana D'Este

Department of Human Anatomy, "Sapienza" University, Roma, Italy

Contact: giorgio.vivacqua@tiscali.it

Our study provides the first immunohistochemical evidence of the presence and distribution patterns in the rat spinal cord of α -Synuclein, a soluble acidic protein well known to be richly expressed in the central nervous system and to be closely related with the pathogenesis of some neurodegenerative diseases including Parkinson and Alzheimer diseases. We used two novel home-made monoclonal antibodies (2E3 and 3D5) recognizing two different epitopes of α -Synuclein [1]. Both antibodies were able to localize α -Synuclein within the nerve terminals, whereas only 3D5 was able to also localize it within the nucleus of the neurons. α -Synuclein-immunoreactive nervous elements were widely recognized along the entire extent of rat spinal cord and in almost all the laminae of gray matter. However, they appeared particularly concentrated within the laminae I, II, VII and X and more scattered in the others. Double immunostaining procedures allowed us to demonstrate the co-localization of α -Synuclein with synaptophysin in the nerve presynaptic terminals, with neuropeptide Y in lamina I, II, IX and X, and its close relationships with tyrosine hydroxylase immunoreactive neurons in lamina VII and X. Interestingly, the α -Synuclein immunoreactive neurons showed poor content of calbindin 28kD. Our findings could help to understand some early clinical symptoms of the neurodegenerative diseases, such as protopathic pain and disautonomic disorders and indicate the spinal cord as a probable start point of them, according to the "ascending theory" of Parkinson's disease [2]. References: 1. Yu et al. *Neuroscience*, 145: 539-555, 2007. 2. Braak et al. *Acta Neuropathol.*, 113 (4) : 421-429, 2007.

P4.12

Calcium binding proteins in the hippocampal formation of the guinea pig (*Cavia porcellus*)

Malgorzata Kolenkiewicz, Anna Robak

University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Contact: malgorzata.kolenkiewicz@uwm.edu.pl

Calcium ions are important intercellular second messengers and they are involved in many neuronal processes. However, concentration of these ions in cell should occur at optimal level because they are toxic and their increase can lead to neuronal death. Neurons have some mechanisms to protect against the toxicity of these ions. Calcium binding proteins (CaBPs) are molecules which bind Ca²⁺ with high affinity and stabilize the concentration of free ions in cell. CaBPs in the hippocampus are mainly presented in the GABAergic interneurons. The aim of this study was to get some information about the distribution of selected CaBPs (parvalbumin – PV; calbindin – CB; calretinin – CR) in the guinea pig hippocampal formation. The studies were carried out on the brains of guinea pigs aged 10

days (P10). The animals were deeply anesthetized and perfused intracardially with 4% paraformaldehyde. Following perfusion, brains were washed twice in the 0.1M phosphate buffer and stored in the 30% sucrose solution until they sunk. Labelling immunofluorescence was made on the 10 μ m thick frozen sections. PV- and CB-immunoreactive (PV-IR and CB-IR) cells and fibres were identified with mouse monoclonal antibodies against PV and CB, whereas CR-immunoreactive (CR-IR) structures were labeled with rabbit polyclonal antibody. It was revealed that in the hippocampal formation all the investigated calcium binding proteins are presented. Some big, triangular and oval-shaped PV-IR neurons were found in the hilus (HL), below the granular layer (GL) and in the pyramidal layer (PL) of the CA3 sector in the hippocampus proper (HP). In GL negative granule cells were surrounded by immunoreactive dots. PV-IR fibres were mainly presented in the dentate gyrus. In the HL there were short and smooth fibres but their direction was not similar. Additionally, few long fibres were perpendicularly gone through the oriens layer (OL) of the hippocampus proper. CB-IR and CR-IR neurons were less numerous and mainly detected in the pyramidal layer. Both kinds of labelled cells were small and oval-shaped. CB-IR neurons were presented in the CA3 sector whereas CR-IR in CA1. Only few smooth fibres were observed in the OL of HP.

P4.13

The cocaine- and amphetamine-regulated transcript (cart) immunoreactivity in the amygdala of the guinea pig

Maciej Równiak¹, Anna Robak¹, Krystyna Bogus-Nowakowska¹, Barbara Wasilewska¹, Mariusz Majewski²

¹Department of Comparative Anatomy, ²Department of Human Physiology, University of Warmia and Mazury, Olsztyn, Poland

Contact: mrowniak@moskit.uwm.edu.pl

Cocaine- and amphetamine-regulated transcript (CART) is a novel neuropeptide involved in multiple physiological functions such as drug reward and reinforcing and mediation of stress response. Although some earlier reports indicate that CART peptides are present in the mammalian amygdala (CA), there is no detailed data concerning the immunoreactivity of this substance in CA of the guinea pig. The animals were deeply anesthetized and perfused intracardially with 4.0% paraformaldehyde in 0.1 M phosphate buffer. Following perfusion, brains were removed from the skulls and post-fixed for 4 h in 4.0% paraformaldehyde, washed twice in 0.1 M phosphate buffer and then stored in 30% sucrose until sectioning. The 10 μ m cryostat sections were processed for the routine double immunofluorescence staining with an antiserum directed against the CART peptide (fragment 61-102) and one of the following substances: neuropeptide Y (NPY), or nitric oxide synthase (NOS), or cholecystokinin (CCK), or somatostatin (SOM), or vasoactive intestinal peptide (VIP), or substance P (SP). The antisera adopted produced robust immunofluorescence staining in all subdivisions of the guinea pig CA and several adjacent forebrain structures. The highest densities of CART immunoreactive (CART-IR) fibers were evident within the central and medial nuclei. Moderate to high expression was found within the large-celled part of the basolateral nucleus and moderate to low levels in the lateral, basomedial and cortical nuclei. CART-peptide positive cell bodies were rarely observed but the most often they were present in the small-celled parts of the basolateral and basomedial nuclei. Single somata were also scattered in the other CA subdivisions. Double labeling studies revealed that in general, there is no coexistence of CART with NPY, NOS, CCK, SOM, VIP or SP in the guinea pig CA neurons. However, single and randomly dispersed CART-IR fibers contain CCK, SOM, VIP or SP.

P4.14

Mitogenic activity in the ventricular and the subventricular zones of rat brain

Faruk Alkan¹, Sibel Kokturk¹, Fatma Kaya Dagistanli², Melek Ozturk², Mumin Uzunalan¹, Bulent Uruuer¹

¹Istanbul University Cerrahpasa Medical Faculty Histology & Embryology

Department, Istanbul, Turkey

²Istanbul University Cerrahpasa Medical Faculty Medical Biology Department, Istanbul, Turkey

Contact: falkan@istanbul.edu.tr

In this study we investigated mitogenic activity in the subventricular and the ventricular zones of the lateral and the third ventricle of the adult rat

brain. A single dose of bromodeoxyuridine (BrdU) (50mg/kg, i.p.) was injected into Sprague Dawley male rats, and a perfusion method was performed for fixation 24 hours after BrdU injection. BrdU antibody was used for immunohistochemistry. BrdU immunopositive cells were counted under the light microscope, ANOVA and Tukey- t tests were used for statistical analysis. Ependymal cells with BrdU immunopositive nuclei were not observed in the ventricular zone at the lateral and the third ventricles. Cells with BrdU immunopositive nuclei were seen in the subventricular zone of the lateral and the 3rd ventricles. The number of BrdU immunopositive cells in the subventricular zone of the lateral ventricle was significantly higher than that in the subventricular zone of the 3rd ventricle ($p < 0.001$). In conclusion, mitotic activity was observed in the subventricular zone of the lateral and the 3rd ventricles. In the subventricular zone of the lateral ventricle cells were more mitotically active than those of the subventricular zone of the 3rd ventricle. We suggest that the subventricular zone of the lateral ventricle contains reserve nerve cells with the potential for further differentiation in the adult rat brain.

P4.15

Low infra red laser light irradiation protects neural cells from oxidative stress: a confocal microscopy analysis on living cells combined with biochemical and morphological studies

Alessandro Giuliani¹, Luca Lorenzini¹, Michele Gallamini², Luciana Giardino³, Laura Calza³

¹DIMORFIPA, University of Bologna, Ozzano Emilia, Italy

²RGM-Medical Devices Division, Genova, Italy

³DIMORFIPA – University of Bologna, Bologna, Italy

Contact: laura.calza@unibo.it

Considerable interest emerged during the last years around the well known notion that biological systems are sensitive to visible light. A great impulse to this old idea was given by the introduction of laser as light source, which offers many benefits, like mono-chromaticity and possibility of transport by means of fibres. In view of clinical application of visible radiation in the far-red to near-infrared region of the spectrum, we explored the reaction of a neural cell line (PC12) to coherent red light irradiation with extremely low energy transfer. We focused on the effect of pulsed light laser irradiation on two distinct biological effects: neurite elongation under NGF stimulus on laminin-collagen substrate, and mitochondrial membrane potential under basal condition and after oxidative stress. We used a 670 nm laser, with extremely low peak power output ($3\text{mW}/\text{cm}^2$) and extremely low dose ($0.45\text{mJ}/\text{cm}^2$). This corresponds to one of the four suggested "active zones" (peak positions between 667.5 and 683.7 nm) for investigations into cellular mechanisms of phototherapy. The total energy is approximately 2000 times less than in photodynamic therapy. We first compared different times (20 sec and 15 min) of irradiation on NGF-induced neurite elongation in PC12, by using the Image Pro Plus software. We found that laser irradiation further stimulates NGF-induced neurite elongation on a laminin-collagen coated substrate (two-way ANOVA and post-hoc test, $p < 0.05$). In order to evaluate the effect of coherent red light irradiation on cell reaction to oxidative stress (10 ml H_2O_2 , final concentration 300 mM), we used laser-confocal microscopy for live recording of mitochondrial membrane potential, using JC1 vital dye. Acquisition started immediately after H_2O_2 addition, simultaneously to red light exposure, and images were taken at 120 sec time intervals for 15 min. Cells were then processed with the Olympus Fluoview 500 Time Course software. The mean intensity on a scale ranging from 0 (black) to 4095 (white) was measured at each time (s) using the fast XY acquisition mode (point scan speed 2). Measurements for photo bleaching were also performed in the same experimental session. The time-dependent decrease of fluorescence intensity from 120 to 960 sec (DY) is then calculated for each cell in the absence (photo bleaching) and in the presence (oxidative stress) of H_2O_2 . Red light irradiation decreases DY after H_2O_2 to control ($-\text{H}_2\text{O}_2$) values, thus suggesting a protective effect of red light radiation on early mitochondrial potential variation due to oxidative stress. This result has been confirmed by a well-established viability assay, e.g. the MTT assay, proving that red light 20 sec laser irradiation at the beginning of H_2O_2 exposure significantly protects the viability of cell culture (Student's t test, $p < 0.05$).

P4.16

Confocal studies of synaptic connections in the first visual neuropil of *Drosophila melanogaster*

Jolanta Górska-Andrzejak, Grzegorz Tylko

Cytology and Histology Department, Institute of Zoology, Jagiellonian University, Kraków, Poland

Contact: j.gorska-andrzejak@uj.edu.pl

The visual system of *Drosophila melanogaster* consists of the compound eye and three optic neuropiles: lamina, medulla and lobula complex. The first visual neuropil or lamina is build of cylindrical columns called cartridges. In each cartridge the photic information is transmitted from photoreceptors to the first order interneurons, the so-called large monopolar cells (LMCs, L1-L5). Every cartridge contains six short photoreceptor terminals (R1-R6) that terminate in the lamina, and two terminals of long photoreceptors (R7, R8) that run beyond lamina to the second neuropil or medulla. In the cartridge, the terminals of photoreceptors (R1-R6) surround axons of their postsynaptic partners, L1 and L2 monopolar interneurons that lie in the centre of the cartridge. Apart from profiles of photoreceptors and L1, L2 monopolar cells, the cartridge comprises also α processes of amacrine cells, β processes of T1 cells as well as processes of L3, L4 and L5 monopolar interneurons. The whole group of profiles is wrapped by epithelial glia. Such regularity of cells' arrangement within each cartridge generates a synaptic organization that is readily accessible to anatomical analysis. In these studies we examined synaptic connections of the first neuropil of the optic lobe behind the fly's compound eye using confocal microscopy. We studied the expression of proteins important for assembling multiprotein complexes at synaptic contacts in the lamina cartridge, a presynaptic active zone protein Bruchpilot (BRP) and a scaffold protein Disc large (Dlg). The first was detected using Mab nc82 (Hybridoma), the latter Mab 4F3 (Hybridoma) and PSD95 (SYMBIOS). To be able to distinguish the part of BRP/Dlg signal that comes from the profiles of the particular cell type of the cartridge we have used GAL4/UAS transgenic lines of *Drosophila* that express green fluorescent protein (GFP) in a cell specific manner; in photoreceptors R1-R6 (RH1-GAL4/UAS-GFP), in monopolar cell L2 (21D-Gal4/UAS-GFP), in monopolar cell L3 (Apterous-GAL4/UAS-GFP), and in epithelial glia (Repo-Gal4/UAS-GFP). Our results revealed different patterns of expression of BRP and Dlg within the lamina cartridge. BRP protein was found only in the active zone of the presynaptic element of the synapse. The majority of BRP signals in the cartridge localized to tetrad synapses of R1-R6 terminals. Dlg protein, on the other hand, was found in both, in pre- and postsynaptic elements of the synapse, and appeared to be more abundant than BRP. We found Dlg in terminals of R1-R6 as well as in axons and dendrites of L2 monopolar neurons. Neither of these two proteins was found in epithelial glia.

P4.17

Effects of prolonged fasting on serum leptin level and brain leptin receptor expression in aged rats

Kotlarz Grazyna

¹Department of Histology and Immunology, Medical University of Gdansk, Gdańsk, Poland

Contact: zgkottl@amg.gda.pl

Leptin is a multifunctional hormone produced predominantly in adipocytes proportionally to the amount of adipose tissue, playing a key role in energetic balance of the organism. During aging there is a tendency to the increase of body weight and white adipose tissue. Regulation of the fuel metabolism is altered in fasted, and fasted-refed animals. The aim of our study was to assess the effect of aging on the expression of leptin receptors in the hypothalamus, serum leptin and serum leptin receptor levels of fasted-refed rats. Methods. Inbred male Wistar rats aged 5 months and 22-24 months old were used. Control rats were fed *ad libitum*; studied groups were fasted for 48 and 96 hours and then refed for 24 hours. Leptin receptors (Ob-Rb) were visualized in brain tissue by the use of IHC on paraffin sections (primary anti-mouse leptin R antibody, R&D). The presence of Ob-R was estimated by Western blotting. Leptin concentration in serum was measured by Mouse Leptin Immunoassay, R&D. Serum levels of leptin receptor protein were measured by mouse ELISA (DuoSet[®] Development System R&D). Results. Normally fed control old rats had 250% higher leptin serum concentration as compared to young adult animals. Fasting for 48 h decreased serum leptin concentration in both age groups. Prolonged fasting for 96 hours did not change serum levels as compared to shorter fasting period neither in young adult rats nor in old ones. Refeeding for 24 h of young rats fasted for 48 or 96 hours increased serum

levels as compared to fasted ones by ca. 300 %. On the contrary, refeeding of old rats that were fasted 96 h led to a high increase of leptin level that reached the value observed in control normally fed old rats. The results of the qualitative analysis of leptin receptor protein by the ELISA test provided very low values (pg/ml). The presence of leptin receptor was shown by IHC mainly in the arcuate (ARN) and ventromedial hypothalamic nuclei (VMN) in young and old rats. Immunopositive reaction was more intense in the 96 hours fasted compared to the 48 hours fasted. The presence of leptin receptor protein (m.w. 120 kDa) was confirmed with Western blotting in a homogenate of hypothalamus in all studied groups. Food restriction increases leptin receptor in the hypothalamus of rat suggesting that adiposity plays a role in the development of leptin resistance associated with aging.

P4.18

Distribution of CART (cocaine- and amphetamine-regulated transcript) and CaBPs (calcium-binding proteins) in the mamillary region of guinea pigs aged ten days postnatally

Anna Robak¹, Małgorzata Kolenkiewicz¹, Maciej Równiak¹, Krystyna Bogus-Nowakowska¹, Mariusz Majewski²

¹Department of Comparative Anatomy, ²Department of Human Physiology, University of Warmia and Mazury, Olsztyn, Poland

Contact: ankar@uwm.edu.pl

The CART system has multiple physiological roles that might transmit opposing messages between different neural circuits depending on the internal and external milieu. Calcium ions play a significant role in a remarkable variety of cellular activities, including transcription, synaptic transmission, metabolism etc. Relatively little attention has been paid to the relations of CART and CBPs neuronal systems in the mamillary region. The purpose of the study was to gain insight about the relationships of these peptides in the mamillary body of guinea pigs at P10 stage of the postnatal life. Five brains were perfused and postfixed in 4% paraformaldehyde in phosphate buffer (pH 7.4), then were cryoprotected in 30% solution of sucrose before cutting into scraps. Frozen scraps were immunostained with a standard fluorescence technique using the primary antibodies against CART, calbindin (CB), parvalbumin (PV) and calretinin (CR), and the species specific secondary antibodies conjugated with fluorochromes FITC or CY3. Five mamillary nuclei were investigated: the medial (MM), lateral (ML) and accessory mamillary (Acc) nuclei, as well as the supramamillary (SM) and posterior part of the tuberomamillary nuclei (TMp). These three calcium-binding proteins were found to be heterogeneously distributed. Neurons expressing high levels of calbindin immunoreactivity were particularly abundant in MM, TMp or TMp/ML. Small neurons displaying immunoreactivity both for CB and CART were especially numerous in MM. PV-immunoreactive neurons occurred mainly in TMp and the most ventral portion of the mamillary body. In contrast to the other two calcium-binding proteins, CR immunoreactivities were almost absent. Both substances (PV and CR) did not colocalize with CART. Fibers displaying moderate intense immunoreactivity for PV were relatively abundant in the ventrolateral and dorso-medial portions of MB, whereas fibers containing CART were observed throughout the studied nuclei. These fibres were of two different morphological types. PV-ir fibres were mostly thick and straight or sometimes slender and wavy, whereas CART-ir fibres were varicose, containing thick irregular varicosities; they run alone (MM) or formed network or a basket-like structure (perimamillary area). The results of the present study suggest that calbindin-D28k and CART participate in some specific aspects of the multifarious role of the mamillary body, whereas CR is unlikely to be involved in a significant manner in functions of the region in young guinea pig.

P4.19

Neurons containing calcium binding proteins in the claustrum and the endopiriform nucleus of the opossum

Katarzyna Majak¹, Beata Domaradzka-Pytel¹, Jan H. Spodnik¹, Ruzanna L. Djavadian², Krzysztof Turlejski², Janusz Moryś¹

¹Dept. of Anatomy and Neurobiology, Medical University of Gdansk, Gdansk, Poland

²Dept. of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland

Contact: kmmajak@amg.gda.pl

To understand the organization of inhibitory circuitries in the claustrum (Cl) and the endopiriform nucleus (En) of the Brazilian short-tailed opos-

sum (*Monodelphis domestica*) the morphological characteristics of neurons containing parvalbumin (PV), calbindin (CB) and calretinin (CR) were investigated. Brains of six adult opossum (both sexes) were single- and double-immunostained for PV, CB and CR and analyzed using a confocal system (BioRad Radiance 2100). The obtained images were reconstructed using the Laser Sharp 2000 v.4 (BioRad) analysis program. The size of cell bodies was measured with Laser Pix 2.0 analyzer (BioRad). In both structures we were able to identify 4 morphological cell types in all three calcium-binding proteins (CaBPs): type 1 – small, round or oval-shaped somata with three to five dendrites of equal size (square area 122.5±20.5, width 10.6±1.2 length 14.5±1.9 µm); type 2 – medium to large multipolar somata with dendrites of variable thickness (square area 211.2 ±58.9 µm², width 14.4±2.2, length 19.2±3.4 µm); type 3 – medium-sized fusiform somata with dendrites emanating from opposite poles (square area 185.7±55.8 µm², width 10.9±1.8, length 23.1±5.4 µm); type 4 – pyramidal medium-sized somata (square area 199.0±62.6 µm², width 14.7±2.4, length 19.9±3.9 µm). The morphology of each cell type did not differ between Cl and En. The highest percentage of colocalization was observed in PV/CB double-immunostaining. In Cl 15% of PV-ir cells were also CB-ir while 17% of CB-ir cells were also PV-positive. The PV/CB colocalization was even higher in EP (16% of PV-ir cells were also CB positive while 22% of CB-ir cells also expressed PV). The smallest percentage of colocalization was present between CB and CR in both structures (approximately 6% of CB-ir in Cl and 10% of CR-ir in En). Our observations provide important baseline information for studies aimed at understanding the complexity of inhibitory circuitries within Cl and En as well as comparison of neurons expressing CaBPs in different mammalian species.

P4.20

GABA-ergic neurons and calcium binding proteins in the rat claustrum and endopiriform nucleus – colocalization study

Edyta Spodnik, Jan H. Spodnik, Sławomir Wójcik, Jerzy Dziewiątkowski, Janusz Moryś

Department of Anatomy and Neurobiology, Medical University of Gdańsk, Gdańsk, Poland

Contact: spoded@amg.gda.pl

Clastrum and endopiriform nucleus are subcortical structures present in mammals. They are interconnected with almost all the cortical areas playing an important role in the integration of different modalities between them. Parvalbumin (PV), calbindin-D28k (CB), and calretinin (CR) are calcium binding proteins (CaBPs), considered to be markers for certain subpopulations of GABA-ergic neurons. Their activity affects flow of information. The aim of this study was to assess the mutual relationships between particular subpopulations of neurons containing CaBPs and GABA-ergic neurons. Neurons containing CaBPs were examined in the claustrum and endopiriform nucleus of the adult rats. After perfusional fixation with paraformaldehyde frozen brains were cut on a cryostat in coronal plane. Then the sections were stained by means of immunohistochemistry for GABA, CaBPs and examined with confocal microscopy. Shape analysis of GABA-ergic neurons revealed presence of three subpopulations. They were classified as round, oval and multiangular. Half of the neurons in the Cl and EN belong to oval ones. Among the remaining neurons of Cl and EN round cells predominated within EN, whereas multiangular neurons within Cl. The relationships between examined subpopulations were complex. The degree of colocalization of GABA and CaBPs is differentiated, depending mainly on the type of calcium binding protein. The highest degree of colocalization characterized PV-ir neurons – 85% of them contained also GABA. The degree of colocalization 63% of CB- and CR-ir neurons with GABA was moderate, reaching the values of 63% and 55%, respectively. In spite of observed double-stained, colocalized neurons, there were present also numerous CB-ir or CR-ir endings on GABA-ergic neurons localized on both perykaria and processes. More numerous PV-ir endings formed characteristic baskets around GABA-ergic neurons. Mutual relationships suggest important role of GABA-ergic and CaBPs neurons in local neuronal circuitry.

P4.21

Resiniferatoxin (RTX)- and tetrodotoxin (TTX)-induced changes in chemical coding of sympathetic neurons in the inferior mesenteric ganglion (IMG) supplying porcine urinary bladder

Agnieszka Bossowska, Joanna Wojtkiewicz, Celina Kowalska, Mariusz Majewski

Department of Human Physiology, Faculty of Medical Science, University of Warmia and Mazury, Olsztyn, Poland

Contact: agnboss@uwm.edu.pl

At present, RTX is used in experimental therapy as a drug which can abolish the activity of afferent neurons involved in abnormal neural circuits causing micturition disorders during various neurogenic diseases. RTX and TTX differ not only by different binding sites to nerve terminals, but also by various mechanisms of pharmacological action. Thus, it may be of great interest to compare the mode of action of both neurotoxins, what could potentially broaden our neurourological armamentarium. To reach this goal, changes in the chemical coding of sympathetic neurons supplying porcine urinary bladder, an animal which can be used as a very good model for human lower urinary tract, have been elucidated in detail after the exposure of the organ to the studied neurotoxins. The study was performed on 18 juvenile female pigs. The retrograde tracer Fast Blue (FB) was injected into the urinary bladder wall in all animals. Three weeks later, bladder instillation of RTX (500 nmoles per animal) was carried out in 6 animals while another group of 6 pigs was treated by TTX instillation (12 µg per animal). Animals of the control group (n=6) were instilled with the vehiculum only. After a week, all animals were sacrificed and the inferior mesenteric ganglia (IMGs) were collected. The neurochemical characterisation of FB⁺ neurons was performed using double-immunofluorescence labelling techniques. In control animals, the vast majority of FB⁺ neurons contained tyrosine hydroxylase/ dopamine β-hydroxylase (TH/DBH) and/or neuropeptide Y (NPY-IR; 95% and 85% of all retrogradely labeled neurons, respectively). A small number of FB⁺ cells also contained somatostatin (SOM), vasoactive intestinal polypeptide (VIP), calbindin (CB) and galanin (GAL; 2.2%, 2%, 1.7% and 1.2%, respectively). After RTX-treatment, a significant decrease in the number of FB⁺ neurons containing NPY and VIP was observed (to 55% and 0%, respectively), while the number of SOM and GAL-IR bladder-projecting sympathetic neurons increased (up to 55% and 13%, respectively). One week after TTX instillation, a significant decrease (to 7.5%) in the number of FB⁺ neurons containing neuropeptide Y (NPY) was observed, while the numbers of SOM-, GAL- or CB-IR neurons increased (up to 53%, 14% and 12% of all FB⁺ neurons, respectively). These preliminary data show that both neurotoxins influence the chemical coding of IMG cells supplying porcine urinary bladder. Thus, it appears that in further studies focusing on the RTX usage in the therapy of neurogenic bladder illnesses, this neurotoxin may also be possibly applied in the cases of disturbed neural control of the detrusor. The observed increase in the number of SOM- or GAL-IR neurons in studied IMGs after TTX instillation may suggest that this neurotoxin may be used in cases of insufficiency of the sympathetic component of the peripheral nervous system supplying the bladder.

P4.22

Calretinin in dopaminergic neurons of the opossum (*Monodelphis domestica*) midbrain nuclei: an immunohistochemical studyIlona Klejbor¹, Beata Ludkiewicz¹, Jan Spodnik¹, Sławomir Wójcik¹, Krzysztof Turlejski², Janusz Moryś¹¹Department of Anatomy and Neurobiology, Medical University of Gdansk, Gdansk, Poland²Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warszawa, PolandContact: klejbor@amg.gda.pl

The dopaminergic (DA) neurons in the ventral tegmental area (VTA) and substantia nigra (SN) are involved in the regulation of motor and motivational aspects of behavior as well as implicated in the emotional and cognitive disturbances associated with various stress-related disorders. Based on analyses of spontaneous behavior it is possible to observe differences between rat and opossum. Opossum showed higher rate of locomotors activity and explored new objects faster. The distinction of these behavioral strategies can reflect the differences in the morphology and function of selected brain systems and also in the expression of dopamine and calretinin (CR), one of calcium-binding

proteins involved in buffering intracellular calcium content. In the present study we used immunohistochemistry to describe the topography of CR-ir and DA-ir neurons as well as to evaluate the degree of CR/TH colocalization in VTA and SN nuclei in the adult gray short-tailed opossum. The material consisted of 9 adult animals. After fixation perfusion brain sections were stained using the antibodies against tyrosine hydroxylase (TH, marker of the dopaminergic cells) and CR and examined by fluorescent microscope BX-51 (Olympus, Japan). We found the presence of CR in all the studied regions. Within VTA nuclei we found round, oval or triangular densely packed CR-ir cells with visible dendrites. CR-ir fibers were long and sometimes showed varicosities. In SN nuclei CR-ir cells were observed mainly in pars compacta (SNc) and in the pars lateralis (SNI), these cells had predominantly polygonal shape with long dendrites. Neuropil showed densely packed CR-ir fibers and terminals. All the studied nuclei of VTA contained mainly round, oval or polygonal cells, and neuropil was rich in the TH-ir fibers and endings. Double labeling showed that most of the neurons in the studied region were TH/CR-ir. It may be concluded that in general the distribution of CR-ir and TH-ir neurons in VTA and SN in opossum and rat was similar but the degree of colocalization of TH/CR was clearly higher in opossum.

P4.23

Forced swim stress changes NGF/c-Fos immunoreactivity in the structures of the limbic system in the aged rats

Ewa Badowska-Szalewska, Tomasz Cecot, Ilona Klejbor, Beata Ludkiewicz, Jan Spodnik, Janusz Moryś

Dept. of Anatomy and Neurobiology, Medical University of Gdansk, Gdansk, Poland

Contact: klejbor@amg.gda.pl

Nerve growth factor (NGF) is the neurotrophin which may contribute to growth, survival and differentiation of neurons in the brain, and promotes repair and remodeling. NGF plays a role not only in the development of the nervous system but also has been implicated as important factor in memory processes and stress response in mature brain. In the aging nervous system, the role of NGF is less clear although aging is associated with a decreased efficiency in the physiological regulation of the response to stress stimuli. The aim of this study was to investigate the influence of acute and repeated stress (forced swim test, FS) on the NGF immunoreactivity in the limbic brain regions including the CA3 regions of the hippocampus, paraventricular (PV), and supraoptic (SO) nuclei of the hypothalamus and medial (MeA), and central (CeA) nuclei of the amygdala in aged rats. 15 Wistar male rats in P-360 (P-postnatal day) were used. Rats were divided into three groups (5 in each group): control (non-stressed), acute FS stress (one time for 15 min), and chronic FS stress (21 days for 15 min daily). Fluorescent double-immunofluorescence staining for NGF and c-Fos protein (marker of neuronal activation) was used. In control rats we found similar, low percentage of NGF/c-Fos colocalized neurons (about 2,5%) in all investigated structures. Single exposition to FS stress caused statistically significant (p<0.05) increase of NGF/c-Fos colocalization in the PV to 23% and SO to 12%. Similar trend was observed in other investigated regions of the limbic system, but it was not statistically important. Under chronic FS exposure statistically significant (p<0.05) increase of NGF/c-Fos colocalization was noted in all studied regions: PV – up to 21%, SO – 8%, CeA – 11%, MeA – 19%, CA3 – 12%. Comparison between acute and chronic FS stress revealed the lack (except of SO) of statistically significant differences. Most prominent changes were noted in PV nucleus of the hypothalamus, which is directly implicated in stress response. Assuming that NGF is expressed by activated neurons which express Fos-protein we conclude that higher colocalization NGF/c-Fos under acute as well chronic FS exposure may suggest stress-evoked plastic changes of the limbic system.

P4.24

Projecting neurons in the rat claustrum and endopiriform nucleus – colocalization study with neurons containing calcium binding proteins

Jan H. Spodnik, Edyta Spodnik, Sławomir Wójcik, Jerzy Dziewiątkowski, Janusz Moryś

Dept. of Anatomy and Neurobiology, Medical University of Gdansk, Gdansk, Poland

Contact: spodnot@amg.gda.pl

Clastrum (Cl) and endopiriform nucleus (EN) are subcortical structures present in all mammals. They play important role in the integrative process-

es within central nervous system. Both structures possess wide reciprocal connections with different cortical areas, CI mainly with neocortex, EN with allocortex. In our studies we use retrograde tracing of Fluoro-Gold (FG) to stain projecting neurons within examined structures after injection of neuronal tracer into the cortical areas. Moreover, we are interested in the localization of calcium binding proteins (CaBPs: parvalbumin (PV), calbindin-D28k (CB), and calretinin, CR) which are considered to be markers for certain subpopulations of GABA-ergic neurons. The aim of this study was to assess the mutual relationships between projecting neurons and interneurons within CI and EN. Tracer was injected into chosen cortical areas by means of iontophoresis. After 5 day of survival period rats were anesthetized and perfused with paraformaldehyde. Frozen brains were cut with a cryostat in coronal plane. Sections were stained immunohistochemically to visualize relationships between projecting neurons (stained by FG) and neurons containing CaBPs. Observations were performed with confocal laser scanning system Radiance 2100. We observed complex relationships between projecting neurons and neurons containing CaBPs. There were numerous CB-ir and CR-ir endings on projecting cells, especially localized on perikarya near the axon origin. More numerous PV-ir endings formed characteristic baskets around projecting neurons. Although we observed almost no colocalisation between CaBPs and projecting neurons the activity of the latter can be still influenced by the presence of numerous (especially PV) endings. This work was supported by grant W-54 of the Medical University of Gdansk.

P4.25

Comparison of ω -conotoxin (CTX) and botulinum toxin-induced changes in the chemical coding of the inferior mesenteric ganglion (IMG) neurons supplying porcine urinary bladder

Joanna Wojtkiewicz, Agnieszka Bossowska, Mariusz Majewski

Department of Human Physiology, Faculty of Medical Sciences,
University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Contact: Joanna.Wojtkiewicz@uwm.edu.pl

ω -conotoxin GIVa (CTX) is one of a wide range of neurotoxins isolated so far from the venom of the marine cone snails that are acting on the mam-

malian cells by modulating the activity of different ion channels. Activities of five conotoxins have been studied so far, and each of them acts on a different target in the affected cell. In this study we used CTX, because it inhibits N-type voltage-dependent calcium channels intimately involved in mechanisms of algnesia, thus evoking pain relief by inducing an analgesic effect. On the other hand, botulinum toxin of the A type (BTX), is one of the most powerful neurotoxins known so far. Recently, BTX has become one of the most effective drugs in the experimental therapy of a range of neurogenic urinary bladder disorders. Although its mode of action on the cholinergic component of the bladder innervation is relatively well-known, there is still a lack of data concerning possible action of this neurotoxin on other components of the peripheral nervous system controlling the functions of the bladder. The present study was aimed at revealing the chemical coding of IMG neurons supplying porcine urinary bladder, after intravesical BTX and CTX treatment. Urinary bladder wall was injected with retrograde tracer Fast Blue (FB) in 18 domestic pigs. After 3 weeks, 12 of them were divided into two experimental group. Group A was injected with BTX by means of a cystoscope, while animals of the group B were injected with CTX in the same manner. IMGs were collected from all animals and processed for a routine immunofluorescence labeling. In control animal, the vast majority of FB⁺ neurons were dopamine β -hydroxylase-immunoreactive (D β H-IR) and/or neuropeptide Y-immunoreactive (NPY-IR) – 95% and 85%, respectively, while a part of these neurons contained also somatostatin (SOM), vasoactive intestinal polypeptide (VIP), calbindin (CB) or galanin (GAL) – 2.2%; 2%; 1.7% and 1.2%, respectively. After BTX treatment, a significant decrease in the number of FB⁺, TH/D β H-, NPY- and/or VIP-IR neurons was observed (to 85%, 29% and 0%, respectively), followed by a slight decrease in the number of GAL-IR perikarya (to 1.0%), while simultaneously the number of SOM- or CB-IR urinary bladder-projecting sympathetic neurons was increased (to 18% and 12%, respectively). One week after injections of CTX into the bladder wall, a significant decrease in the number of FB⁺ IMG neurons containing NPY, SOM, VIP or CB were observed (to 66%, 12%, 0% and 6.8%, respectively), while the number of SOM-IR neurons was increased (to 12%). We have shown that treatment of the urinary bladder with BTX or CTX is able to profoundly change the neurochemical coding of noradrenergic (sympathetic) limb of peripheral micturition reflexes. Thus, it appears possible, that these neurotoxins may be used in the therapy of the overactivity of the sympathetic component of the urinary bladder innervation.

P4.26

Large-scale chromatin remodeling and DNA methylation in immature bovine oocyte during the later phase of growth and differentiation

Valentina Lodde, Silvia C. Modina, Federica Franciosi, Irene Tessaro, Alberto M. Luciano

Department of Animal Sciences, University of Milan, Milan, Italy

Contact: valentina.lodde@unimi.it

In mammals, the oocyte nucleus or germinal vesicle (GV) exhibits a distinctive chromatin configuration that is subject to dynamic changes during oogenesis [1]. This process is critical to confer the female gamete with developmental competencies. In spite of its biological significance, little is known concerning the mechanisms regulating large-scale chromatin structure in gametes [1]. Recent studies revealed that epigenetic effects by means of DNA methylation have an important role in the process of chromatin condensation and nuclear organization, besides gene expression regulation [2,3]. DNA methylation involves the transfer of a methyl group to the 5 position of cytosine residues within CpG dinucleotides. This process is catalyzed by DNA methyltransferases (Dnmts), which comprise a family of proteins involved in the establishment and maintenance of DNA methylation patterns. Since in cow we recently characterized the morphological transitions in the GV during the later phases of oocyte growth (from GV0 to GV3 stages), in which chromatin progressively condenses and the transcriptional activity decreases [4,5], the present study is designed to provide mechanistic insight into the complex relationship between large-scale chromatin structure, transcriptional silencing and global DNA methylation, which are crucial for the attainment of the final oocyte differentiation. Indirect immunofluorescence with an antibody to 5-methyl cytosine was carried out to evaluate and compare the degree of DNA methylation with the increase in chromatin condensation in immature bovine oocytes. Moreover the cellular distribution of Dnmt1, which is involved in the maintenance of DNA methylation patterns, was evaluated by indirect immunofluorescence. Samples were analyzed by confocal laser scanning microscopy after nuclear counterstaining with Propidium Iodide or Hoechst 33342. Semi quantitative analysis of DNA methylation levels was conducted on digitalized images. Our data indicate that DNA methylation is already established in GV0 stage oocytes. However, a slight increase of DNA methylation levels was observed in the transition from GV0 to GV1 oocyte, in which the major drop of transcriptional activity was also detected [5], suggesting a possible role of DNA methylation in oocyte chromatin remodeling and silencing. Dnmt1 protein was present throughout the cytoplasm in all the oocytes analyzed, while nuclear staining was not observed. This could indicate that Dnmt1 is not engaged in DNA methylation during this period. Ongoing distribution analyses of Dnmt3a and Dnmt3b should further clarify the role of Dnmts in the bovine oocyte (*L'OREAL-Unesco for women in science granted*). References: 1. De La Fuente R. Dev Biol 2006; 292: 1-12. 2. Reik W, et al. Science 2001; 293: 1089-93. 3. Robertson KD, et al. Nat Rev Genet 2000; 1: 11-9. 4. Lodde V, et al. Mol Reprod Dev 2007; 74: 740-9. 5. Lodde V, et al. Mol Reprod Dev 2008; doi:10.1002/mrd.20824.

P4.27

Keratinization of outer root sheath cells is prevented by contact with inner root sheath of rat hair follicles

Justyna Niderla-Bielińska, Ewa Jankowska-Steifer, Stanisław Moskalewski

Medical University of Warsaw, Department of Histology and Embryology, Warsaw, Poland

Contact: justynan@ib.amwaw.edu.pl

The purpose of the present study was to elucidate why keratinocytes of the outer root sheath (ORS) do not keratinize *in situ*. Two possibilities were considered – inhibition of keratinization is caused by contact of ORS with inner root sheath (IRS) or supply of keratinization promoting factors from the surrounding tissues to the ORS is insufficient. ORS cells from mid seg-

ments growing *in vitro*, in a medium with low Ca²⁺ content, formed monolayers, which after elevation of Ca²⁺ produced multilayers with cells containing keratohyalin-like granules. Ca²⁺ or EGF treatment upregulated expression of involucrin, filaggrin and Kdap. Culture of mid segments of hair follicles in low calcium culture medium under conditions preventing cell spreading increased expression of filaggrin and Kdap, but downregulated expression of involucrin. Stimulation by Ca²⁺ further increased expression of filaggrin and Kdap, but had no effect on the level of involucrin expression. EGF stimulated expression of filaggrin only. It is concluded that IRS exerted an inhibitory effect on the expression of involucrin, an essential component of the cornified envelope, thus preventing keratinization of ORS cells *in situ*. On the other hand, improved access of nutrients or promoting factors of keratinization to the mid segment of hair follicles augmented expression of filaggrin and Kdap, proteins engaged in the differentiation of keratinocytes but not involved in its terminal phase.

P4.28

Response to prednisone in relation to NR3C1 intron B polymorphisms in childhood nephrotic syndrome.

Grzegorz Zalewski¹, Anna Wasilewska², Lech Chyczewski¹, Walentyna Zoch-Zwierz²

¹Medical University of Białystok, Department of Clinical Molecular Biology, Białystok, Poland

²Medical University of Białystok, I Department of Paediatrics, Białystok, Poland

Contact: gregzal@wp.pl

Background: Different time required to cessation of proteinuria in nephrotic syndrome (NS) in children represents one aspect of variation in response to glucocorticoid (GC) treatment. Polymorphism of glucocorticoid receptor gene (NR3C1) is postulated to partially explain differences in clinical presentation as well as reaction to treatment in GC-treated diseases. Material and methods: 118 children diagnosed with NS who initially responded to oral GC treatment (steroid responsive nephrotic syndrome – SRNS group) and 136 healthy children were genotyped for three intron B single nucleotide polymorphisms of NR3C1, namely Bcl I (C/G), rs33389 (C/T) and rs33388 (A/T). In the SRNS group three-marker haplotype analysis of NR3C1 was performed in relation to response to prednisone represented as time to proteinuria resolution (TPR) considered as categorical and ordinal variable. Results: Distribution of individual polymorphisms and three-marker haplotypes was similar in healthy children and SRNS patients (all p values >0.05). GTA haplotype was associated with higher GC sensitivity as determined by TPR. It was found to be more prevalent in early (response ≤ 7 days) than in late (response > 7 days) prednisone responders (27.7% vs 14.5%, hap-score = -2.22, p=0.05 adjusted for biopsy results). Conclusions: The study is in accordance with previous report of association of intron B haplotypes with GC sensitivity. Moreover, the data show the distribution of GC polymorphisms in the population of north-eastern Poland.

P4.29

Role of cyclic AMP response element-binding protein in the duct cell differentiation in mouse submandibular gland

Shoichi Iseki, Sunisa Keattikunpairoj, Natthiya Sakulsak, Masa-aki Nakaya, Tomohiko Wakayama

Department of Histology and Embryology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

Contact: siseki@med.kanazawa-u.ac.jp

The submandibular gland (SMG) of mice has a marked sexual dimorphism in which a duct portion called the granular convoluted tubule (GCT) is developed preferentially in males with puberty. Administration of testosterone to female mice readily causes conversion of striated duct (SD) cells into GCT cells, but the underlying molecular mechanisms are unclear. Cyclic AMP response element-binding protein (CREB) is a transcription factor functioning in the downstream of a variety of signal transduction pathways. In the present study, we examined the expression of CREB and its activated form, phosphorylated CREB (p-CREB), in the mouse SMG by

use of Western blotting and immunohistochemistry. Immunoreactivity for both CREB and p-CREB was higher in the female than male gland, and localized to the nuclei of intercalated duct cells and a subpopulation of SD cells. In contrast, GCT cells in males appeared negative for CREB or p-CREB. The levels of CREB and p-CREB in the SMG were increased with castration in males and decreased by repeated administration of testosterone to females or to castrated males. Within 15 min after a single administration of testosterone to females, many SD cells newly gained nuclear immunoreactivity for p-CREB, which was lost as the cells converted to GCT cells by 48 h. These results suggest that the transcription factor CREB plays significant role in the androgen-dependent differentiation of the duct system in the mouse SMG.

P4.30

Expression pattern of RET finger protein in developing and young adult mice

Hiroyasu Umakoshi¹, Mayumi Jijiwa², Masahide Takahashi²

¹Nagoya University School of Medicine, Nagoya, Japan

²Department of Pathology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Contact: umakoshi.hiroyasu@d.mbox.nagoya-u.ac.jp

RET Finger Protein (RFP) belongs to the large B-box RING finger protein family and is known to become oncogenic by fusion with RET tyrosine kinase. RFP is reported to be expressed in various tumor cell lines and normal testes, function as a strong transcription repressor in mammalian cells, and contribute to diverse cellular processes such as apoptosis, development, and ubiquitination. Its *in vivo* function, however, is largely unknown. To investigate the role of RFP *in vivo*, the mice lacking RFP (designated 'RFP KO mice') were generated. The RFP KO mice were born normally and could survive at least 6 months after birth. In the gross findings and shapes of major organs, there were not any apparent differences between RFP KO mice and wild type mice. Histological analysis of various tissues of RFP KO mice did not show any obvious abnormalities. To clarify the RFP expression pattern in mice, we performed western blotting and immunohistochemistry using tissues from both RFP KO mice and wild type mice. Western blot analysis demonstrated that RFP is expressed not only in testes but also some other organs such as thymus, brain and bone marrow. Placenta and organs from embryos (E13.5, 15.5 and 17.5) were also investigated, and it was found that RFP expression in embryonic organs were stronger than in adult mice. Immunohistochemical analysis revealed that in testes RFP was expressed in perinuclear cap-like structure of primary spermatocytes. Moreover, we discovered that RFP was expressed in epithelial cells in thymus, neurons in various parts of central and peripheral nervous systems, and myeloid cells in bone marrow.

P4.31

Distribution and localization of wolframin in adult human tissues

Maria De Falco¹, Angela Lucariello², Luca De Luca², Lucrezia Manente², Rosa Tedesco², Anna Sellitti¹, Vincenza Laforgia¹, Antonio De Luca²

¹Dept. of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples "Federico II", Naples, Italy

²Dept. of Medicine and Public Health, Section of Anatomy, Second University of Naples, Naples, Italy

Contact: antonio.deluca@unina2.it

Wolfram syndrome is an autosomal recessive disorder characterized by diabetes insipidus, diabetes mellitus, optic atrophy, and deafness, thereby known also with the acronym of DIDMOAD. Genetic analysis has demonstrated that mutations in the WFS1 gene are associated with the Wolfram syndrome. This gene is also responsible for dominant forms of low-frequency hearing loss and has been associated also with increased risk for other phenotypes. The WFS1 gene encodes a transmembrane glycoprotein of endoplasmic reticulum (ER) consisting of 890 amino acids. It has been demonstrated that disruption of the WFS1 gene in mice causes progressive β -cell loss in the pancreas and impaired stimulus-secretion coupling in insulin secretion. Since the physiological function of this protein remains totally unknown, in the present study, we used immunohistochemistry to determine the localization of wolframin in a panel of different human adult tissues. First, we have characterized wolframin antibody in order to demon-

strate its specificity. Then, we have observed that wolframin was ubiquitously expressed in many organs although with different tissue distribution and expression levels. In particular, a variable positive staining was observed in both simple or stratified epithelium, with a slight wolframin expression in the basal layer of the skin compared to a more intense immunopositivity for this protein in other stratified epithelia such as oesophagus and cervix. In normal placenta there was a modulation of wolframin throughout pregnancy with a strong level of expression during the first trimester and a moderate level in the third trimester of gestation. A moderate wolframin expression was showed also in hepatocytes and in the endocrine portion of the pancreas. Also the adrenal glands and the kidney showed a moderate level of wolframin immunopositivity. In conclusion, on the basis of wolframin distribution in several human tissues we may hypothesize that this protein may have important roles in cell proliferation and in the maintenance of cellular homeostasis.

P4.32

CYP2B1/2, CYP3A1 and CYP3A2 immunolocalization in normal and regenerating livers of rats of different age

Aleksandra Bryzek, Danuta Plewka, Piotr Czekaj, Ryszard Wiaderkiewicz

Department of Histology, Medical University of Silesia, Katowice, Poland

Contact: metabolizm@slam.katowice.pl

Cytochromes P450 are the key element of mixed function oxidase system, which participates in the metabolism of exogenous compounds. The aim of the study was to show to which extent age, induction, chemical damage and regeneration may modify localization of CYP2B1/2, CYP3A1 and CYP3A2 within the liver. Study was performed on male Sprague-Dawley rats presenting 11 age categories: fetuses (16-, 18-, 20-day old), newborns, adolescent animals (0.5-, 1-, 2-month old), mature ones (4-months old) and aged ones (20-, 24- and 28-month old). Within particular age groups 3 experimental subgroups were separated, namely: one control subgroup (C) and two inductor ones (PB and Dex), and – in adult rats – one subgroup of experimental hepatocarcinogenesis (CDE). Rats from PB and Dex subgroups were administered twice classical inducers of cytochrome P450 isoform: phenobarbital (80 mg/kg of b.w.) and dexamethasone (30 mg/kg of b.w.), respectively. Rats from CDE subgroup were fed *ad libitum* for three weeks with choline-deficient ethionine-supplemented diet, which injures hepatocytes and stimulates liver regeneration process depending on non-differentiated cells. Localization of individual P450 isoforms within the liver acinus was analyzed immunohistochemically. CYP-positive cells were located in hepatoblasts in the late phase of fetal life. Zone-like expression of CYP2B1/2 and CYP3A1 was formed during young life and sexual maturation. Zone 2 and 3 of the liver acinus was the most important localization of these isoforms appearance within the age range from 0.5 to 4 months; zone 3 – in old rats. Zone 3 of the liver acinus was the only localization of CYP3A2 appearance within the age range from 0.5 to 28 months. In livers sampled from rats fed with CDE diet CYP3A1 was located within the 2 and 3 zone, whereas CYP2B1/2 and CYP3A2 expression was observed in all zones of the liver acinus. Expression of CYP3A1 and CYP3A2 was heterogenous within the zones. It was concluded that local effects of P450 isoforms expression in regenerating rat liver can be associated with disappearance of zone-like localization of CYP2B1/2 and CYP3A2. Supported by KBN, Grant no 3P05D03623.

P4.33

Pathomorphological evaluation of an Alzheimer's disease model induced by sodium azide in rats

Mihály Albert

EGIS PLC, Budapest, Hungary

Contact: albert.mihaly@egis.hu

One of the most prevalent dementias is Alzheimer's disease (AD), in which the patients suffer from a progressive, debilitating learning and memory disturbance. The exact aetiology of AD is still obscure. Insufficient mitochondrial function is one of the possible causes that has been suspected to lead to AD. Na-Azide (NaN₃) inhibits the electron transport chain in mitochondria causing severe disturbance in the cellular energy production. Therefore, NaN₃ can elicit hypoxia, increase free radical production and glutamate release leading to excitotoxicity and neuronal degeneration. The

NaN₃-induced AD-like condition is as yet a rarely used, newly developed model of AD. The aim of this study was to characterize further histological consequences induced by treatment with NaN₃ in order to delineate its suitability for testing memory-enhancing effects of drug candidates. Adult, male SPRD rats, weighing 300 g, were treated with NaN₃ at 30 mg/kg/day for 31 days using osmotic minipumps. Memory and learning abilities were tested with behavioural tests (Morris water maze, open field), and detailed histopathology was performed at the termination of the study. The morphological alterations in the brain were compared to vehicle treated controls. Light microscopy revealed normal structure in the brain of control animals. Neuronal degeneration (lack of Nissl substance, shrunken neurons, neurofibrillary tangle-like structures) and necrosis were seen in the cortical and hippocampal areas in the rats treated with NaN₃. Amyloid protein, which is characteristic for AD, was not detectable with the Congo red stain. Microtubule associated protein 2 expression, a specific marker of neurons, was lower in the neurons of rats treated with azide. Ultrastructural changes were detected in the neurons (mitochondria and microtubules) and glial cells in the NaN₃ treated rats suggesting some degree of disruption of blood-brain barrier. Significant loss of the neurons was detected in the hippocampal CA1 and CA3 regions. Ki67 immunohistochemistry, to evaluate neurogenesis, demonstrated a decreased rate of cellular proliferation in the dentate gyrus in the animals given azide. Decreased learning and memory functions were accompanied with the morphological alterations in the NaN₃-treated animals. Nearly all changes in the central nervous system induced by NaN₃ resemble those observed in human AD. The model of dementia caused by inhibition of mitochondrial energy production mimics the central features of AD including the histological picture, learning and memory dysfunctions, so it seems suitable for testing nootropic agents.

P4.34

Role of TNF-alpha in pulmonary hypertension induced by monocrotaline in rats

Ivan Kocić, Katarzyna Sztornowska, Iga Wapniarska

Department of Pharmacology, Medical University of Gdańsk, Gdańsk, Poland
Contact: iga112@amg.gda.pl

Summary: Tumor necrosis factor alpha (TNF-alpha) is a pleiotropic inflammatory cytokine involved in a variety of reactions associated with human diseases. The objective of the study was to determine whether the serum concentration of TNF-alpha changes during pulmonary arterial hypertension (PAH). PAH leads to pathological changes in the cardiovascular system and current research has shown overproduction of inflammatory proteins in this condition. Experiments were performed on Wistar rats (body weight 180-220 g). In Group 1 (male) and Group 2 (female) rats were treated by monocrotaline (i.p., 60 mg/kg b.w.), and in Group 3 (male control) and Group 4 (female control) they were treated by an equivalent volume of normal saline solution (n=40). After 4 weeks serum concentration of TNF-alpha was measured by enzyme-linked immunosorbent assay (ELISA). No statistically significant changes in the level of TNF-alpha were observed between the study groups. Our results indicate that PAH does not increase serum concentration of TNF-alpha, at least in rats treated with monocrotaline.

P4.35

Level of p53 and p21 protein in pterygium cells

Anna Lekawa¹, Halina Antosz²

¹Department of Human Genetics, Medical University of Lublin;
²First Department of Ophthalmology and

²Department of Human Genetics, Medical University of Lublin, Lublin, Poland
Contact: annalekawa@wp.pl

Introduction: Pterygium is a lesion of unknown pathogenesis, derived from conjunctiva. It was proved that its occurrence is connected with UV radiation exposure. It appears with various recurrence rate after surgery. Pterygium was investigated to establish whether it has characteristics of transformed cells at the level of p53 and p21. P53 is a suppression gene of neoplastic transformation and its expression increases after DNA damage. P53 activates p21 protein, which is an inhibitor of cell growth cycle. The accumulation of mutated p53 is considered to be a marker of increased proliferative cellular activity. Aim: The aim of this research was to estimate the level of p53 and p21 protein in pterygium tissue. Materials and methods: 20

pterygium tissues and 6 conjunctiva tissues were used as an investigated material and comparative material respectively. Tissues were drawn from patients operated in the First Department of Ophthalmology, Medical University of Lublin, Poland. The tissues under research were stained immunohistochemically with the use of monoclonal antibodies against human proteins p53 clone DO-7 and p21^{Waf1/Cip1} clone SX118 (DAKO Cytomation). The percentage of positively marked objects from 500 counted cells was estimated. Results: The expression of p53 and p21 protein was investigated in the basal layer of pterygium and conjunctiva epithelium. Immunohistochemical testing showed the difference between the investigated tissues. The level of p21 protein in pterygium tissue in comparison to its level in conjunctiva tissue was significantly lower. It was observed, that in the pterygium tissue simultaneously with high expression of p53, the low expression of p21 occurred.

P4.36

Collagen-chondroitin sulphate-hydroxyapatite porous composites: a histochemical approach

Otilia Zarnescu¹, Oana Craciunescu², Mirela Balan², Lucia Moldovan²

¹Department of Animal Biology, Faculty of Biology, University of Bucharest, Bucharest, Romania

²National Institute of Research and Development for Biological Sciences, Bucharest, Romania

Contact: otilia@bio.unibuc.ro

Biomaterials used for regenerative medicine are designed to mimic the structure, mechanical properties, and biologic behavior of native tissue and their extracellular matrix. They provide three-dimensional architecture for the cells to reconstitute into new tissues with appropriate structure and function. Collagen-hydroxyapatite scaffolds were used in regenerative medicine due to their biocompatibility, osteoconductivity and structural similarity to the inorganic component of bone. Aim of our study was to describe by histochemical and immunohistochemical methods the distribution of collagen, hydroxyapatite and chondroitin sulphate inside three new variants of collagen-based biodegradable composites. In order to fabricate the composite scaffolds, a solution of collagen type I (0.8%) was mixed with a solution of chondroitin sulphate (1%) and hydroxyapatite powder, in weight ratios of 1:0.5:1 (variant 1), 1:0.5:2 (variant 2) and 1:0.5:4 (variant 3). These composites were constructed as three-dimensional scaffolds with microporous structure by integrating hydroxyapatite powder in a gel mixture of collagen-chondroitin sulphate. In order to show the micro-structure of three porous composites we used von Kossa method to stain hydroxyapatite, immunofluorescence microscopy for detection of chondroitin sulphate, and picosirius red staining in combination with polarization microscopy for collagen demonstration. Based on polarization color, collagen represented major component of the porous composites. Immunofluorescence showed a random distribution of chondroitin sulfate among collagen fibers, some collagen fibers being associated with more chondroitin sulphate than others. Depending on the ratio between collagen : chondroitin sulphate : hydroxyapatite, von Kossa histochemical staining showed a progressive loading of collagen-chondroitin sulphate bundles with hydroxyapatite. The hydroxyapatite deposits were of different sizes. Work was supported by Romanian PN2 Program, BIOSTEM 61-012/2007.

P4.37

Basal cell marker revealed by RAM11 antibody: delayed expression during epithelial regeneration in rabbits

Grzegorz J. Lis¹, Joanna Zarzecka², Jan A. Litwin¹, Mariusz Gajda¹, Tadeusz Cichocki¹

¹Department of Histology, Jagiellonian University Medical College, Krakow, Poland
²Department of Conservative Dentistry and Endodontics, Institute of Stomatology, Jagiellonian University Medical College, Krakow, Poland

Contact: mmlis@cyf-kr.edu.pl

Objective: RAM11 is a monoclonal IgG₁ antibody developed by immunization of mice with rabbit alveolar macrophage extract. The antibody binds a cytoplasmic, so far unknown antigen and has been used for identification of macrophages in rabbit tissues. We previously demonstrated that RAM11 reacted with basal cells of stratified squamous epithelia of rabbit skin, oral mucosa, and esophagus. This study compares RAM11 immunoreactivity in basal cells of normal and regenerating epithelium in rabbit

oral mucosa. Methods: 6 µm paraffin sections were prepared from oral wounds surgically created in rabbits (New Zealand White), and excised within intact margin in the course of spontaneous healing process on days 1, 3, 7, and 14 (results presented in this abstract are part of a larger study on epithelial regeneration). Some sections were stained routinely with HE, the others were used for immunohistochemistry. The following primary mouse monoclonal antibodies were used: RAM11, anti-keratin 5.8, anti-S100 protein (subunit A and B) and anti-PCNA. Goat anti-mouse Cy-3-conjugated antiglobulins were employed as secondary antibodies. Cell nuclei were counterstained with DAPI. Sections were examined with Olympus BX50 light/fluorescence microscope. Results and conclusions: In normal oral mucosal epithelium, RAM11 immunoreactivity was revealed by almost all cells located in the basal layer. The signal was seen as small fluorescent granules located preferentially in the supranuclear region. However, no RAM11-positive cells were observed in the regenerating epithelium examined on days 1 and 3 of wound healing. A weak immunofluorescence first appeared on day 7 in single basal cells, with no particular relation to the distance from the intact epithelium. The number of RAM11-positive basal cells on day 14 increased as compared to day 7, however, still more than a half of the cells stained negatively. In both, normal and regenerating/newly formed epithelia, expression of keratin 5, a marker of the basal layer of human oral epithelium, was observed only in the intermediate layer. Basal cells with nuclear expression of PCNA were keratin 5-negative. Nuclear expression of PCNA was present in almost all basal and parabasal cells in both intact and regenerating epithelium showing that RAM11 immunoreactivity was not related to the proliferative character of basal cells. The immunostaining for S100 protein expressed in many cell types including melanocytes and phagocytic cells (subunit A) was occasionally found in the cells of intermediate layer, but only in the intact epithelium. The basal epithelial cells were always negative. Our observations demonstrate that expression of the antigen recognized by RAM11 antibody begins during maturation of the basal epithelial cells and seems not to be associated with either melanocytes or antigen-presenting cells, hence it can be regarded as basal cell marker only in matured stratified squamous epithelia.

P4.38

Seasonal lectin binding pattern in thumb pad of the frog (*Rana ridibunda*)

Engin Kaptan, Sehnaz Bolkent

Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey
Contact: engkaptan@istanbul.edu.tr

Thumb pad is one of the most common secondary sexual characteristics in frogs. Although it has been known that anuran amphibian skin has affinity to several lectins, there is no report regarding the lectin binding affinity of the thumb pad or its structural components. In this study, the localization and seasonal variations of specific carbohydrate moieties of glycoconjugates in both epidermis and dermis of the frog thumb pad were investigated at light microscopic level using lectin histochemistry and discussed in different points of view. In the current study, we compared four groups of frogs, namely active, prehibernating, hibernating and posthibernating. Five horseradish peroxidase-conjugated lectins were employed. It was found that *Conavlia ensiformis* (Con A) and *Arachis hypogaea* (PNA) lectins gave no significant reaction on both epidermal layers and dermal glands (so-called breeding glands) of the thumb pad. *Dolichos biflorus* (DBA), *Triticum vulgare* (WGA) and *Ulex europaeus* (UEA-I) lectins gave positive reactions on both epidermal layers and dermal glands. Besides, these three lectins bound specifically to certain secretory cells in the glands and the distribution of these cells and epithelial lectin reactions exhibited seasonal changes. Generally, epidermal lectin binding showed highly dense affinity in the posthibernating period. DBA and WGA specific cells in the gland decreased gradually until the posthibernating period. Several UEA-I specific cells were evident in the posthibernating period. These findings point out that glycoconjugates binding DBA, WGA and UEA-I lectins may be a result of seasonal adaptations related to reproductive activities. Moreover, the presence of the specific lectin binding cells indicates that the glands contain heterogeneous secretory materials. These cells maybe produce the substances playing a role in breeding.

P4.39

Antigen presenting cell-lymphocytes clusters formation in H-2d mice infected with ectromelia virus

Lidia Szulc, Małgorzata Gieryńska, Marek Niemiałtowski

Division of Immunology, Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Warsaw, Poland
Contact: lidiaszulc@gmail.com

Lymphocytes-APC (antigen presenting cell) clusters serve as the initial activation of T cells during adaptive immune response and they are the site of mutual interactions between lymphocytes' subsets. These interplays include CD4⁺ T cell help for CD8⁺ T or B cell differentiation, commitment of naive T cells to T_h1 or T_h2, and suppression of effector T cells by regulatory CD4⁺ T cells. The physical contact zone (cell membranes) between APC and lymphocytes leads to immunological synapse formation. Our studies were focused on the phenotype of effector cells (NK, T CD8⁺/CD4⁺, and B cells) forming "rosettes" with APC (DC, M ϕ) in genetically susceptible BALB/c (H-2^d) mice infected with a highly pathogenic Moscow strain of ectromelia virus (ECTV-MOS). Multicellular clusters were received using enzyme digestion of mouse draining lymph nodes (DLN) and spleen. They were analyzed in fluorescence (Olympus BX60 ColorViewIII), confocal (Olympus IX81 FV1000) and scanning electron (FEI Quanta 200) microscopes. Our data showed that the number of clusters increased twofold in DLN and spleen at 7th dpi, therefore their formation was clearly enhanced by the ongoing immune response. Among all clusters isolated on 2nd and 7th dpi from BALB/c splenocytes, ECTV-MOS specific clusters reached 15% and 47%, respectively. Different populations of effector cells were involved in cluster formation, including NK cells, neutrophils, CD4⁺/CD8⁺ T cells and CD19⁺ B cells. It suggests the recognition of ECTV-MOS antigens by effector mechanisms of innate and adaptive immune response. The percentage of neutrophils within clusters that expressed ECTV-MOS specific antigens increased at 7th dpi, which is the hallmark of acute inflammation. Different T cell subsets (CD4⁺ and CD8⁺) co-localized within individual clusters, where CD4⁺ effector T cells help in the generation of an optimal ECTV-specific CD8⁺ CTL response. During infection the percentage of effector cell populations in clusters has changed. An increase in the percentage of antigen-specific cytotoxic CD8⁺ T cells producing IFN- γ at 7th dpi can be the results of their direct involvement in the elimination of target cells (APC) infected with ECTV-MOS. Grant No 2 PO5A 050 30 from the Ministry of Sciences and Higher Education (for MN).

P4.40

The influence of diet supplementation with selenomethionine and vitamin E on the morphological picture of rabbits' organs in experimental atherosclerosis

Barbara Stawiarska-Pięta¹, Ewa Szafłarska-Stojko¹, Ewa Birkner², Ewa Grucka-Mamczar², Jolanta Zalejska-Fiolka², Anna Maj¹, Marta Knapik¹

¹Department of Pathology, Faculty of Pharmacy, Silesian Medical University in Katowice, Sosnowiec, Poland

²Department of General Biochemistry, Faculty of Medicine, Silesian Medical University in Katowice, Zabrze, Poland

Contact: bspjeta@o2.pl

Atherosclerosis is characterized by regressive and progressive changes in the intima and tunica of arteries. Recent studies indicate that in the pathogenesis of atheromatosis, significant role is played by oxidation processes. Oxidative free radicals oxidize low density lipoproteins (LDL) that may damage blood vessel endothelium. Vitamins A, E, and C, as well as methionine, cysteine, and co-enzyme Q, help in protecting organism from free radical oxidation. The aim of the study was to assess the influence of selenomethionine and vitamin E on the development of pathomorphological changes in the internal organs of rabbits in experimental atherosclerosis. The study was performed on 30 male rabbits of the New Zealand strain with the initial body weight of 3000g \pm 50g. The animals were divided into 5 groups: a control group and four study groups. Rabbits in groups I and II were fed a cholesterol-supplemented diet (0.5 g cholesterol/rabbit/day). Additionally, animals of the group II were supplemented with selenomethionine (12.5 mg/kg bw/24h) and vitamin E (10 mg/kg bw/24h). Animals in group III were fed a higher cholesterol-supplemented diet (2.0 g of cholesterol/rabbit/day). Animals in group IV were fed the same high-choles-

terol diet and were also given selenomethionine (12.5 mg/kg bw/24h) and vitamin E (10 mg/kg bw/24h). Control group received regular GLK fodder. The experiment lasted for three months. Concentrations of plasma lipids were monitored monthly. At autopsy, blood was collected for biochemical analyses, and the aorta and liver were collected for histopathological evaluation. The pathomorphological changes in the organs were assessed in paraffin sections stained with hematoxylin and eosin. The presence of fat in the atheromatous lamellas of aorta and in hepatocytes was confirmed in cryosections stained with Sudan III for the detection of neutral fat. In the doses used selenomethionine and vitamin E have preventive influence on the atherogenic processes in the aorta and on the regressive changes (steatosis) occurring in the liver of rabbits fed both with high- and low-cholesterol diet. However, the preventive effect was stronger in the group of rabbits on low-cholesterol diet. In this case only small atheromatous plaques and no fatty degeneration in liver were observed.

P4.41

Mouse model of the DNA damage response

Pavla Lužná, D. Kylarová, S. Takáčová, V. Divoký, E. Kašpárková, E. Nežhybová, L. Píterková, J. Ehrmann

Olomouc, Czech Republic

Contact: pavla.luzna@tiscali.cz

DNA replication stress is thought to protect the cell from precancerous processes leading to tumour development. It involves activation of the DNA damage checkpoint pathway involved in cell cycle arrest, apoptosis or senescence. The most prolific DNA damage is caused by double-strand breaks. They are detected by the checkpoint kinases ATM, ATR and Chk2, and the ATM molecule phosphorylates histone H₂AX. These molecules activate other proteins which, in turn, determine the fate of the cells. The aim of this study was to set up a DNA model of mouse bone marrow cell damage, induced by ionizing radiation and to use it as a positive control in further experiments. Material and methods: Three groups of normal C57/BL6 male mice were irradiated by a total dose of 5 Gray of ionizing radiation. The mice were then sacrificed at four time points (1, 3, 6 and 24 h). Femoral bones were then removed and decalcified. A three-step indirect immunohistochemistry method was used to investigate the levels of active forms of proteins known to play a crucial role in the DNA damage response pathway (ATM, H₂AX). Finally, TUNEL assay and active caspase-3 staining was performed to visualize apoptotic cells. Results: We found different levels of expression of the selected proteins at different time points following irradiation; the highest level of phosphorylated histone H₂AX was detected one hour after irradiation. The expression of ATM was also highest one hour after irradiation. The highest number of apoptotic cells were detected 3 hours after irradiation by both TUNEL and active caspase-3 staining. Conclusion: The results confirm the usefulness of this model for the DNA damage studies in mouse bone marrow. Acknowledgement: This work was supported by grant MSM 6198959205.

P4.42

Histochemistry and nuclear magnetic resonance as tools for evaluating meat quality

Manuela Malatesta¹, Federica Perdoni², Barbara Cisterna², Marco Biggiogera³, Elena Nicolato¹, Marco Fontanella¹, Carlo Zancanaro¹

¹Dipartimento di Scienze Morfologico-Biomediche, Sezione di Anatomia e Istologia, University of Verona, Verona, Italy

²Dipartimento di Biologia Animale, Laboratorio di Biologia Cellulare e Neurobiologia, University of Pavia, Pavia, Italy

³Dipartimento di Biologia Animale, Laboratorio di Biologia Cellulare e Neurobiologia, University of Pavia, and Istituto di Genetica Molecolare del CNR, Pavia, Italy

Contact: manuela.malatesta@univr.it

Food safety is universally recognized as a public health priority which requires a global approach, from production to consumption [1]. Increasing efforts are ongoing to improve the quality of meat as an important component of the human diet. It is known that the nutritional and sensory quality of meat may be influenced by multiple interacting factors before slaughter including cattle diet [2,3]. When evaluating the outcome of different food on meat quality, several compositional and structural parameters must be considered [4,5]. Special relevance is given to the muscle fibre composition, as well as the amount and distribution of fat and connective tissue,

which are major determinants of the whole organoleptic characteristics of meat. The aim of our study was to identify differences in structure and composition of meat of pigs fed on two different diets by combining histochemistry and nuclear magnetic resonance (NMR). The staining procedures for the histological components (connective tissue fibres, lipids, muscle fibre types), allow for fine structural characterization of meat, while NMR is an established methodology to investigate its composition and structure [e.g. 6,7]. In the spectroscopy mode (MRS), NMR provides data on the chemical composition, whereas, in the imaging mode (MRI), it allows to localize spatially the tissue signal, thus enabling morphological description of meat. Twenty pigs (Large White hybrid) were grown from the 5th to the 9th month of age according to two nutritional protocols: ten animals were fed a standard commercial fodder, and other ten a fodder made of vegetables cultivated with an inorganic fertilizer with a very low content of N, P, and K which proved to increase nutrients in the crop (Bio-Pre srl, Vicomosciano, CR, Italy). After slaughtering, several muscles or muscle groups for wholesale cut were isolated, enclosed under vacuum in a plastic bag at 4°C and rapidly examined by MRI. Muscle tissue fragments were also frozen or fixed and paraffin embedded to be processed for histochemistry as well as for MRS analyses. Using this combined procedure, differences in connective tissue, water and fat content and distribution were detected between the two animal groups, suggesting that a relationship may exist between the different feeds and the organoleptic features of pork meat. Acknowledgement: This work was supported by University of Verona (Joint Project 2007) and Regione Lombardia (Bando 241 / 2006, Id. 411298). References: 1. OIE Animal Production Food Safety Working Group Rev Sci Tech 25:823, 2006; 2. Wood JD et al. Proc Nutr Soc 58:363, 1999; 3. Olsson V, Pickova J. Ambio 34:338, 2005; 4. Jeremiah LE et al. Meat Sci 65:1013, 2003; 5. Purslow PP. Meat Sci 70:435, 2005; 6. Monzilos M et al. Magn Reson Imaging 23:745, 2005; 7. Fantazzini P et al. Magn Reson Imaging 23:359, 2005.

P4.43

The effect of hypometabolizing molecules on transcription as shown by a new two-step pulse-chase method

Alessandro Spedito¹, Manuela Malatesta², Marco Biggiogera³

¹Dipartimento di Biologia Animale, Laboratorio di Biologia Cellulare e Neurobiologia, University of Pavia, Pavia, Italy

²Dipartimento di Scienze Morfologico-Biomediche, Sezione di Anatomia e Istologia, University of Verona, Verona, Italy

³Dipartimento di Biologia Animale, Laboratorio di Biologia Cellulare e Neurobiologia, University of Pavia, and Istituto di Genetica Molecolare del CNR, Pavia, Italy

Contact: manuela.malatesta@univr.it

DADLE (D-Ala-D-Leu Enkephalin) and DALE (D-Ala-Leu Enkephalin) are two synthetic opioids capable of inducing a hypometabolic state in cell cultures. Their structural difference causes a quick degradation for DADLE inside the cell, and a slower one for DALE. HeLa cells were treated as follows: control cells; 48 hours treatment with the peptides (10⁻³ M) in the culture medium; 48 hours treatment with the peptides (10⁻³ M) followed by 48 hours recovery (DADLE recovery, DALE recovery). Our experiments focus on the nuclear metabolism, in particular on transcription. Our previous data demonstrated that the transcriptional activity decreases after 48 hours treatment with either DADLE or DALE, while, after recovery, the two peptides show different results. We observed also that, after DADLE recovery, the cells restored their transcriptional ability; on the contrary, after DALE recovery, the cells showed the same transcriptional level as during the treatment. The aim of this research was to evaluate the amount of incorporation of two RNA precursors after DADLE and DALE treatment by an ultrastructural immunocytochemical method. We added chlorouridine to the medium culture for 15 min and, after 15 min of incubation with fresh medium, we added iodouridine for further 15 min. Afterwards, two anti-BrdU antibodies, recognizing specifically chlorouridine or iodouridine, were used. Statistical analysis was carried out by counting the number of double-labelled perichromatin fibrils. The presence of only one precursor on the fibrils indicates that transcription (for that specific fibrils) took place in less than 15 minutes, whereas the presence of both precursors shows that transcription took place for at least 15 minutes or longer. Consequently, an increased number of double-labelled fibrils indicates a transcriptional slowing down. The results showed that, after both DADLE and DALE treatment, the amount of double-labelled fibrils increased as compared to the controls. In the control cells we observed 22% of double-labelled fibrils while, after treatment with the peptides, we found 62,5% (DADLE) and 58,4% (DALE). Conversely, after the recovery period we

obtained different results for the two peptides: after DADLE recovery the amount of double-labelling was 37%, while after DALE recovery was 57.4%. These results confirm our previous data, showing that both peptides are capable of inducing transcriptional slowing down. Moreover, the recovery data indicate that cells can restore their transcriptional activity after DADLE treatment, but not after DALE. This is probably due to the different metabolism of the peptides: cells are incapable of degrading DALE and so its effect may last longer than DADLE's; therefore, 48 hours recovery could not be sufficient to restore the transcriptional activity.

P4.44

Annual reproductive cycle in two free living populations of three-spined stickleback (*Gasterosteus aculeatus* L.): patterns of ovarian and testicular development

Ewa Sokolowska

Institute of Oceanology of Polish Academy of Sciences, Sopot, Poland

Contact: ewasokol@iopan.gda.pl

The annual reproductive cycle in two wild populations of three-spined stickleback was studied. Sticklebacks from the Dead Vistula river (Martwa Wisła) (brackish water) and the Oliva Stream (Potok Oliwski) (freshwater) were exposed to annual environmental changes in their natural habitats. Ovaries and livers (females), and testes and kidneys (males) were collected during 1-2 years. The gonadosomatic (GSI), hepatosomatic (HSI), nephrosomatic (NSI) indices, kidney epithelium height (KEH) and size of oocytes were calculated. The number of mature oocytes and percentage of ovulating females were determined during the spawning season. Histological changes in the ovaries and testes were described throughout a year. Annual reproductive cycles were similar in both populations of sticklebacks. This is the first histological and morphological study carried out throughout a year, simultaneously in two wild populations of three-spined sticklebacks inhabiting different environments. An improved scale of gonadal development in conjunction with the determined indices and fecundity give a comprehensive description of the reproductive cycle. These new observations, in combination with previously reported features, provide a universal scale that can be successfully applied to distinguish all phases of gametogenesis in sticklebacks in different habitats.

P4.45

Bilateral changes of IL-6 protein levels in the rat dorsal root ganglia associated and non-associated with nerve injury

Zuzana Veselková, Radim Jančálek, Ilona Klusáková, Ivana Svíž enská, Lumír Trenčanský, Petr Dubový

Department of Anatomy, Division of Neuroanatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Contact: zvesel@med.muni.cz

Peripheral nerve injury results in neuroimmunologic responses that may be integral to the development and maintenance of neuropathic pain. Recent data have demonstrated that cytokines may be strongly implicated in the generation of pain states at both peripheral and central nervous system sites. This study sought to evaluate both spatial and temporal changes of IL-6 protein levels in ipsi- and contralateral lumbal (L4-L5) and also within cervical (C6-C7) dorsal root ganglia (DRG) after the lesion of peripheral nerves. A commercially available ELISA test (BioSource, CA, USA) was used for quantitative analysis of IL-6 protein. Male Wistar rats (weighing 250-300 g) were divided into four groups: i/ rats which underwent aseptic triple unilateral ligation of sciatic nerve (ScNL; n=12); ii/ rats operated on ligation of L4-L5 spinal nerves (SNL; n=12), all operated animals were left to survive for 1, 3, 7 and 14 days; iii/ no nerve lesions were produced in animals of na?ve control group (n=9); iv/ sham operated group (n=12) with periods of survival 3 and 14 days. One day after ScNL, significantly increased levels of IL-6 protein were measured in both the ipsilateral and contralateral cervical and lumbal DRG. The DRG from other periods of survival displayed just mild increase in IL-6 protein levels close to the control (na?ve group) baseline level. After the SNL operation, IL-6 protein was significantly increased from post-operation day 1, and rose up to 14 days not only in ipsi- and contralateral L4-L5 DRG, but also in ipsi- and contralateral C6-C7 DRG. The obtained data provide evidence for changes of IL-6 protein levels not only in the DRG associated with damaged nerve

(L4-L5), but also in those non-associated with nerve injury (C6-C7) in two distinct rat experimental neuropathic pain models. Stimuli for bilateral increase of IL-6 protein in the DRG after unilateral nervous lesion are probably of systemic character.

P4.46

Morphology and function of cells demonstrating expression of beta estrogen receptor in lateral lobe of rat prostate under hyperprolactinaemia induced with risperidone

Maria Laszczyńska¹, Karolina Chorzępa-Nowicka¹, Marcin Wylot², Małgorzata Piasecka¹

¹Laboratory of Histology and Developmental Biology, Pomeranian Medical University, Szczecin, Poland

²Department of Histology and Embryology, Pomeranian Medical University, Szczecin, Poland

Contact: sphbr@sci.pam.szczecin.pl

The latest research demonstrates that not only androgens but also prolactin (PRL) and estradiol (E₂) have a strong influence on the physiology and pathology of human and rat prostate. It was shown that both a high level of PRL and E₂ may cause serious disorders of the function of prostate and induce hyperplasia of this organ. The discovery of β estrogen receptor (ER β) in human and rat prostate as well as the demonstration of its antiproliferative action permitted further understanding of the hormone regulation mechanisms in this gland. Because of the similarity of lateral lobe of rat prostate to human prostatic gland, this organ is often used for experimental purposes. The aim of our study was to demonstrate the influence of a new generation of neuroleptic, i.e. risperidone-induced hyperprolactinemia, on the morphology and function of ER β -expressing cells in the lateral lobe of rat prostate. Moreover, changes in PRL, E₂ and testosterone (T) serum concentrations in the study group were compared to those in the control group of rats. The experiment was conducted on three-month-old, sexually mature, male Wistar rats. The animals were divided into two groups: the control one (C) and the study one (R). The (R) group animals were administered risperidone (Risplept, Janssen Pharmaceutica, Belgium) for 14 days in the dose of 0,3 mg/kg m.c. in order to induce hyperprolactinemia, while (C) group rats were administered a placebo. Immunoenzymatic method was used to determine medium level of PRL, T, E₂ in serum. Polyclonal rabbit antibodies (Affinity BioReagents, USA) and ABC rabbit Staining System (Santa Cruz Biotechnology, USA) were used to demonstrate ER β expression in prostate cells. The results of the examinations showed an essential increase in PRL and E₂ serum concentration in the risperidone group and a simultaneous decrease in T concentration. Immunolocalization and expression of ER β was found in secretory cells of glandular epithelium, stroma fibrocytes, and in endothelial cells of blood vessels of the rat prostatic gland. The animals of (R) group demonstrated changes in the morphology of epithelial cells of lateral lobe with a small increase in ER β expression. They were manifested mainly in the form of local hyperplasia of glandular epithelium. Based on the obtained results, one can state that PRL exerts trophic influence on prostate. Moreover, PRL increases ER β expression in epithelial cells of lateral lobe of the rat prostate.

P4.47

CB1 cannabinoid receptors in the central nervous system of *Podarcis sicula*

Rossana Favorito¹, Luigia Cristino², Maria Consiglio Grimaldi¹, Ida Ferrandino¹

¹Department of Biological Sciences, Section of Evolutionary and Comparative Biology, University Federico II, Naples, Italy

²Institute of Cybernetics E. Caianiello, Consiglio Nazionale delle Ricerche, Pozzuoli, Naples, Italy

Contact: rossana.favorito@unina.it

The endocannabinoid system is an important intercellular signaling system in which are involved cannabinoid receptors, endocannabinoids (endogenous transmitters) and different enzymes (McPartland et al., 2002). Two types of cannabinoid receptors have been characterized, CB₁ and CB₂ receptors. While CB₂ receptors are mainly expressed by cells of the immune system, CB₁ receptors are expressed in many brain regions where they have a relevant neuromodulatory role (Felder and Glass, 1998). The

endocannabinoid system appears to be phylogenetically ancient because its homologs are expressed in the genome of many organism. The phylogenetic distribution of this system is yet poorly known and its components apparently evolved separately in different epochs (McPartland, 2004). Genes encoding orthologues of the mammalian CB₁ receptor have been identified in fishes, amphibia and birds, indicating that CB₁ receptors may occur throughout the vertebrates (Elphick and Egertová, 2001). To our knowledge few is known in regard to CB₁ receptors in reptilia. In the present work we have studied the occurrence and distribution of the CB₁ receptors in the brain of the reptile *Podarcis sicula*. The study was performed on 6 µm serial section of *P. sicula* brains embedded in paraffin and processed by the ABC technique with the use of the polyclonal antiserum anti-CB₁ receptor at the working dilution of 1/800. CB₁-immunoreactivity (IR) was observed in all regions of *P. sicula* CNS. The immunoreactivity appeared specific, however, the distribution was different between several brain areas. CB₁-IR was more abundant in the optic chiasma, in the transitional area between diencephalon and mesencephalon, and in the optic tectum, where we in particular observed CB₁(+) fibres. Few CB₁-IR structures were also observed in the telenchephalon, confined in the central area, and in the cerebellum. These preliminary data prove the occurrence of CB₁-receptors also in the lizard brain and confirm the hypothesis that the CB₁ receptors of the other vertebrates' species have a structural homology with that of mammals (Cesa et al., 2002).

P4.48

Biological responses in MG-63 osteoblast-like cell line cultured on sol-gel derived bioglass layers

Elżbieta Menaszek¹, Anna Ścisłowska-Czarnecka², Katarzyna Cholewa-Kowalska³, Maria Łączka³

¹Department of Cytobiology and Histochemistry, Jagiellonian University, Kraków, Poland

²Department of Anatomy, Academy of Physical Education, Kraków, Poland

³Department of Technology of Glass and Amorphous Coating, AGH University of Science and Technology, Kraków, Poland

Contact: mfmensz@cyf-kr.edu.pl

The objective of this study was to investigate the effect of a new generation bioglass (CaO-SiO₂-P₂O₅ system) on osteoblast-like cells' activity. Bioactive glasses prepared by sol-gel techniques are very promising materials for bone tissue engineering because of their favourable biological properties allowing to form direct bindings between the ceramics and bone. Materials and methods: Human osteoblast-like MG-63 cells were cultured on two types of sol-gel derived bioglasses: A2 containing 54 mol% CaO/40 mol% SiO₂/6 mol% P₂O₅, and S2 containing 16 mol% CaO/80 mol% SiO₂/4 mol% P₂O₅. Cells were seeded at a density of 1.0 × 10⁵ cells/ml/ well in 24-well tissue culture plates containing sterile, round (15 mm in diameter) glass coverslips coated with the A2 or S2 bioglass. Single coatings of the A2 and S2 material were applied onto coverslips using a dip-coating method. Coverslips with an inert silica (SiO₂) layer and clean coverslips without any coating were used as references. After 3 and 7 days of culture, behaviour of cells grown on the examined and control surfaces was compared through the determination of: cell viability (MTT test) and adhesion (CV colorimetric assay), alkaline phosphatase activity (histochemical assay), presence of active matrix metalloproteinases (zymography analysis), collagen type I production (immunoassay), and mineralization of extra-cellular matrix (Alizarin Red S staining). Cell morphology and properties of biomaterials' surfaces were analysed with optical microscope and scanning electron microscope (SEM). The results of tests performed after 3 and 7 days of culture proved better ability of bioglass layers to stimulate cell adhesion and activity, compared to control SiO₂ coatings and clean coverslips. Differences between cultures performed on A2 and S2 coatings indicate higher bioactivity of S2 bioglass. MG-63 cells cultured on S2 coatings showed higher ALP activity and more mineralization than those cultured on A2 coatings. The absence of metalloproteinase-9, and the presence only of an inactive form of metalloproteinase-2 in all samples may indicate that bioglasses didn't evoke inflammation processes nor extracellular matrix degradation. Conclusion: Bioactive A2 and especially S2 sol-gel derived glasses are promising materials as implant coatings or scaffolds for bone tissue engineering as they not only provide an osteoconductive and osteoproduktive substrate, but also stimulate the activity of bone cells.

P4.49

Changes of p63 expression pattern during morphogenesis of the mouse submandibular gland

Sachiko Matsuura¹, Akio Kikuta²

¹Department of Oral Histology, School of Dentistry, Matsumoto Dental University, Shiojiri, Japan

²Department of Anatomy, School of Medicine, University of Occupational and Environmental Health, Kita-Kyushu, Japan

Contact: smatsu@po.mdu.ac.jp

p63 is a member of the p53 tumor-suppressor gene family and is highly expressed in several types of tumor cells of human salivary glands. p63 is also known to be expressed in the basal cells of the stratified epithelium of the skin and mucosa and is reported to be essential for a number of aspects of ectodermal differentiation during embryogenesis. Organs derived from the stratified epithelium of oral mucosa or skin, such as teeth, hair follicles and mammary glands, are absent in p63-deficient mice. Roles of p63 in the development of salivary glands, which develop from oral mucosa, however, have not yet been clarified. Here, we describe the immunohistochemical expression pattern of p63 during the morphogenesis of the mouse submandibular glands (SMG). The oral mucosa surrounding the tongue and the SMG were removed from ICR mice from embryonic day 12 (E12) to postnatal day 14 (P14), and were fixed in 70% ethanol or 4% paraformaldehyde. Some mice were given BrdU 2h before the sacrifice to detect the proliferation pattern. The fixed specimens were embedded in O.C.T. compound and frozen. Serial cryostat sections were cut and the immunohistochemistries were done using the following primary antibodies: against p63, BrdU, smooth muscle actin (SMA), marker of myoepithelial cells, Mi and mSMG C, markers of secretory cells in SMG. The expression of p63 was detected immunohistochemically in nuclei of the basal epithelial cells of the oral mucosa and the invaginating epithelium of the primordium of SMG from E12 mice. At E14, the terminal buds (TB), including prospective region of the intercalated duct (ICD) and acini, became morphologically distinguishable from the ducts, while both the basal cells of the duct and TB expressed p63. Until E14, no immunostaining with other antibodies except for anti-BrdU was observed in the epithelium of the SMG primordium, showing no sign of specialization in the SMG. After E15, elongated ducts and increased small TB were evident resulting from the proliferation and branching, however, labeling for p63 was restricted to the basal cells of ducts and TB. In addition, p63-unexpressing cells in TB increased in number day by day. In the TB, immunolabeled cells with anti-SMA, anti-mSMG C and anti-M1 antibodies first appeared at E15, E16 and E17, respectively, showing the start of the functional specialization of TB, and ICD and acinar structures were also gradually constructed during these stages. p63 expression pattern in the ducts did not change during the embryonic periods. Postnatally, p63 immunolabeling in ducts and TB decreased, and at P14, it was observed only in the ICD, occasional acinar cells and proximal excretory ducts. No relationship between p63 expression and BrdU labeling was noted through the examined periods. These results demonstrate that the disappearance of p63 expression occurs parallel to the appearance of differentiation markers in the development of the acini during the morphogenesis of the mouse SMG.

P4.50

The effect of melatonin on survival of ultraviolet-A and ultraviolet-B irradiated human dermal fibroblasts and melanoma cells

Iłona Izykowska¹, Aleksandra Piotrowska¹, Marek Cegielski¹, Marzena Podhorska-Okolow¹, Maciej Zabel², Piotr Dziegieł¹

¹Department of Histology and Embryology, University School of Medicine, Wrocław, Poland

²Department of Histology and Embryology, Medical University, Poznań, Poland

Contact: ilona@hist.am.wroc.pl

Ultraviolet radiation reaching the earth surface and affecting human skin includes UVA (92%) and UVB (8%) spectrum. One of the natural defense systems is pineal-hormone melatonin (Mel), which was shown to be produced also in skin. Mel has an antioxidative activity and acts as a free radical scavenger. *in vivo*, Mel applied on patients' skin could suppress erythema. *in vitro* studies showed that preincubation with Mel of keratinocyte, fibroblast and leukaemia cell lines protected against radiation-induced apoptosis. The aim of our study was to investigate the effect of melatonin

on UVA- and UVB-irradiated human skin fibroblasts and melanoma cells in an *in vitro* model. Fibroblasts (NHDF-Normal Human Dermal Fibroblasts, Cambrex Bio Science) and melanoma cells (BM-Bridegroom Melanoma, Polish Academy of Sciences, Wrocław) were pretreated with various concentrations of Mel (Sigma): 10^{-3} , 10^{-6} and 10^{-9} M followed by UVB irradiation at dose of 60 mJ/cm² (Ultraviolet Lamp, 312 nm, Cole-Parmer) and UVA irradiation at dose of 15 J/cm² (High-Intensity Longwave Ultraviolet Lamp, 365 nm, Cole-Parmer). The number of living cells binding sulforhodmine B (SRB) was evaluated after 24 hours by SRB colorimetric assay (100% cells unirradiated and untreated with Mel). Mel added at the concentration of 10^{-6} M prior to UVB-irradiation of fibroblasts significantly increased the number of living cells compared with cells without Mel ($p < 0.05$). This corresponds to an increase from 55% to 63%. Other tested concentrations (10^{-3} , 10^{-9} M) had no protective effect. Preincubation of UVB-irradiated melanoma cells with Mel at the concentration of 10^{-9} M increased the number of surviving cells up to 43% compared with other concentration and cells without Mel (31%) ($p < 0.05$). Only the highest concentration of Mel showed a protective effect on fibroblast exposed to UVA and significantly increased the number of living cells up to 95% ($p < 0.05$) compared with untreated cells (86%). In contrast, melatonin's protective effect on melanoma cells was obtained at the concentration of 10^{-6} (98%) and 10^{-9} M (94%) compared with untreated control (85%) ($p < 0.05$). Multiple actions of Mel on fibroblasts and melanoma cells subjected to UVA and UVB irradiation may be connected with multiple expression of melatonin receptors.

P4.51

Left ventricular cardiomyocyte response to myocardial ischemia

Elena Stalioraityte, Dalia Pangonyte, Reda Ziuraitiene, Danute Kazlauskaitė, Jolita Palubinskiene, Lina Utkiene

Laboratory of Cardiac Pathology, Institute of Cardiology, Kaunas University of Medicine, Kaunas, Lithuania

Contact: ziuraitred@yahoo.com

The aim of the study was to detect changes in the left ventricular cardiomyocyte size and shape in response to chronic ischemia and loss of cardiac tissue (myocardial infarction) during the course of ischemic heart disease (IHD). Object and methods. Heart specimens of 85 males who died suddenly out-of-hospital (within 6 hours of the onset of the terminal event) due to IHD were investigated. Cases were divided into two groups: preinfarction IHD group – 53 decedents with the first fatal acute attack (aged 48.6±2.9 yrs) and 32 decedents of postinfarction IHD group (aged 51.7±2.9 yrs), who had a postinfarction scar detected on autopsy. Seeking to evaluate the impact of ischemia as an isolated factor, examination was performed only on decedents who had no clinically or morphologically defined disease which could increase the heart load and clinical symptoms of heart failure. Control group consisted of 29 males of similar age (mean 46.0±3.1 yrs) who succumbed to external causes. Left ventricular cardiomyocyte dimensions (diameter and length) were estimated by image analysis system (motorized Olympus BX61 microscope, Media Cybernetics Evolution QEi camera and ImagePro AMS software), and their cross-sectional area and volume were assessed. Results. We have found cardiomyocyte hypertrophy in the preinfarction IHD group already. The cardiomyocyte volume was increased by 32.0 percent in comparison with the same index in control group, and cross-sectional area, length and diameter – by 17.2, 12.5 and 8.2 percent, respectively. In the postinfarction IHD group, all studied cardiomyocyte parameters did not differ significantly from the analogous indices in the preinfarction IHD group ($p > 0.05$). Cardiomyocyte hypertrophy was related to the increase of the left ventricular weight and endocardial surface area. Correlation between the parameters of cardiomyocytes and the left ventricular weight, as well as endocardial surface area, was determined. Cardiomyocyte diameter and length in the control group was related to the left ventricular weight ($R=0.35$, $p < 0.05$ and $R=0.43$, $p < 0.05$, respectively), but no significant correlation was found in the other groups. Different correlation was found between the cardiomyocyte diameter and endocardial surface area: in the pre- and postinfarction IHD groups it was negative ($R=-0.47$, $p < 0.05$ and $R=-0.33$, $p < 0.05$, respectively), but there was no such significant correlation in the control group. There was no correlation between the cardiomyocyte length and left ventricular endocardial surface area. No correlation was found between the cardiomyocyte parameters and the postinfarction scar size. Conclusions. Left ventricular cardiomyocyte hypertrophy occurs prior to the first myocardial infarction. In postin-

farction myocardium, cardiomyocyte size did not differ significantly from that in preinfarction group at least prior to the appearance of congestive heart failure syndrome.

P4.52

The lacrimal outflow ducts exfoliative cytology of healthy persons

Katarzyna Wesolowska, Andrzej Zieliński

Department of Cytophysiology, Histology and Embriology, Medical University of Lodz, Lodz, Poland

Contact: simbik@op.pl

Tears and conjunctiva cytology is an examination used in ophthalmology mainly for diagnosis of dry eye syndrome. It is a fairly simple and little invasive method of examination. In up to date guidelines for diagnosis of allergic disease of eyes, presented by Polish Allergic Association and Polish Ophthalmology Association, cytodiagnosis of conjunctiva was ranked as additional examination. However, up to now there are no proper cytograms of lacrimal ducts. The aim of the study was to determine proper cytogram of outflow lacrimal ducts of healthy people, what would allow to use the cytodiagnosis of tears in identification of other eye disease including allergic disease. Patients and methods: Examinations were made on healthy persons who, by general and ophthalmologic examinations as well as by additional examinations, were found to be free from diseases which could have influenced results of research. Material for cytological examination of tear liquid was collected from conjunctival sac in amount of approximately 2 µl by use of micropipette tubes. Tear liquid was spread on glass slides, left for drying, and stained according to Giemza's method. Then following cells were counted by the use of meander method: epithelial cells, neutrophils, eosinophils, basophils, lymphocytes and monocytes. These cells were cytomorphologically examined. The collected material was stained with pinocyanol erythrosinate in order to precisely determine the amount of mast cells in each sample. Results: In the samples the epithelial cells of superficial and deep layers were observed. Among superficial layer cells, flat cells in form of cornified cells were noted. Second very characteristic type of recognized epithelial cells were columnar cells originating probably from lacrimal ducts. Less numerous, other cuboid and less regular cells originated from deeper layers of epithelium. Epithelial cells appeared singly or as cell clusters of different number of cells. Small amount of cells with stained nucleus only were noted. Besides of epithelial cells always present in collected material, in some samples neutrophils and lymphocytes were observed. There were no monocytes, basophils and eosinophils. Neutrophils mostly had three-segmental nucleus, in smaller number of cells double- and four- segmented nucleus were also observed. On the basis of obtained results the frequency of the appearance of epithelial and inflammatory cell's was calculated. Conclusion: The obtained data can be useful for exfoliative cytodiagnosis of eye conjunctiva and lacrimal ducts.

P4.53

Histamine deficient zebrafish – a step towards understanding the interactions between the histaminergic and other neurotransmitter systems

Maria Sundvik, Hisaaki Kudo, Pertti Panula

Neuroscience Center and Institute of Biomedicine, University of Helsinki, Helsinki, Finland

Contact: maria.sundvik@helsinki.fi

Although the basic properties of the brain histaminergic system are known, the development of the neurons and the exact functions and molecular mechanisms of the interactions with other neurotransmitter systems are still poorly understood. Diseases in which the histaminergic system has been implicated include Alzheimer's disease, Parkinson's disease, schizophrenia and epilepsy. We analysed the location of histamine producing cells in the hypothalamus of larval zebrafish. This neuronal population was visualized by histidine decarboxylase (HDC) whole mount *in situ* hybridization from 2 dpf onwards, and by histamine (HA) immunocytochemistry from 5 dpf. This group of neurons has been suggested to develop later than we now observed. The result suggests that expression of HDC mRNA precedes the synthesis of histamine by this enzyme, and other enzymes expressed earlier, like L-DOPA decarboxylase, do not produce detectable histamine. The

number of histamine positive neurons was significantly decreased after the injection of HDC morpholino oligonucleotide, as detected with histamine immunocytochemistry. RT-PCR results from 1 dpf, 3 dpf and 5 dpf showed that the expression of HDC in zebrafish larvae was significantly decreased and almost completely blocked in the HDC morpholino-oligonucleotide treated fish at all three time-points, whereas no change in expression levels of HDC could be observed in the control samples. The decrease in histamine-ir neurons and fibers was accompanied with a behavioral change observed at 5 dpf. Larval zebrafish swam more slowly, and the turn angle of fish increased significantly compared to control fish. Both the reduction in HA-ir neuron number and change in behavior were specific for the HDC morpholino-oligonucleotide, and could not be detected with the HDC mismatch morpholino-oligonucleotide. Taken together, we present a model in the zebrafish larvae that shows a significant decrease in histamine neurons and thereby a model for histamine deficiency in zebrafish. Supported by the Academy of Finland, Sigrid Juselius Foundation and the Helsinki Biomedical Graduate School.

P4.54

Ultrastructural study of microfungi under heavy metal contamination

Bożena Sosak-Świdarska

Centre of Ecological Research, Polish Academy of Sciences, Warsaw, Poland

Contact: sozologia@tlen.pl

The ability of microfungi to survive and grow in the presence of heavy metals is significant. Mechanisms of metal resistance may include extracellu-

lar precipitation, complexation, transformation of metal species, biosorption to cell walls and pigments, decreased transport and intracellular compartmentation. Electron microscopy analysis (Joel JEM 1200 Ex, Carl Zeiss Jena) was used to determine the location of melanin granules in microfungal cells. The location of cadmium, cuprum, lead and zinc was investigated in *Cladosporium cladosporoides* cells. The fungal cells equilibrated with a 1 mM concentration of metals were then fixed in 3% glutaraldehyde in 0.1 mM sodium phosphate buffer, pH 7.2. Dehydration was completed using a graduated series of ethyl alcohol and propyleneoxide. Cells were embedded in Epon 812 and cell sections were unstained. Chemical analysis of structure and property of melanins were determined by EPR (SE/X, Radiopan) and IR (Specord M-80, Carl Zeiss Jena) techniques. Cadmium, cuprum and zinc form discrete black granules and electron microscopy analysis indicated that these metals were also associated with cell walls and intracellular structures. Localization of lead was clearly evident. Lead granules were found in cell walls and in intracellular structures, most likely-vacuoles, which have an important role in the regulation of the cytosolic concentration of metal ions. We know numerous examples of fungal structures that are extremely resistant to heavy metal stress and it is significant that most of these resistant structures contain black pigments which have been described as melanins. Microfungi able to accumulate high amounts of heavy metals can be used as biosorbents for removing metals from industrial wastes and for environmental remediation.

Index of Authors

- Adamec Milos P4.7
Adamek Agnieszka P2.51
Adjobo-Hermans Merel L004
Adriaensen Dirk L085
Adrych Krystian P2.57, P2.58
Akbuga Julide P3.53
Akhter Noor L012
Akita Masumi P3.19, P3.54
Aktar Fadime P3.43
Aktaş Abit P2.2
Albert Mihály P4.33
Albertini Roberta P4.8
Alberto Strada P3.64
Alessandra Colonna P3.64
Alessandro Mauro P3.64
Alfayate M.C. P3.29
Alkan Faruk P4.14
Allegra A. P2.44
Alvaro Tomas P2.40, P2.64
Amarelli Cristiano P3.3
An Sucai P2.14
Anastasi G. L024
Andrea Brioschi P3.64
Andres Sarah A. L013
Antony Claude P2.39
Antosz Halina P4.35
Aoki Daisuke L016
Aoyagi Hidekazu P2.36
Arbak Serap P3.52, P3.53
Arco A. P2.45
Arda-Pirincci Pelin P2.21
Arena S. L023
Arendt Thomas L102
Aşan Esin P1.14
Atilla Pergin P2.6, P2.27
Atmaca Soner P1.2
Augoff Katarzyna P3.41, P3.42
Augustin Ewa P1.36, P2.22
Aviram Michael P3.22
Aytar Hamit P1.13
Baczyńska Dagmara P3.40
Badowska-Szalewska Ewa P4.23
Bagriyanik Alper P1.2
Bajko Ewelina P3.26, P3.27
Bakker Annette L096
Balaban Yasemin P1.14
Balan Mirela P4.36
Balcerska Anna P1.38
Balwierz Adriana P3.13, P3.16
Banachowska Ewa P1.58
Bancone Ciro P3.2, P3.3, P3.4
Bańbura Marcin W P3.35
Barletta Claudia P1.4
Bartel Hieronim P3.57
Barun Sureyya P2.25
Bas Serap Sancar P1.56
Basiji David A. L044
Baskin Denis L002
Battistelli Serafina P1.9
Baykara Basak P1.2
Bayrak Bertan Boran P1.17
Beksac Sinan P2.6
Bellafiore M. P4.10
Bengtsson Niclas E. L038
Bernardi Paolo L022
Bese Tugan P2.24
Bickmeyer Ulf P2.41
Biczysko Wiesława P3.55, P2.51
Biela-Witkowska Ewa P2.48
Bielecka Maria P2.34
Bień Ewa P2.8
Bierla Joanna P2.9, P2.13, P2.20, P3.10
Biesaga Beata P1.31
Biggiogera Marco L082, P1.9, P4.42, P4.43
Bilińska Barbara P1.60, P1.61
Bilir Ayhan P1.24, P3.53
Biltekin Burcu P3.53
Birkner Ewa P4.40
Biyiksiz Pelin Coştur P4.5
Blanco David L017
Blevins James L002
Bogus Katarzyna P3.6
Bogus-Nowakowska Krystyna P4.13, P4.18
Bolkent Sehnaz P1.8, P1.10, P1.17, P1.22, P1.54, P1.57, P2.21, P4.38
Bolkent Sema P1.53
Bonaldo Paolo L022
Bonomini Francesca P3.22
Borsani Elisa P4.8
Borska Sylwia P1.44
Bosch Ramon P2.40, P2.63, P2.64
Bossowska Agnieszka P4.21, P4.25
Bothmann Hendrick L096
Bottone Maria Grazia L082
Boucek Petr P4.7
Bovin Nicolai L030, P1.41
Bozkurt Betul P1.24
Braun Thomas L040
Brezillon Stephane L062
Brodrick Zachary L012
Brouns Inge L085
Brown Gary L038
Bruschetta D. P2.44
Bryzek Aleksandra P3.6, P4.32
Brzóska Małgorzata M. P1.51
Budka Herbert L104
Budzyńska Monika P1.59
Buijs Ruud L026
Bulc Michał P1.65, P1.66
Burda Jozef P2.15, P2.16
Burdikova Zuzana P3.63
Buscemi M. P3.23, P4.10
Bzowska Małgorzata P2.18, P2.26
Cagatay Enbe P2.24
Cai Wenqin P1.1
Cakar Nur P2.6
Caliendo Sabrina P1.4
Calza Laura P4.15
Canberk Yurdagul P3.43
Capaldo Anna P1.4
Capodanno Simona P1.23
Cappello F P3.23
Carpio Diego P1.26
Casanan N. P3.29
Casini Alessandro P2.59
Castaldo Clotilde P3.2, P3.3, P3.4
Cavallotti Ivan P3.20
Cecchetti Alessandra P2.59
Cecot Tomasz P4.23
Cegielski Marek P3.11
Celejewska Natalia P2.48
Celio Marco L091
Cemil Berker P1.12
Cevirgen Banu P1.12
Chang Yan-Zhong P2.53, P2.56
Chen Dong P3.8
Chen Peng-Hui P1.1
Chen Yu-Chia L055, P4.6
Chi Bom Chae P2.55
Childs Gwen L012
Chmielewska Anna P3.35
Chojnowski Grace P2.68, P3.59
Cholewa-Kowalska Katarzyna P4.48
Chorzępa-Nowicka Karolina P4.46
Chybicki Janusz P2.58
Chydzewski Lech L112, P4.28
Cichocki Tadeusz P4.37
Cichoń Tomasz P1.34
Cichorek Mirosława P1.42, P2.30
Ciereszko Renata P1.55, P2.17
Ciesielska Urszula P1.47
Cilaker Serap P1.2
Cisterna Barbara P4.42
Čížková Dana P3.21
Claassens Oswaldien P2.68
Clarke Margaret L051
Cobellis Luigi P3.20
Cobos-Correa Amanda P2.42
Colacurci Nicola P3.20
Coleman Raymond L108, P3.22
Colombari Romano P1.26
Consolo A. L023
Contuk Gazi P3.50
Coppola Gabriele P3.20
Couchman John L009, L065
Craciunescu Oana P4.36
Crescenzo Raffaella P1.23
Cristino Luigia P4.47
Cubells Laia L057
Cui Cheng L072
Cutroneo G. P2.44
Cymerys Joanna P3.35
Czajka Arkadiusz P2.51
Czapla Leszek P3.42
Czech Urszula P3.16
Czekaj Piotr P3.6, P4.32
Czekaj Tomasz M. P3.6
Czirok Andras L072
Czykier Elżbieta P1.67
Dagistanli Fatma Kaya P1.29, P1.52, P2.24, P4.14
Daglioglu Suzan P2.2
Dalçik Cannur P4.5
Dalçik Hakki P4.5
Danielisova Viera P2.15, P2.16
Darzynkiewicz Zbigniew L080, P2.48
De Angelis Antonella P3.2
De Falco Maria P1.4, P1.23, P3.20, P4.31
De Luca Antonio P1.23, P3.20, P4.31,
De Ponte F. P2.44
De Proost Ian L085
De Robertis Alessandra L096
De Solorzano Carlos Ortiz L017
De Witt Hamer Philip L045
Decossas Marion L083
Dembinska-Kieć Aldona P3.13, P3.16, P3.17, P3.18
Dembowska Bożena P1.38
Demir Ramazan P1.62, P3.28, P3.56
Demirogullari Billur P2.25
Demoura Sophia P2.10, P3.44
Demura Tatiana P1.18, P1.19, P1.20
Demurtas Paolo P1.25, P1.26, P3.5
Demusiak Monika P1.36
Deng Qi-Yue P1.1
Denir Secnur P3.53
D'Este Loredana Giorgio Vivacqua P4.11
Dettlaff-Pokora Agnieszka P2.33
Dezawa Mari P3.12, P3.14
Di Mauro D. P2.45
Di Meglio Franca P3.2, P3.3, P3.4
Dias James A. L006
Dieker Jürgen L083
Divoký V. P4.41
Djavadian Ruzanna P4.19, P3.7

- Dobashi Yoh P2.37
 Dobrohotova Juliya P1.19
 Dobrovolskaya O. P1.46
 Dobrowolski Sebastian P2.58
 Dobrucki Jurek L081, P2.48, P3.62
 Dobrucki Lawrence W. L074
 Dolgun Anil P2.25
 Dolińska-Krajewska Barbara P1.21
 Domagala Teresa P3.31
 Domaradzka-Pytel Beata P4.19
 Dominik Paweł P1.34
 Domorakova Iveta P2.15
 Dong Chao P3.38
 Dong Zhi-yong P3.8
 Dongguan Hui Xue P3.8
 Dosescu Julie L047
 Drag-Zalesińska Małgorzata P1.47, P1.44
 Drożdż Katarzyna P3.15
 Drożyńska Elżbieta P1.38, P2.8
 Duan Xiang-Lin P2.53, P2.56
 Dubovy Petr P1.11, P4.9, P4.45
 Dubowska Joanna P2.58
 Duca A. L023
 Duda M. P1.60
 Dudek Wojciech P3.16
 Dulak Jozef P3.24, P3.25, P1.34
 Durak Rumeysa P3.52
 Dusza Luiza P1.58, P1.59
 Dyduch Grzegorz P3.17, P3.18
 Dzierzbicka Krystyna P2.54
 Dziewiątkowski Jerzy P2.32, P4.20, P4.24
 Dzieciol Janusz P1.15A, P3.65
 Dzięgiel Piotr P1.21, P1.44, P1.47, P2.52, P3.15, P1.45, P3.11
 Egashira Noboru L066
 Egeblad Mikala L094
 Ehrmann Jiří P4.41, 3.21
 Eiki Yuka P3.54
 Ekerbicer Nuran P1.32
 Ekinci Saniye P2.27
 Emanuelli Costanza P3.24
 Engel Ulrike L051
 Enrich Carlos L057
 Ercan Feriha P3.49, P3.50, P3.51
 Erdem Selcuk P1.24
 Ergen Ali P2.23
 Ergun Ertan P1.13
 Erkanli Gozde P3.49
 Erlbruch Andrea P2.39
 Ersoy Yasemin P3.51
 Escriva Patricia P2.40, P2.63, P2.64
 Falcone Italia P1.23
 Favalaro A. L024
 Favorito Rossana P1.16, P4.47
 Fedorowska Natalia P4.6
 Fekete Csaba L027
 Feret Dorota L078
 Fernandez-Garcia Ignacio L017
 Feron Olivier L076
 Ferrandino Ida P1.16, P4.47
 Festa F. L024
 Figiel Izabela P3.33
 Filip Stanislav P3.21
 Filipek Anna P3.33
 Filipkowski Robert K. L041
 Fliers Eric L026
 Florek Ewa P3.55
 Floris Carlo P1.25, P3.5
 Fontanella Marco P4.42
 Fornai Francesco P2.59
 Förstermann Ulrich L075
 Fraczek Monika P3.60
 Franciosi Federica P4.26
 Friedl Peter L092
 Fuchinoue Fumi L014
 Fujii Ritsuko P2.61, P4.2
 Fujii Yasuhisa P3.38
 Fujimoto Toyoshi L059
 Fujita Keiko P3.19
 Fujiwara Katsuji L053
 Fukudome Takayasu P1.5
 Fulceri Federica P2.59
 Funaya Charlotta P2.39
 Furukawa Koichi P1.30
 Gackowska Lidia P3.36, P3.37, P3.39, P3.55
 Gadella Jr. Theodorus W.J. L004
 Gaeta R. P2.45
 Gajda Mariusz P2.4, P4.37
 Galas Jerzy P1.60, P2.48
 Gallamini Michele P4.15
 Gao Fulu P1.28, P2.35
 Gatta V. L024
 Gavrilovic Jelena L063
 Gay Flaminia P1.4
 Gączarzewicz Dariusz P3.60
 Gedikoglu Gökhan P1.14
 Gendek Ewa P2.5, P3.47
 Gendek-Kubiak Hanna P2.5, P3.47
 Gerbino A P3.23, P4.10
 Gerisch Günther L051
 Gezginci Selda P1.17
 Gezginci-Oktayoglu Selda P1.8, P1.54
 Giampieri Silvia L093
 Giardino Luciana P4.15
 Gieryńska Małgorzata P4.39
 Gilmour Darren L095
 Giordano Cinzia L096
 Giuliani Alessandro P4.15
 Glac Wojciech P2.3
 Godlewski Janusz P1.43, P2.58, P2.9, P2.13, P2.20, P3.35
 Goedhart Joachim L004
 Gökcan Hale P1.14
 Gölgeci Fazile Akyuz P2.24
 Gołąb Aleksandra P2.57
 Gomulkiewicz Agnieszka P1.45
 Gonca Süheyla P4.5
 Goto Junpei P2.50
 Gottlieb Miroslav P2.15, P2.16
 Górka Paweł P2.20
 Górska-Andrzejak Jolanta P4.16
 Grabiec Marta P3.7
 Grabowski Krzysztof P3.41, P3.42
 Grajkowska Wiesława P1.38, P2.8
 Grażyna Kotlarz P4.17
 Grechukhina Olga P1.20
 Grembecka Beata P2.3
 Grewal Thomas L057
 Grimaldi Maria Consiglio P1.16, P4.47
 Grossenbacher-Zinchuk Olga L068, L091, P2.61
 Grube Achim P2.41
 Grucka-Mamczar Ewa P4.40
 Grygalewicz Beata P1.27
 Grzanka Alina P3.34, P3.36, P3.37, P3.39
 Grzanka Dariusz P3.34, P3.36, P3.37, P3.39
 Gultomruk Mine P3.43
 Gungor Tayfun P2.25
 Gunzer Matthias L098
 Gursoy Devrim P2.25
 Güvenç Tolga P4.3
 Haftek Marek L064
 Halstead Sue P4.4
 Halstead Susan K P2.43
 Hamaguchi Michinari L019
 Hamaloglu Erhan P1.14
 Han Dai P2.12
 Han Fang P1.3, P1.15, P2.31
 Hanako Kajiya L066
 Haney Anessa L012
 Hansel Donna L015
 Harrison Keith P2.68
 Hasçelik Gülsen P1.14
 Hata Natsumi P1.30
 Havrdova Terezia P4.7
 Hayashi-Takanaka Yoko L079
 Hayek Tony P3.22
 Hejmej Anna P1.61
 Helmin-Basa Anna P3.37, P3.39
 Hill Caroline L093
 Hirasaka Katsuya P2.50
 Hishikawa Yoshitaka P2.14
 Homma Taku P1.40
 Houqi Liu P3.1
 Huo Xia P2.12
 Ignatowska-Jankowska Bogna P1.7
 Ikonen Elina L060
 Inan Sevinc P1.32
 Inceli Meliha Sengezer P1.56
 Inci Kubilay P2.23
 Ironside James W. L104
 Irving John P2.68
 Iseki Shoichi P4.29
 Ishige Toshiyuki L014
 Ito Mikako P1.5
 Ito Yoshinori P2.62
 Itoh Johbu L007, L105, P2.46
 Itoh Yoshiko L007, P2.46
 Iwasaki Shin-Ichi P2.36
 Izdebska Magdalena P3.36, P3.39
 Izycki Dariusz P3.11
 Izycka-Świeszewska Ewa P1.38, P2.8
 Izykowska Ilona P1.21, P3.11, P4.50
 Jackson Dean L078
 Jaen Joaquin P2.40, P2.63, P2.64
 Jagoda Ewa P1.47
 Jancelek Radim P4.9, P4.45
 Janczak Dariusz P3.15
 Jankowska-Steifer Ewa P4.27
 Jankowski Maciej P1.7
 Jankun-Woźnicka Małgorzata P2.47
 Jaskiewicz Kazimierz P2.65
 Jaśkiewicz Janusz P3.9
 Jaśkiewicz Kazimierz P3.45
 Jazwa Agnieszka P3.24, P3.25
 Jerzemowska Grażyna P2.60
 Jiang Hong-Hui P1.35
 Jiang Yufeng L099
 Jijiwa Mayumi P3.30
 Joost Hans P3.17
 Józkwicz Alicja P3.24, P3.25, P1.34
 Juszczyk Jacek P2.51
 Kaczmarek Elżbieta P3.55
 Kaczmarek Leszek L041
 Kadomatsu Kenji P2.38
 Kageyama Haruaki L025, L028
 Kaibuchi Kozo L056
 Kaihong Ji P3.1
 Kaleczyc Jerzy L088, P2.7, P2.67
 Kalinowski Leszek L077
 Kalsbeek Andries L026
 Kamińska Barbara P1.58
 Kaneko Katsuji P3.19, P3.54
 Kaner Gultekin P1.29
 Kaptan Engin P1.10, P1.56, P4.38
 Kara Beyhan P1.57
 Karabulut Ebru P3.43
 Karabulut-Bulan Omur P1.22
 Karahan Siyami P4.3
 Karakaya Jale P1.32
 Karatug Ayse P1.10
 Karnak Ibrahim P2.27
 Kartanowicz Dorota P2.58
 Karwacki Zbigniew P2.32
 Kasacka Irena P1.49, P1.50
 Kašpárková E. P4.41

- Kasprzak Aldona P1.63, P1.64, P2.51, P2.52
 Kaszuba Marek P3.31
 Kaszubowska Lucyna P2.33
 Kataoka Fumio L016
 Kato Ikuo P2.9
 Katoh Ryohei L018
 Kaur Hitchintan L047
 Kawasaki Tomonori L018
 Kaymaz Figen P1.12, P1.13
 Kaymaz Memduh P1.12, P1.13
 Kazlauskaitė Danute P3.48, P4.51
 Keattikunpairoj Sunisa P4.29
 Keidar Shlomo P3.22
 Kerr Li Alan D. L013
 Khudoerkov Rudolf P4.1
 Kiec-Wilk B P3.13
 Kikuchi Kentarou L014
 Kikuta Akio P4.49
 Kim Wan-Seop P2.55
 Kimura Hiroshi L079, P2.62
 Kitada Masaaki P3.12, P3.14
 Kitamura Kunio P3.54
 Klejbor Ilona P4.22, P4.23
 Klepacka Teresa P1.38
 Kleszczyński Konrad P2.19
 Klimaschewski Lars L086
 Klings Karl-Walter P2.41
 Kluetzman Kerri L006
 Klusakova Ilona P4.9, P4.45
 Kmiec Zbigniew P2.57
 Knapik Marta P4.40
 Kobierska-Gulida Grażyna P1.38
 Kocabaş Yasemin P3.52
 Kocić Ivan P4.34
 Köck Matthias P2.41
 Kogan Evgenija P1.20, P1.18, P1.46, P2.10, P3.44
 Koji Takehiko L011, L110, P2.14
 Kojima Iori P2.38
 Kokturk Sibel P4.14
 Kolář Zdeněk P3.21
 Kolehmainen Juha L055
 Kolenkiewicz Małgorzata P4.12, P4.18
 Kolondra Adam P3.41
 Kołtan Sylwia P1.38
 Komorowski Andrzej P1.37
 Kondej Karolina P3.9
 Kondo Tetsuo L018, P1.33
 Kondo Yuji P1.30
 Kondraciuk Beata P1.6
 Konopa Jerzy P1.36, P2.22
 Koprowska Joanna P1.36
 Korkusuz Petek P1.14, P3.46
 Korybalski Bartłomiej P2.58
 Koryciak-Komarska Halina P3.6
 Kotil Tugba P3.43
 Kotula-Balak Małgorzata P1.61
 Kotula-Horowitz Katarzyna P3.31
 Kotunia Anna P2.9, P2.13, P2.20
 Kovalenko N. P1.46
 Kowalska Celina P4.21
 Kowalski Zygmunt P2.20
 Kowianski Przemysław P2.32
 Kozakowska Magdalena P3.25
 Kraszewska Olga P1.55, P2.17
 Kravcukova Petra P2.15
 Kreier Felix L026
 Krejci Eric P1.5
 Kruczak Anna P1.31
 Krueer Traci L. L048
 Kruszewski Wiesław P3.45
 Krysiak Elżbieta P1.27, P1.39
 Krzyściak Wirginia P2.28
 Krzyżanowski Bartosz P3.31
 Kubinova Lucie P3.63
 Kucharzewska Paulina P3.24, P3.25
 Kucia Magdalena L044, L106
 Kudo Hisaaki P4.6, P4.53
 Kulig Andrzej P3.57
 Kurmyshkina Olga P1.41
 Kurpisz Maciej P3.60
 Kuwahara Atsukazu P2.9
 Kuzak Mateusz P3.62
 Kylarová Dana P4.41, P2.29
 La Barbera G. P3.23
 La Marca G. P3.23
 Lackowska Bożena P1.34
 Laforgia Vincenza P1.4, P1.23, P4.31
 Lakomy Miroslaw P2.7
 Lansford Rusty L072
 Lassota Peter L067
 Laszczyńska Maria P3.60, P4.46
 Lavik Erin L069
 Lebelt Agnieszka P1.15A, P3.65
 Lechan Ronald M. L027
 Ledóchowski Paweł P2.3
 Lee Sang Hwa P2.55
 Leja Justyna P3.24, P3.25
 Lejeune Marylene P2.40, P2.63, P2.64
 Lekawa Anna P4.35
 Lemancewicz Dorota P1.15A, P3.65
 Lemke Krzysztof P1.34
 Lentini S. P2.45
 Lenzi Paola P2.59
 Leska Anna P1.58, P1.59
 Lewczuk Bogdan P1.65, P1.66
 Leyla Sati P1.62
 Li He L099
 Li Huige L075
 Li Quanwen L047
 Li Wei P2.49
 Li Xiao-Ming P2.31
 Li Xin P2.35
 Li Xinmin L103
 Li Zhen P1.48, P2.49
 Liberski Paweł P. L101, L104
 Lichtman Jeff W. L001
 Lietzau Grażyna P2.32
 Likus Wirginia P3.6
 Lillard James W. L044
 Lim So Dug P2.55
 Lipari A. P4.10
 Lipari D. P3.23
 Lipari E Farina P3.23, P4.10
 Lis Grzegorz J P4.37
 Little Charles L072
 Litvinova Natalia P1.19
 Litwin Jan A. P4.37
 Litwiniec Anna P3.34
 Liu Hong P1.3
 Liu Jia-mei P3.8
 Liu Jing-Ze P2.56
 Liu Li-Yun L090
 Liu Sheng-Hong P1.35
 Liu Xiao-Zhi P2.53
 Liu Yu-Qian P2.56
 Lodde Valentina P4.26
 Lodererova Alena P4.7
 Lopez Carlos P2.40, P2.63, P2.64
 Lorenzini Luca P4.15
 Lucariello Angela P3.20, P4.31
 Luciano Alberto M P4.26
 Ludkiewicz Beata P4.22, P4.23
 Lužná Pavla P4.41
 Łakomy Miroslaw P1.43, P2.67
 Łączka Maria P4.48
 Łukaszyk Andrzej P1.63, P1.64
 Mach Monika P3.40
 Maciejewski Jarosław L039
 Madri Joseph A. L069
 Maeda Tetsuyo P1.40
 Magaouda E. P2.44
 Magaouda L. P2.45
 Magdalan Jan P1.21
 Magnoni Letizia L096
 Maj Anna P4.40
 Majak Katarzyna P4.19
 Majewski Mariusz L089, P4.13, P4.18, P4.21, P4.25
 Malatesta Manuela P1.9, P4.42, P4.43
 Malicka-Błaszkiewicz Maria P3.40
 Mall Marcus P2.42
 Malecki Krzysztof P1.37
 Manente Lucrezia P1.23, P3.20, P4.31
 Manfait Michel L050
 Manna Chanchal Kumar P1.68
 Maquart Francois-Xavier L062
 Maraldi Nadir M L022
 Markova Anya P1.46
 Marsh Brad L054
 Marszalek Andrzej P3.55, P3.39
 Martinez-Pomares Luisa L029
 Matsuno Akira L105
 Matsuura Sachiko P4.49
 Matteoni Silvia L096
 Mattingly Raymond L047
 Maxia Cristina P1.25, P1.26, P3.5
 Maya-Mendoza Apolinar L078
 Mazur Agnieszka P1.34
 Mazurkiewicz Joseph E. L032, L006
 Mazzarella Giuseppe P2.1
 Mcconnell Gail P2.48
 Mcdonald Donald M. L071
 McGonigal Rhona P4.4
 Mele Valentina P3.3
 Meloni Marco P3.24
 Menaszek Elżbieta P4.48
 Meng Xiao-ting P3.8
 Meola Giovanni L020
 Merlini Luciano L022
 Micali A. L023
 Mietelska Anna P3.33
 Mikołajczyk Tomasz P2.28
 Milardi D. P2.45
 Minarowski Łukasz L112
 Minerba Luigi P1.25, P1.26, P3.5
 Mir Shahryar L045
 Miraglia Rita P3.2, P3.3, P3.4
 Miśkowiak Bogdan P1.6
 Mittag Anja L102
 Miyake Kanae P2.62
 Mochizuki Kunio L018
 Modena Silvia C P4.26
 Moin Kamiar L047
 Moiseeva Ekaterina P1.41
 Mokry Jaroslaw P3.21
 Moldovan Lucia P4.36
 Moll Jacek P3.57
 Moll Jadwiga P3.57
 Moll Ronald P1.18
 Montagnani Stefania P3.2, P3.3, P3.4
 Montuenga Luis M. L017
 Monzon-Mayor M P3.29
 Morawski M. L102
 Mori Elisa L096
 Morone Nobuhiro L056
 Morton Gregory L002
 Moryś Janusz P1.42, P2.32, P4.19, P4.20, P4.22, P4.23, P4.24
 Mosch Birgit L102
 Moskalewski Stanisław P4.27
 Moś-Rompa Anna P2.22
 Motyl Tomasz P3.10
 Muftuoglu Sevda P1.32, P2.23, P2.25, P2.27
 Mulder Jan L046

- Muller Sylviane L083
 Multhaupt Hinke L009, L065
 Munoz Arrate L017
 Murakami Eriko L014
 Murata Eiko P3.19, P3.54
 Murtas Daniela P1.25, P1.26, P3.5
 Musolino C. P2.44
 Mutlu Ozgur P1.10
 Myśliwska Jolanta P2.33
 Myśliwski Andrzej P2.33, P2.54, P3.9
 Na Chan-Hyun P2.55
 Nagler Arnon L043
 Nakae Yoshiko P2.50
 Nakanishi Yohko L014
 Nakaya Masa-aki P4.29
 Nakazawa Tadao L018
 Nałęcz Adam P2.65, P3.45
 Nazikoglu Aysegul P2.6
 Nechamen Cheryl L006
 Nemethova Miroslava P2.15, P2.16
 Nemoto Norimichi L014, P1.40
 Neuhuber Winfried L087
 Nezhybová E. P4.41
 Nicolato Elena P4.42
 Niderla-Bielińska Justyna P4.27
 Niemiałtowski Marek P4.39
 Niemiec Joanna P1.31, P1.37
 Niesiołowska Kaja P1.37
 Niewiadomska Grazyna P3.33
 Nikawa Takeshi P2.50
 Nishiyama Kana P2.62
 Nishiyama Yukihiro P2.62
 Niu Siyun P2.35
 Nobuki Nakamura L018, P1.33
 Nodera Makoto P3.54
 Nomura Hiroyuki L016
 Nowak-Ziatyk Dorota P2.22
 Nowicki Michał P1.6
 Nozaki Naohito L079
 Nurzynska Daria P3.2, P3.3, P3.4
 Nynca Anna P1.55, P2.17
 Obana Yukari P1.40
 Ocalewicz Konrad P2.47
 Oh Eok-Soo L009
 Ohni Sumie L014
 Ohno Kinji P1.5
 Ohno Nobuhiko P3.38, P3.58
 Ohno Shinichi P3.58
 Oinuma Toshinori P1.40
 Okada Takeshi P1.5
 Olejniczak Karolina P1.63, P1.64, P2.51, P2.52
 Ooi Akishi P2.37
 Opalka Marek P1.58
 Orhan Nurcan P1.57
 Orta Alberto L017
 Ortyń William E. L044
 Oruc Ozgur P2.23
 Osamura Robert Y. L037, L066, L007, L105
 Ostalska-Nowicka Danuta P1.6
 Ostasov Pavel P3.63
 Ostrowska Alina P1.21,
 Ostrowska Halina P1.49, P1.50
 Ostrowski Maciej P3.36
 Oteri G. P2.44
 Ozat Mustafa P2.25
 Ozer Erdener P1.2
 Özgür Figen P3.46
 Ozogul Candan P1.2
 Oztay Fusun P1.17, P1.57
 Ozturk Melek P1.52, P2.11, P2.24, P4.14
 Paciello Orlando L021
 Pajares Maria J L017
 Palubinskiene Jolita P3.48, P4.51
 Pangonyte Dalia P3.48, P4.51
 Panula Pertti L055, P4.6, P4.53
 Paparelli Antonio P2.59
 Papież Monika P2.18, P2.26, P2.28
 Partyka L. P3.13
 Pasaoglu Aydin P1.13
 Patrzalek Dariusz P3.15
 Pazyńska Galina P1.41
 Pekcetin Cetin P1.2
 Pellegatta Serena L096
 Pellicciari Carlo L082, P1.9
 Pengo Thomas L017
 Pepperkok Rainer L003
 Perdoni Federica P1.9, P4.42
 Perek Danuta P1.38
 Peri G. P3.23, P4.10
 Perra Maria Teresa P1.25, P1.26, P3.5
 Peters Eva L034
 Petroff Brian P1.55
 Pęksa Rafał P3.45
 Piasecka Joanna P2.17
 Piasecka Małgorzata P3.60, P4.46
 Pidsudko Zenon P1.43
 Piegari Elena P3.2
 Pienczk Jerzy P2.8
 Pieńkowska-Grela Barbara P1.27
 Pietrzak Piotr P2.9, P2.13, P2.20
 Piukuła Michał P3.9
 Piljić Alen P2.39
 Pilat-Marcinkiewicz Barbara P1.51
 Pinar Asli P1.14
 Pintelon Isabel L085
 Pińkowski Roman L107
 Pio Ruben L017
 Piotrowska Aleksandra P1.21
 Piras Franca P1.25, P1.26, P3.5
 Pisani A. L023
 Piskorowska Joanna P1.27, P1.39
 Piszcz Jarosław P1.15A, P3.65
 Piszczek Łukasz P2.19
 Piterková L. P4.41
 Plewka Danuta P4.32
 Plucińska Karolina P1.7, P2.60
 Podhorska-Okolow Marzena P3.11, P1.21,
 P1.45, P3.15
 Podlasz Piotr P2.7, P2.67, P4.6
 Polus Anna P3.13, P3.16, P3.17, P3.18
 Pons Luis E. P2.64, P2.40, P2.63
 Priyadarshini Madhusmita L055
 Prusik Magdalena P1.66
 Przybylska-Gornowicz Barbara P1.65, P1.66
 Przybyszewska Wiesława P2.51, P2.52
 Puzzolo D. L023
 Qing Zhang P1.30
 Rabczynski Jerzy P3.41, P3.42
 Radwańska Agata P3.40
 Rafińska Ludmiła P1.63, P1.64
 Raggiaschi Roberto L096
 Rapoport Eugenia P1.41
 Ratajczak Janina L106
 Ratajczak Katarzyna P3.42
 Ratajczak Mariusz L106, L042, L044
 Ratajska Anna P1.34
 Razny Urszula P3.18, P3.17
 Rezza S. L024
 Rezzani Rita P3.22, P4.8
 Rice Frank L033
 Richard Derek J P3.59
 Rizzo G. P2.45
 Robak Anna P2.17, P4.12, P4.13, P4.18
 Robinson J. Paul L036
 Rodella Luigi F P3.22, P4.8
 Rodriguez Marta P2.64
 Romano Gianpaolo P3.2, P3.4
 Romano Veronica P3.2, P3.3, P3.4
 Romero-Aleman M P3.29
 Rongish Brenda L072
 Rosa Gasco Maria P3.64
 Rossi Marco L096
 Roth Jürgen L031
 Roth Kevin L008, L109
 Równiak Maciej P4.13, P4.18
 Ruchała Marek P1.63, P1.64
 Ruciński Marcin P1.63, P1.64
 Runci M. P2.45
 Rupp Angie P2.43
 Ryohei Katoh P1.33
 Ryś Janusz P1.37
 Rzepko Robert P2.65, P3.45
 Sadowska Jolanta P1.61
 Sahai Erik L093
 Sai Xiao Rei P3.32
 Saitoh Sei P3.38, P3.58
 Sakulsak Natthiya P4.29
 Salerno Massimiliano L096
 Sallinen Ville P4.6, L055
 Salvado Maria Teresa P2.64
 Salvado Teresa P2.40
 Sameni Mansoureh L047
 Sano Makoto P1.40
 Santin Giada P1.9
 Santoro G. L023
 Santos E. P3.29
 Sara Calderoni P3.64
 Sati Leyla P3.28, P3.56
 Sato Yoko P2.14
 Saudek Frantisek P4.7
 Sawicki Bogusław P1.51
 Sawiński Piotr P1.63, P1.64
 Schaefer Liliana L061
 Schneider Gabriela P3.33
 Schultz Carsten L005, L070, P2.39, P2.42
 Schwartz Michael L002
 Sciarrillo Rosaria P1.4
 Scott Edward W. L038
 Scovassi Anna Ivana L082
 Seget Monika P3.55
 Seki Toshimi L014
 Selamat Widawilis Binte P4.2
 Sellitti Anna P1.4, P1.23, P4.31
 Seval Yasemin P1.62, P3.28, P3.56
 Shi Yuxiu P1.3, P1.15, P2.31
 Shibata Yasuaki P2.14
 Shindo Hiroaki P3.19, P3.54
 Shioda Seiji L025, L028
 Shono Masayuki P2.50
 Sidor-Kaczmarek Justyna P1.42
 Sienkiewicz Waldemar P1.43, P2.7, P2.67
 Sieroń Aleksander L P3.6
 Sierpniowska Aleksandra P1.34, P3.24, P3.25
 Sikorska Beata L104
 Sikorski Aleksander F P3.41
 Şimşek Halis P1.14
 Sinischalchi E Nastro P2.44
 Sinusas Albert L073
 Sirigu Paola P1.25, P1.26, P3.5
 Sixt Michael L097
 Składanowski Andrzej P2.19
 Skrzypek Klaudia P1.34
 Skwarska Anna P2.22
 Sliwa A. P3.13
 Sloane Bonnie L047
 Slominski Andrzej L035
 Słomczyńska M P1.60
 Smolarczyk Ryszard P1.34
 Smoliński Dariusz P3.34
 Sokmenser Lale P2.25
 Sokołowska Ewa P4.44,
 Sokołowska Małgorzata P1.34
 Solakoglu Seyhun P3.43
 Sommer Claudia P4.7
 Sosak-Świdarska Bożena P4.54

- Sowiński Jerzy P1.63, P1.64
 Spedito Alessandro P4.43
 Speranza G L024
 Spodnik Edyta P4.20, P4.24
 Spodnik Jan H. P1.42, P4.19, P4.20, P4.24, P2.32, P4.22, P4.23
 Stachura Jerzy P3.17, P3.18
 Stalioraityte Elena P3.48, P4.51
 Stanisławowski Marcin P2.57, P2.58
 Stawiarska-Pięta Barbara P4.40
 Stefanię Rosita P2.1
 Stefanowicz Joanna P2.8
 Stępień Aleksandra P3.37
 Stoward Peter J. P2.50
 Stuppia L. L024
 Sugitani Masahiko P1.40
 Sujka Patrycja P2.52
 Sukhikh Gennadij P1.19
 Sun Zhijian P1.48
 Sunagawa Keishin L014
 Sundvik Maria L055, P4.53
 Susumu Nobuyuki L016
 Suzuki Atsushi L016
 Suzuki Shioto P2.37
 Suzuki Yumi P1.5
 Svizenska Ivana P4.9, P4.45
 Svoboda Petr P3.63
 Szaflarska-Stojko Ewa P4.40
 Szala Stanisław P1.34
 Szaryńska Magdalena P2.54
 Szczeklik Andrzej P3.31
 Szczepański Mariusz P3.37
 Sztornowska Katarzyna P4.34
 Szuba Andrzej P3.15
 Szulc Lidia P4.39
 Szurowska Edyta P2.8
 Szymańska Hanna P1.27, P1.39
 Ścisłowska-Czarnecka Anna P4.48
 Śliwińska-Jewsiewicka Agnieszka P2.47
 Tabarowski Zbigniew P2.4
 Tabola Renata P3.41, P3.42
 Tadao Nakazawa P1.33
 Taha Hevidar P3.24
 Takáčová S. P4.41
 Takahashi Masahide P4.30
 Takamatsu Tetsuro L049, L053
 Takeda Shin'ichi P1.5
 Takekoshi Susumu L066, L105
 Takenoya Fumiko L025, L028
 Takumi Toru P2.61, P4.2
 Tamada Yutaka L016
 Tan Huiping L099
 Tanaka Hideo L053
 Tang Chi L078
 Tanyel Cahit P2.27
 Tapul Leyla P1.24
 Tarnok Attila L102, L111
 Tasar Celik P2.23
 Tatar Gonca P1.14
 Tedesco Rosa P4.31
 Terada Nobuo P3.38, P3.58
 Teresa Salvado P2.63
 Tessaro Irene P4.26
 Tetsu Yamane P1.33
 Thatcher Brendan L002
 Thiele Christoph L058
 Thomas Richard L006
 Timmermans Jean-Pierre L085
 Tokuda Noriyo P1.30
 Tomas Barbara P2.64
 Tomas Lvaro P2.63
 Tomonori Kawasaki P1.33
 Tong Ye-Qing P1.35
 Tosik Dariusz P3.57
 Trembecka Dominika P3.62
 Trenčanský Lumír P4.45
 Trimarchi F. L023
 Trojanek Oanna P2.42
 Trojnar Weronika P1.7, P2.60
 Trzonkowski Piotr P2.54, P3.9
 Tsuda Hiroshi L016
 Tucholska Anna P3.35
 Tuna Sevilcan P1.24
 Tunçbilek Gökhan P3.46
 Tuncdemir Matem P2.24, P2.11
 Tunici Patrizia L096
 Tuong Vuong P2.10, P3.44
 Turan Pinar P3.52
 Turan Suna Ozbas P3.53
 Ture Durukan P1.12
 Türk Neslihan P1.53
 Turlejski Krzysztof P3.7, P4.19, P4.22
 Turowska Agnieszka P3.35
 Tylko Grzegorz P3.61, P4.16
 Ugalde Jorge P1.25, P3.5
 Ugorski Maciej P1.45
 Ugur Yesim P2.6
 Ulloa-Aguirre Alfredo L006
 Umakoshi Hiroyasu P4.30
 Umay Cenk P3.49
 Umemura Shinobu L066
 Unanyan Ara P1.20
 Urbanek Ksymena P3.6
 Uruluer Bulent P4.14
 Uslu Bahar P3.53
 Usukura Jiro L056
 Utkiene Lina P4.51
 Uzel O. P3.49
 Uzunalan Mumin P4.14
 Vaccarino G L024
 Vahakangas Elisa P3.24, P3.25
 Valentino B. P3.23, P4.10
 Valentino F. P3.23
 Valiante Salvatore P1.4, P1.23
 Van Noorden Cornelis L000, L045
 Van Weeren Laura L004
 Vatansver Seda P1.32
 Veselková Zuzana P4.45
 Vinarov A. P1.46
 Vinoly R. P3.29
 Voronkov Dmitry P4.1
 Voska Ludek P4.7
 Wachulska Małgorzata P2.30
 Wakayama Tomohiko P4.29
 Walter Glenn A. L038
 Wan Yu-Jui Yvonne P3.18
 Wang Hai-Tao P1.15, P2.56
 Wang Li-Yan P1.1
 Wang Weixi L099
 Wang Xiao-Li P1.35
 Wang Yu P2.61
 Wapniarska Iga P4.34
 Wardowska Anna P2.54
 Wasilewska Anna P4.28,
 Wasilewska Barbara P4.13
 Wasilewska Ewa P2.34
 Wasowicz Krzysztof P2.7
 Waś Halina P1.34
 Watanabe Noriko L014
 Watanabe Takashi L056
 Wator Lukasz P3.18
 Wąsowicz Krzysztof P2.67
 Wąsowska Barbara P2.17, P2.34, P3.26, P3.27
 Wątor Lukasz P3.17
 Węgrowski Yanusz L062, P3.40
 Wehr Bernhard P2.68
 Weier Heinz-Ulli L010
 Wesołowska Katarzyna P4.52
 Whiteford James L065
 Wiaderkiewicz Ryszard P4.32
 Widlak Piotr L084
 Wierzbicki Piotr P2.57, P2.58
 Williams Diana L002
 Willison Hugh J P2.43, P4.4
 Winnicka Anna P2.7
 Wittliff James L. L013, L048
 Włodarski Tomasz P2.48
 Wojnar Andrzej P1.45
 Wojtkiewicz Joanna P4.21, P4.25
 Wong Loo Chin P4.2
 Woszczyński Marek P1.39
 Wójcik Krzysztof P2.48, P1.42, P2.32, P4.20, P4.22, P4.24
 Wrona Danuta P2.3, P2.66
 Wrzesińska Justyna P2.20
 Wu Wan L106
 Wu Yan P1.35
 Wurdinger Tom L045
 Wylot Marcin P4.46
 Wypych Joanna P2.57
 Wysocka Joanna P1.31
 Wysocka Teresa P1.44, P1.47
 Xiao Lan L103
 Xu Xijin P2.12
 Yamada Kumiko P3.54
 Yamane Tetsu L018
 Yamauchi Yohei P2.62
 Yanardag Refiye P1.10, P1.17, P1.57
 Yanes C. P3.29
 Yegen Berrak P3.52
 Y?ld?z F?r?zan P4.5
 Yilmazer Selma P1.29, P1.53
 Yla-Herttua Seppo P3.24, P3.25
 Yoneda Atsuko L009, L065
 Yoshida Mizuko P2.50
 Yoshimura Toshiro P1.5
 Yue Fengming P2.35
 Zabel Maciej P1.44, P1.45, P1.47, P2.51, P2.52, P3.11
 Zabielski Romuald P2.9, P2.13, P2.20
 Zadonskaya Juliya P1.19
 Zagorska Anna P3.24, P3.25
 Zalejska-Fiolka Jolanta P4.40
 Zalewski Grzegorz P1.67, P4.28
 Zamir Evan L072
 Zancanaro Carlo P4.42
 Zapala B P3.13, P3.30
 Zarnescu Otilia P4.36
 Zarzecka Joanna P4.37
 Zdanov D. P1.46
 Zeng Yuan-Shan L100
 Zeybek Dilara P1.32, P2.23
 Zhang Yanbo L103
 Zhang Yanhui P1.3
 Zhang Yinong L099
 Zhang Yuanqiang P1.48, P2.49
 Zhao Jie P1.48, P2.49
 Zhi-Long Liu P1.35
 Zhiuraitiene Reda P3.48
 Zhou Guo-Min L090
 Zielińska-Chomej Katarzyna P2.30
 Zieliński Andrzej P4.52
 Zimmermann Bernhard L052
 Zinchuk Vadim L068, L091, P2.61
 Ziobro Marek P1.31
 Ziuraitiene Reda P4.51
 Zoch-Zwierz Walentyna P4.28
 Zuba-Surma Ewa K. L044, L106
 Zudaire Isabel L017