FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 46, No. 2, 2008
pp. 171-176

Expression of connexin 26 in endometrial adenocarcinoma - analysis of correlations with some anatomoclinical features

Tomasz Lesniewicz[1], Luiza Kanczuga-Koda[2], Marek Baltaziak[1], Mariola Sulkowska[1], Ryszard Rutkowski[3], Mariusz Koda[1], Stanislaw Sulkowski[1]

Departments of: [1]General and [2]Medical Pathomorphology, [3]Respiratory Diagnostics and Bronchofiberoscopy, Medical University of Bialystok, Bialystok, Poland

Abstract: Alterations of gap junctional intercellular communication appear to play a role in the development and progression of cancer. Gap junction channel is composed of two connexons - hexameric units formed of transmembrane proteins called connexins (Cxs). The aim of the study was to evaluate the expression and localization of Cx26 in 73 cases of endometrial cancers and to estimate the relationships between expression of this protein and selected anatomoclinical features. The control group consisted of 20 sections of normal endometrium in various menstrual cycle phases, obtained from premenopausal women. In the normal endometrium punctate, membrane-associated immunoreactivity for Cx26 was observed. 54 of 73 endometrial cancers showed Cx26 expression, but 46/54 (85%) immunopositive sections revealed cytoplasmic localization for Cx26 with granular or occasionally diffuse immunostaining pattern. In addition, part of Cx26-positive tumours showed mixed: cytoplasmic and membranous staining pattern and focally also nuclear or perinuclear immunostaining was present. In 21/54 (39%) of Cx26-positive cases weak staining pattern was seen, however in 33/54 (61%) cancers strong reaction was noted. We did not find relationship between Cx26 expression and patients' age, histological type of cancer and histological grade, nevertheless we observed positive association between Cx26 expression and tumour size ($p=0.037$). In conclusion, our results suggest that transformed malignant cells continue to produce Cx26, which are probably not assembled into functional gap junction channels, but could still play other roles in endometrial cancer cells.

Key words: Connexin 26 - Gap junction - Endometrial cancer - Immunohistochemistry

Introduction

Gap junctions are intercellular membrane channels connecting directly the cytoplasm of adjacent cells for the exchange of inorganic ions, second messengers and small metabolites (<1kDa) [1]. Gap junctional intercellular communication (GJIC) is involved in tissue development, maintenance of tissue homeostasis and morphogenesis and it affects cell differentiation and proliferation by regulation of signal transduction and cell cycle [2]. Gap junction channels are constructed of assemblies of oligomeric proteins called connexins (Cxs). These proteins are synthesized, joined in hexamers, called connexons, and aligned with the connexon of the adjacent cell membrane to form a channel [3]. Gap junctional communication is subject to regulation by numerous substances, such as growth factors, Ca^{2+} and hormones, also involved in the synthesis and recruitment of connexins [4,5]. Connexins are encoded by different genes and until now more that 20 connexins have been recognized in human [6]. It has been shown that Cx26 and Cx32 are expressed in human endometrial glandular epithelium, whereas Cx43 is present in endometrial stromal cells [7]. Expression of Cx26 and Cx32 changes significantly during the menstrual cycle: weak expression in the proliferative phase, markedly elevated during ovulation and most pronounced in the mid-secretory phase.

Correspondence: S. Sulkowski, Dept .of General Pathomorphology, Medical University of Bialystok, Waszyngtona Str. 13, 15-269 Bialystok, Poland; tel.: (+4885) 7485945, fax.: (+4885) 7485944, e-mail: sulek@zeus.amb.edu.pl

Abbreviations: Cx26 - connexin 26, GJIC - gap junctional intercellular communication

Folia Histochem Cytobiol. 2008:46(2): 171 (171-176)
doi: 10.2478/v10042-008-0026-3

There is substantial evidence that interruption of gap junctional communication is an important step ahead towards malignant transformation [8-10]. Most normal cells have functional intercellular communication, while most, if not all, tumour cells have dysfunctional GJIC [11,12]. Decreased communication via gap junctions may be an important event in the oncogenesis. Reversely, reconstitution of gap channels has been shown to restore normal phenotypes and to retard tumour cell growth. It was thus suggested that genes encoding connexins could play a tumour suppressing role [13,14]. Endometrial cancer is worldwide the most often diagnosed gynaecological malignancy and its frequency is increasing in the developed countries. It has been reported that during endometrial carcinogenesis, loss of GJIC may occur as a result of decreased expression and aberrant localization of Cxs [15]. Nevertheless, knowledge about possible role of Cxs in human endometrial carcinogenesis is very limited. Furthermore, correlation between connexin expression and anatomoclinical features in endometrial cancer has not yet been investigated. Consequently, the aim of the present study was to evaluate expression and localization of Cx26 by immunohistochemistry in normal endometrium and in endometrial cancer specimens as well as to estimate the relationships between assessed connexin and selected anatomoclinical features.

Material and methods

Patients and tissue specimens. Tissue samples were obtained from 73 women treated surgically for primary endometrial cancer. The patients' age ranged from 37 to 83 years (mean 61.9 years). Tumour samples were collected shortly after tumour removal, fixed in 10% buffered formaldehyde solution for 48h and then embedded in paraffin blocks at 56°C according to standard procedures. 5 μm sections were cut from the specimens and stained with haematoxylin-eosin. The diagnosis was based on the World Health Organization (WHO) classification of endometrial tumours. Our study included 67 endometrial cancers classified histopathologically as endometrioid adenocarcinoma and 6 as endometrioid adenocarcinoma with squamous metaplasia. 12 (16.4%) cases were classified as G1 grade (well-differentiated adenocarcinoma), 50 (68.5%) cases as G2 (moderately differentiated adenocarcinoma) and 11 (15.1%) cases as G3 (poorly differentiated adenocarcinoma). Staging was done according to the International Federation of Gynaecology and Obstetrics (FIGO) system. This resulted in 5 IA cases, 30 - IB, 27 - IC, 7 - IIA, 3 - IIB, and 3 - IIIA.

Twenty sections of normal endometrial tissues in various phases of the menstrual cycle (proliferative and secretory), obtained from premenopausal women undergoing hysterectomy for myoma uteri or early invasive sqamous cell carcinoma of the cervix (IA1 by the FIGO classification) were the control group.

Immunohistochemistry. Paraffin-embedded tissue sections were subjected to immunostaining using goat polyclonal Cx26 antibody (Santa Cruz Biotechnology, USA) in dilution rate 1:100. Primary antibody was diluted in PBS with 1.5% normal blocking serum. A streptavidin-biotin-peroxidase complex technique was used to reveal antibody-antigen reactions (LSAB kit, Dako, Denmark). Immunohistochemistry was performed as described previously [16]. Slides were counterstained with haematoxylin. Following immunohistochemical controls were performed: normal endometrium was used as a positive control [7]; negative control included omission of the primary antibody. The evaluation of immunostaining for studied protein was analyzed in 10 different tumour fields and the mean percentage of tumour cells with positive staining was evaluated. The expression of Cx26 in cancer samples was classified using a scale: 0 (negative cases) less than 10% of positive cells; 1+ with immunoreactivity ranging from 10% to 50% of positive cancer cells; 2+ with over 50% of positive cells.

Statistical analysis. The relationship between Cx26 expression and studied clinicopathological features was evaluated with the χ^2 (Chi-square) test. Probabilities of $p<0.05$ were considered statistically significant.

Results

Expression and localization of Cx26 in normal endometrium and endometrial cancer

In normal human endometrium mainly intercellular granular localization of immunostaining pattern was observed only in endothelial cells of endometrial glandular epithelium, but not in the surrounding stroma or myometrium. The expression of Cx26 was most intense in the late proliferative phase and in the early secretory phase of the menstrual cycle (Fig. 1). The studied marker was not detected in control samples, where immunostaining was performed with the omission of the primary antibody.

In the endometrial cancer 54 of total 73 samples (74%) were positive for Cx26. In 21/54 (39%) of Cx26-positive cases weak staining pattern was seen, however in 33/54 (61%) cancers there was a strong reaction. Immunohistochemical analysis revealed cytoplasmic localization of Cx26 in 46/54 (85%) immunopositive sections with granular or occasionally diffuse immunostaining pattern (Fig. 2A, 2B, 2C). In these cases sporadically and focally membranous staining was present. The samples that showed diffuse cytoplasmic reaction for Cx26 were carcinomas in G3 grade (Fig 2A, C). In 8/54 (15%) of Cx26-positive tumours, classified as G1 grade, mixed reaction: cytoplasmic and punctate, membrane-associated was seen. (Fig. 2D) Focally nuclear or perinuclear immunoreactivity for Cx26 was also noted in some samples (Fig. 2A, 2C).

Correlations between Cx26 expression and anatomoclinical features

To study the relationship between Cx26 and anatomoclinical features such as: histological grade (G1, G2 and G3), tumour size, histopathological type (adenocarcinoma and adenocarcinoma with squamous metaplasia) and age (≤60 and >60), a total number of 73 cases of endometrial cancer were examined.

Folia Histochem Cytobiol. 2008:46(2): 172 (171-176)
doi: 10.2478/v10042-008-0026-3

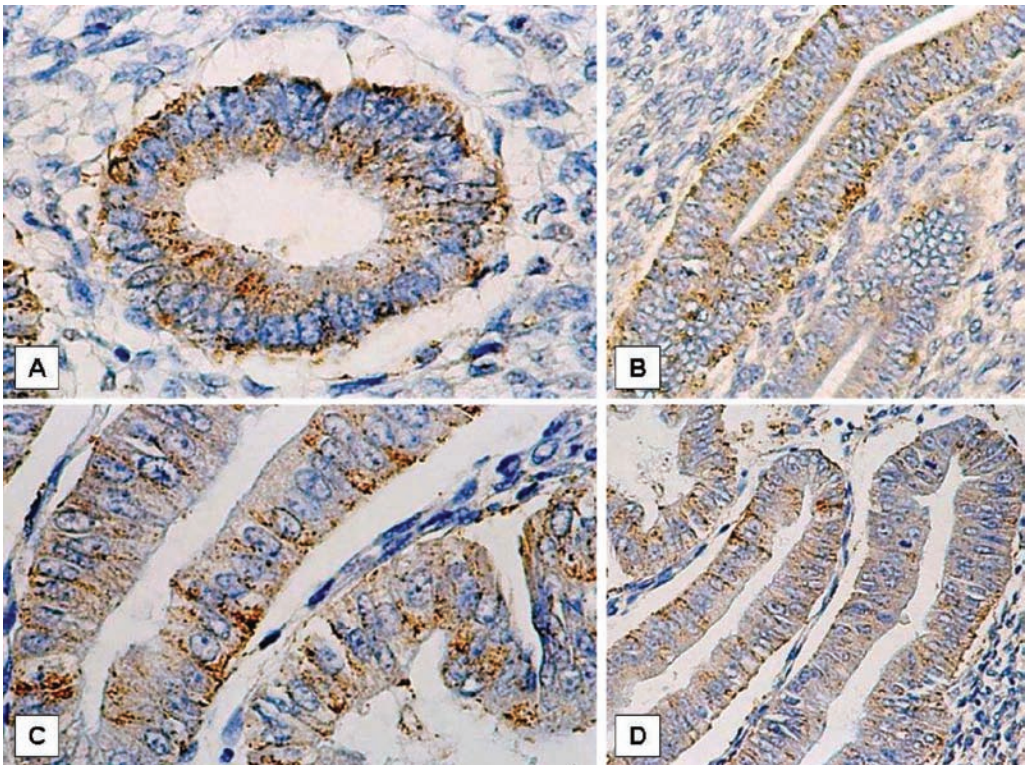

Fig. 1. Expression and localization of Cx26 in normal endometrium.(**A, B**) Mainly intercellular localization of the granular immunostaining pattern only in the endothelial cells of endometrial glandular epithelium in late proliferative phase of the menstrual cycle. (**C, D**) Immunopositive granular deposits of Cx26 distributed between epithelial cells of endometrium in early secretory phase of the menstrual cycle.

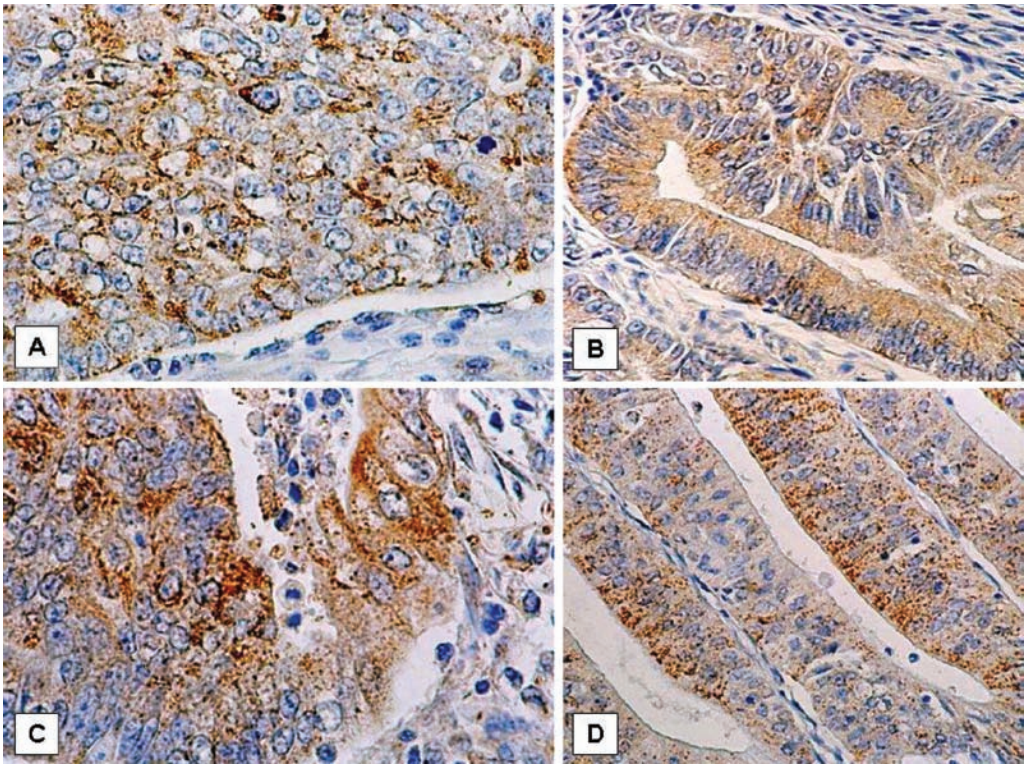

Fig. 2. Expression and localization of Cx26 in endometrial cancer. (**A**) Strong cytoplasmic immunostaining of Cx26 in endometrial cancer cells, focally perinuclear immunoreactivity. (**B**) Strong cytoplasmic immunostaining of Cx26 in the majority cells of G2 grade endometrial cancer. (**C**) Diffuse or granular cytoplasmic reaction in G3 endometrial cancer, focally also nuclear or perinuclear immunostaining. (**D**) Punctate Cx26 immunostaining densely distributed mainly between cancerous cells.

Folia Histochem Cytobiol. 2008:46(2): 173 (171-176)
doi: 10.2478/v10042-008-0026-3

The expression of Cx26 was not associated with age, histological grade and histopathological type of tumour. However, there was an indication for positive correlation with statistical significance between Cx26 expression and tumour size (p=0.037), although positive immunostaining for Cx26 appeared more frequently in advanced tumour stages (cancer invading more than halfway into the myometrium or from the body of the uterus to the cervix) than in tumours which spread less than halfway through the myometrium.

Discussion

In the present study we examined immunohistochemically the expression of Cx26 in normal human endometrium and in the endometrioid adenocarcinoma of the uterus. We observed mainly membranous granular immunostaining for this protein in endothelial cells of normal endometrial glandular epithelium, but not in the surrounding stroma or myometrium. These results are compatible with the results of the previous study by Saito *et al.* [7], who analyzed the distribution of Cx26 and Cx32 in human endometrial glandular epithelium as well as changes in the expression of these proteins during the reproductive cycle.

Carcinogenesis is a multistage process and different pathways can lead to cancer [17]. Decreased expression of Cxs and alterations in GJIC correlate with tumourigenesis [18-20]. Connexins are typically localized in the cell membrane and normally show a punctate pattern of expression [21,22]. Aberrant localization of connexins was revealed in several types of tumours in our and other studies [15,20,23,24] and it might result in the intercellular communication loss via gap junction channels. In the present study, we demonstrated changes in the Cx26 expression and localization, which manifested in decreased expression and mainly cytoplasmic localization of this protein in the endometrial cancer cells. Disturbed expression and subcellular localization of this protein in our study is consistent with previous observations made by Saito *et al.* [15], who showed that during endometrial carcinogenesis suppressed expression and aberrant localization of Cx26 and Cx32 may occur at relatively early stages.

Our previous observations [20,25] and present results suggest that cells may depend on the disturbances in the synthesis and subcellular localization of Cx26, but mechanisms responsible for these changes are still little known. Only one connexin gene mutation in human tumours has been described so far (advanced stages of human sporadic colon cancer) [26]. On the other hand, there is growing body of evidence suggesting that connexin genes could be inactivated by hypremethylation of their promoter regions [27]. It is probable that this epigenetic inactivation of connexin genes may be one of the causes of decreased expression of connexins in the endometrial cancer, however it requires detailed studies.

Another important problem, which warrants further explanation, is the aberrant subcellular (cytoplasmic or nuclear) localization of connexins in cancerous cells, observed also in this study. It has been well documented that cell-cell adhesion is necessary for the formation of functional gap junction channels [8]. Cadherins and catenins are involved in the creation of adherens junctions. Dysfunction of these proteins may result in the decreased membrane expression of Cxs. It has been reported that β-catenin was localized in the nucleus in endometrial hyperplasia and cancer [28]. Additional observations made by Shih *et al.* [29] have shown that the expression of E-cadherin and cytoplasmic β-catenin in endometrial carcinomas decreased compared to normal proliferative endometrial glands. Moreover, the expression of E-cadherin and cytoplasmic β-catenin tends to be reduced in histologically high-grade tumours, compared to low-grade tumours. Additionally, we have recently demonstrated that during colorectal carcinogenesis altered expression and localization of connexins, E-cadherin and β-catenin is a common phenomenon. Furthermore, we noted the statistically significant correlation between expression of examined connexins and adhesion proteins (data not shown). This may suggest close relations between these proteins. Additionally, it is possible that changes in the expression of adhesion proteins might contribute to the incorrect localization of connexins in the cancerous cells.

Cx26 protein accumulates in the cytoplasm of cancer cells and plays a role different from its physiological one. Krutovskikh *et al.* [30] discovered that subcellular localization of Cx43 in tumour cells could play a role in the regulation of tumour growth. Recently, Olbina *et al.* [31] showed that changes in protein sequence of the second extracellular region of Cx43 prevent incorporation of the protein into the plasma membrane, but do not decrease its ability to inhibit the growth of tumour cells in vitro. They concluded that regulation of cellular growth by Cx43 does not necessarily require well-functioning gap junctions. It is possible that connexins in the intracellular (cytoplasmic or nuclear) compartment may control tumour progression modulating expression of the genes responsible for cell growth regulation, differentiation and apoptosis as well as other functions of cancerous cells. These findings show that connexins localized in the cytoplasm and between cells can play different roles in the cell signalling pathways. Several reports described growth suppression in the absence of functionally coupled channels [32,33]. For example, Qin *et al.* [32] found that tumour-suppressing properties of connexins in breast cancer cell lines were independent from gap

Folia Histochem Cytobiol. 2008:46(2): 174 (171-176)
doi: 10.2478/v10042-008-0026-3

junction communication. They suggested that it could be a result of down-regulation of genes involved in tumour growth, such as the gene encoding fibroblast growth factor receptor-3 [32]. Furthermore, Zhang *et al.* [33] revealed a gap junction-independent suppression of tumour growth. They demonstrated that Cx43 in osteosarcoma cell line inhibited expression of S phase kinase-associated protein 2 (Skp2), which was responsible for p27 ubiquitination. Zhang *et al.* [33] supposed that Cx43 localized in cytoplasmic or nuclear cell compartment could indirectly take part in the degradation of this protein regulating the expression of other genes. Moreover, Cx43 expression was associated for example with an increased expression of cyr61, an immediate early gene encoding a cysteine-rich heparin-binding protein, which seems to be involved in several cellular pathways including growth and differentiation [34].

In the present study we have also analyzed the correlation between Cx26 expression and some clinicopathological features. We found that in advanced tumour stages (cancer spread more than halfway through the myometrium or from the body of the uterus to the cervix), more Cx26 positive cases were present than in tumours which spread less than halfway through the myometrium. It is hard to comment on these findings because statistical significance was not strong and an association between Cxs expression and anatomoclinical features in endometrial cancer has not yet been investigated. Nevertheless, in our recent studies on connexin and adhesion proteins expression in colorectal cancer, we observed an analogous correlation between Cx32 expression and tumour size, but only in the subgroup of patients without metastases to lymph nodes (data not shown). On the other hand, it is important to note that in malignant cells synthesized Cxs are localized mainly in cytoplasm, which was observed also in this study. In this instance, it may suggest that increased expression of Cx26 in the most advanced stages of cancer might be a result of long-term development of cancerous cells and longer period of protein synthesis and/or decreased tumour cell turnover because of disturbed apoptosis. We suppose that Cxs, despite its well known role in the stimulation of apoptosis, could also interact with antiapoptotic proteins. Recently, in our immunohistochemical study on Cx26 and apoptotic markers expression in colorectal cancer, we revealed the positive correlation between Cx26 and antiapoptotic protein - Bcl-xL [35]. The association between Cx26 and Bcl-xL expression in colorectal cancer cells might partly explain this theory, but additional functional studies on the role of connexins in these processes are required.

In conclusion, results of our study may suggest that endometrial cancer cells continue to produce connexin 26, which is probably not assembled into functional gap junction channels, however it still plays an important roles in the transformed malignant cells.

References

[1] Kumar NM, Gilula NB. The gap junction communication channel. *Cell.* 1996;84:381-388.

[2] Yamasaki H, Naus CC. Role of connexin genes in growth control. *Carcinogenesis.* 1996;17:1199-1213.

[3] Martin PE, Evans WH. Incorporation of connexins into plasma membranes and gap junctions. *Cardiovasc Res.* 2004;62: 378-387.

[4] Huang R, Lin Y, Wang CC, Gano J, Lin B, Shi Q, Boynton A, Burke J, Huang RP. Connexin 43 suppresses human glioblastoma cell growth by down-regulation of monocyte chemotactic protein 1, as discovered using protein array technology. *Cancer Res.* 2002;62:2806-2812.

[5] Ozog MA, Bechberger JF, Naus CC. Ciliary neurotrophic factor (CNTF) in combination with its soluble receptor (CNTFRalpha) increases connexin43 expression and suppresses growth of C6 glioma cells. *Cancer Res. 2002;*62: 3544-3548

[6] Sohl G, Willecke K. Gap junctions and the connexin protein family. *Cardiovasc Res.* 2004;62:228-232

[7] Saito T, Oyamada M, Yamasaki H, Mori M, Kudo R. Co-ordinated expression of connexins 26 and 32 in human endometrial glandular epithelium during the reproductive cycle and the influence of hormone replacement therapy. *Int J Cancer.* 2004;73:479-485

[8] Trosko JE, Ruch RJ. Cell-cell communication in carcinogenesis. *Front Biosci.* 2004;15:208-236

[9] Trosko JE, Ruch RJ. Gap junctions as targets for cancer chemoprevention and chemotherapy. *Curr Drug Targets.* 2002;3:465-482

[10] King TJ, Bertram JS. Connexins as targets for cancer chemoprevention and chemotherapy. *Biochim Biophys Acta.* 2005; 1719:146-160

[11] Mesnil M, Asamoto M, Piccoli C, Yamasaki H. Possible molecular mechanism of loss of homologous and heterologous gap junctional intercellular communication in rat liver epithelial cell lines. *Cell Adhes Commun.* 1994;2:377-384

[12] Cesen-Cummings K, Fernstrom MJ, Malkinson AM, Ruch RJ. Frequent reduction of gap junctional intercellular communication and connexin43 expression in human and mouse lung carcinoma cells. *Carcinogenesis.* 1998;19:61-67

[13] Mehta PP, Perez-Stable C, Nadji M, Mian M, Asotra K, Roos BA. Suppression of human prostate cancer cell growth by forced expression of connexin genes. *Dev Genet.* 1999;24: 91-110

[14] Mesnil M. Connexins and cancer. *Biol Cell.* 2002;94:493-500

[15] Saito T, Nishimura M, Kudo R, Yamasaki H. Suppressed gap junctional intercellular communication in carcinogenesis of endometrium. *Int J Cancer.* 2001;93:317-323

[16] Koda M, Sulkowski S, Garofalo C, Kanczuga-Koda L, Sulkowska M, Surmacz E 2003; Expression of the insulin-like growth factor-I receptor in primary breast cancer and lymph node metastases: correlations with oestrogen receptors alpha and beta. *Horm Metab Res.* 35:794-801

[17] Farber E. The multistep nature of cancer development. *Cancer Res.* 1984;44:4217-4223

[18] Wilgenbus KK, Kirkpatrick CJ, Knuechel R, Willecke K, Traub O. Expression of Cx26, Cx32 and Cx43 gap junction proteins in normal and neoplastic human tissues. *Int J Cancer*. 1992;51:522-529

[19] Hanna EA, Umhauer S, Roshong SL, Piechocki MP, Fern-

Folia Histochem Cytobiol. 2008:46(2): 175 (171-176)
doi: 10.2478/v10042-008-0026-3

strom MJ, Fanning JD, Ruch RJ. Gap junctional intercellular communication and connexin43 expression in human ovarian surface epithelial cells and ovarian carcinomas *in vivo* and *in vitro*. *Carcinogenesis.* 1999;20:1369-1373

[20] Kanczuga-Koda L, Sulkowski S, Koda M, Sulkowska M. Alterations in connexion 26 expression during colorectal carcinogenesis. *Oncology.* 2005;68:217-222

[21] Bruzzone R, White TW, Paul DL. Connections with connexins: the molecular basis of direct intercellular signalling. *Eur J Biochem.* 1996;238:1-27

[22] Kanczuga-Koda L, Sulkowski S, Koda M, Sobaniec-Lotowska M, Sulkowska M. Expression of connexins 26, 32 and 43 in the human colon - an immunohistochemical study. *Folia Histochem Cytobiol.* 2004;42:203-207

[23] Defeijter AW, Matesic DF, Ruch RJ, Guan X, Chang CC, Trosko JE. Localization and function of the connexin 43 gap-junction protein in normal and various oncogene-expressing rat liver epithelial cells. *Mol Carcinog.* 1996;16:203-212

[24] Kanczuga-Koda L, Sulkowski S, Lenczewski A, Koda M, Wincewicz A, Baltaziak M, Sulkowska M. Increased expression of connexins 26 and 43 in lymph node metastases of breast cancer. *J Clin Pathol.* 2006;59:429-433

[25] Kanczuga-Koda L, Sulkowski S, Tomaszewski J, Koda M, Sulkowska M, Przystupa W, Golaszewska J, Baltaziak M. Connexins 26 and 43 correlate with Bak, but not with Bcl-2 protein in breast cancer. *Oncol Rep.* 2005;14:325-329

[26] Dubina MV, Iatckii NA, Popov DE, Vasil'ev SV, Krutovskikh VA. Connexin 43, but not connexin 32, is mutated at advanced stages of human sporadic colon cancer. *Oncogene.* 2002;21:4992-4996

[27] Piechocki MP, Burk RD, Ruch RJ. Regulation of connexin32 and connexin43 gene expression by DNA methylation in rat liver cells. *Carcinogenesis.* 1999;20:401-406

[28] Nei H, Saito T, Yamasaki H, Mizumoto H, Ito E, Kudo R. Nuclear localization of beta-catenin in normal and carcinogenic endometrium. *Mol Carcinog.* 1999;25:207-218

[29] Shih HC, Shiozawa T, Miyamoto T, Kashima H, Feng YZ, Kurai M, Konishi I. Immunohistochemical expression of E-cadherin and beta-catenin in the normal and malignant human endometrium: an inverse correlation between E-cadherin and nuclear beta-catenin expression. *Anticancer Res.* 2004;24;3843-3850

[30] Krutovskikh VA, Troyanovsky SM, Piccoli C, Tsuda H, Asamoto M, Yamasaki H. Differential effect of subcellular localization of communication impairing gap junction protein connexin43 on tumour cell growth *in vivo*. *Oncogene.* 2000;19:505-513

[31] Olbina G, Eckhart W. Mutations in the second extracellular region of connexin 43 prevent localization to the plasma membrane, but do not affect its ability to suppress cell growth. *Mol Cancer Res.* 2003;1:690-700

[32] Qin H, Shao Q, Curtis H, Galipeau J, Belliveau DJ, Wang T, Alaoui-Jamali MA, Laird DW. Retroviral delivery of connexin genes to human breast tumour cells inhibits in vivo tumor growth by a mechanism that is independent of significant gap junctional intercellular communication. *J Biol Chem.* 2002; 277:29132-29138

[33] Zhang YW, Kaneda M, Morita I. The gap junction-independent tumor-suppressing effect of connexin 43. *J Biol Chem.* 2003;278:44852-44856

[34] O'Brien TP, Lau LF. Expression of the growth factor-inducible immediate early gene cyr61 correlates with chondrogenesis during mouse embryonic development. *Cell Growth Differ.* 1992;3:645-654

[35] Kanczuga-Koda L, Sulkowski S, Koda M, Skrzydlewska E, Sulkowska M. Connexin 26 correlates with Bcl-xL and Bax proteins expression in colorectal cancer. *World J Gastroenterol.* 2005;11:1544-1548

Submitted: 29 March, 2007
Accepted after reviews: 11 October, 2007

Folia Histochem Cytobiol. 2008:46(2): 176 (171-176)
doi: 10.2478/v10042-008-0026-3