FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 45, No. 2, 2007
pp. 81-86

STAT3, HIF-1α, EPO and EPOR - signaling proteins in human primary ductal breast cancers

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Abstract: STAT3 upregulates expression of HIF-1 induced EPO. Receptor EPOR was reported to activate STAT3. Our study was aimed at demonstration of tissue immunoreactivities of those proteins and determination of their relationships in reference to clinicopathological variables of breast cancers. We detected STAT3, HIF-1α, EPO and EPOR in specimens of 76 human, female, ductal breast cancers by immunohistochemistry. STAT3 was detected in 38 of 76 cancers (50%). HIF-1α was found in 55 cases (72%). EPO positive tumors comprised 89% of all the cancers (68 cases). EPOR was also visualized in 55 cases (72%). Anti-HIF-1α and anti-STAT3 stained nuclei and cytoplasm of breast cancer cells in diffuse and finely granular fashion. Strong membranous expressions of EPO and EPOR were distributed in cytoplasmic and membranous granularity or diffuse staining. STAT3 correlated with HIF-1 in general ($r=0.4012$, $p<0.0001$) and in different patients' subgroups. STAT3 was significantly associated with EPO and EPOR in all the cancers ($r=0.2370$, $p=0.039$ and $r=0.3336$, $p=0.003$, respectively). Besides a correlation between STAT3 and EPOR in node negative ones, STAT3 wasn't related to EPO and EPOR in remaining subgroups. HIF-1α correlated with EPO and EPOR in most of analyzed groups. Immunoreactivity to EPO generally was associated with EPOR ($r=0.3520$, $p=0.002$). Statistically analyzed distributions of the proteins reflected functional dependences among STAT3, HIF-1α, EPO and EPOR in cellular signal conduction.

Key words: Signal transduction - Breast cancer - Hypoxia - Chemotherapy

Introduction

Signal pathways cross themselves. Some factors exert multiple actions and their effects converge in regard to changing environmental intracellular contributions in breast cancer development. Hypoxia is a common cause of rearrangement of protein expression in breast cancer [10]. In pathological conditions like inflammation or cancer hypoxia induces synthesis of subunit α of HIF-1 (Hypoxia-inducible factor). HIF-1α binds with constantly expressed HIF-1β and the complete molecule of HIF-1 can perform its functions [30]. In normoxia HIF-1 is not activated due to lack of α subunit HIF-1. HIF-1 induced proteins like GLUT1 (Glucose transporter 1) or leptin in colorectal cancers and estrogen dependant cancers like endometrial cancer and breast cancer [10,19,25,29,30,31]. Breast cancer also involves upregulation of transcriptional agents like STAT3 (Signal transducer and activator of transcription), STAT3 activator - EPOR (Erythropoietin receptor), and a HIF-1 downstream protein - EPO (Erythropoietin) [1,17,21,22]. There are serious expectations that mentioned proteins are involved in the same chain of interactions of cellular signaling for a number of reasons.

Significance of a powerful transcriptional factor, STAT-3 is discussed in development of breast cancer. This transcriptional factor becomes activated during transmission of various intracellular signals both in physiological and pathological processes [7]. STAT-3 (particularly activated form: tyrosine-phosphorylated STAT-3) was detected in various breast cancer cell lines under activation of EGFR (epidermal growth factor receptor) [22]. STAT3 can also be recruited by Src (Rous sarcoma) and JAK (Janus kinases) tyrosine kinase, if EGFR signaling is abolished but activation of STAT3 remains less intensified in result [11]. Constitutively activated STAT3 was regarded as oncogenic and was found to correlate with cyclin D1 in primary breast cancers. In addition, it was elucidated that cyclin D1 is a target gene for regulatory transcriptional activity of STAT3 which strongly suggests involve-

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ment of STAT3 in favoring of cell proliferation and survival [14]. Anyway, functional knockout of STAT3 resulted in restriction of apoptosis of mammary epithelium and impairment of breast involution after weaning in genetically modified mice [8]. Moreover STAT-3 seemed to silence expression of cyclin D1 in liver fetal development [20]. At present an opinion predominates that STAT3 prolongs cancer cell lifespan and amplifies growth by contribution to proliferation of the mammary neoplasm [11,23]. HIF-1 is hypoxia inducible factor that induces transcription of cytoprotective proteins in malignant cells in hypoxic conditions. HIF-1α predicts poor prognosis breast cancer [9,18]. HIF-1 and STAT3 actions have been associated indirectly with each other. One of linking factors was HGF (hepatocyte growth factor). HGF induced HIF-1α in MCF-7 (Human breast adenocarcinoma cell line - low invasive breast carcinoma) cell line, but transcription of HIF-1α failed to be switched on by HGF in MDA-MB-231 cells (Human Caucasian breast adenocarcinoma cell line - highly invasive breast carcinoma) and - moreover - alpha subunit of HIF-1 probably underwent degradation after exposure to HGF [27]. c-Src (cellular Src) kinase interacted with HGF promoter leading to transcription of this factor in breast carcinoma cells. Next, c-Src kinase lifted the tyrosine 705 phosphorylation and DNA binding affinity of STAT3. STAT3 and Src synergically augmented transcription of HGF. The presented signaling loop uncovered one link between HIF-1 and STAT-3 in breast cancer [7]. HIF-1 and STAT-3 relationship was more evidently highlighted by interference of STAT-3 transcription with a small-molecule inhibitor and resultant downregulation of HIF-1 and VEGF (vascular endothelial growth factor) that delayed tumor growth and angiogenesis [32].

EPO and EPOR are induced by hypoxia in breast cancer and could contribute to increased survival rate of tumor cells via counteraction to hypoxic injury [1]. EPO counteracts outflow of cytochrome c from mitochondrion by upregulation of Bcl-xL. EPO prevents apaf-1 complex dependent activation of caspase 9 and 3 by inhibition of binding cytochrome c to apaf-1 and cyt-c in cytoplasm. EPO stimulates proliferation of blood red cells and cancer cells. EPOR generates signals for cell growth in both non neoplastic and non erythroid cells, for instance endothelial and intestinal cells [24]. A chain of functional dependence exists between EPO and STAT3, too. Serine and tyrosine phosphorylation of STAT-3 were induced by EPO [13]. Furthermore, Tyr432 residue on human EPOR was recognized as a region required for STAT-3 activation [17]. HIF-1 induced transcription of EPO [28]. EPO overexpression correlated with HIF-1α and was proved fatal in endometrial cancer, which like breast cancer was also estrogen dependent neoplasm [3]. The outlined ties encouraged us to compare expressions of STAT-3, HIF-1α, EPO and EPOR in human breast cancers. Thus, we intended to detect, if immunohistochemistry uncovered linkages between two transcriptional agents: STAT3, HIF-1, STAT3 activator - EPOR, and HIF-1 dependant expression of EPO.

Materials and methods

Tissue samples of breast ductal cancers were obtained from 76 women. 32 patients underwent preoperative chemotherapy before tumor resection. These human studies have been performed in agreement with the ethical standards laid down in the 1964 Declaration of Helsinki and its latest revision in 2000 (the approval by the ethics committee of Medical University of Bialystok). All the subjects expressed their informed consent before inclusion in the study.

The biopsy material was stored in 10% buffered formaldehyde solution for 48 hours, and then embedded in paraffin blocks at 56°C. Diagnosis was stated on the base of 5 μm thick, haematoxylin and eosin stained specimens and standard histopathologic parameters of breast cancers were determined (including AJCC/UICC TNM (American Joint Committee on Cancer/Internationale Contre le Cancer Tumor Node Metastasis) stage, tumor type and grade of differentiation G).

Immunohistochemical evaluation. The cancers were stained by immunohistochemistry. Tissue sections were 3-5 μm thick and mounted on 3-aminopropyltriethoxysilane-coated slides, dewaxed in xylene and rehydrated through graded alcohols to phosphate-buffered saline (PBS). The slides were kept in 2% hydrogen peroxide for 10 min to eliminate endogenous peroxidase activity. Prior to visualization of HIF-1α and STAT-3 protein, tissue sections underwent incubation with blocking serum for one hour in case of HIF-1α, EPO and EPOR, whereas the incubation lasted 1 and half an hour in case of STAT-3. Before incubation in blocking serum, microwaves were used for EPO antigen retrieval for 3 minutes. Selected proteins were detected with specific anti-HIF-1α rabbit polyclonal IgG (sc-10790), anti-STAT3 (sc-7179) rabbit polyclonal IgG, anti-EPO (H-162) and anti-EPOR, (C-20) (Santa Cruz Biotechnology, Inc.). For STAT3, HIF-1α, EPO and EPOR the primary antibodies were diluted at 1:500, 1:400 1:150 and 1:200 solution, respectively. The specific antibodies reacted with sections overnight at 4°C except for EPOR slides which were incubated for 2 hours. The color reaction in tissues was established with En Vision method after 7 minutes of exposure to DAB in HIF-1 evaluation, 6 minutes for EPO, 5 minutes for EPOR and 10 minutes for STAT3. Sections were counterstained with haematoxylin, dehydrated, cleared and mounted. In negative controls the primary antibodies were omitted. Stained specimens of colorectal cancer were positive controls.

Scoring and statistical analysis. The results were analyzed with Spearman's rank correlation test. All the statistical results with $p<0.05$ were assumed to be significant. We applied 3-grade scoring system as follows: grade 0 if there was less than 10% immunoreactive cancer cells; grade 1 if immunoreactive cancer cells ranged from 10 to 50%; grade 2 if 50% malignant cells were immunoreactive. The immunohistochemical stainings were assessed by two pathologists in 10 high power fields of each tumor in light microscopy and the mean rate of tumor positive cells was determined. Each of the proteins was analyzed alone with Chi-square Pearson's test to pursue statistically significant differences of their immunoreactivities in regard to variability of each chosen clinicopathological trait. The grade of histological differentiation and tumor staging were not assessed in primary tumors of patients given chemotherapy due to subsequent damage of the cancer cells. In result, expressions of proteins were not compared and analyzed statistically between either moderately (G2) and poorly (G3) dif-

ferentiated cancers or larger (T2) and smaller (T1) ones due to severe chemotherapy induced destruction of primary tumors. Thus statistics covered only patients without chemotherapy in comparison between different G or T status.

Results

Characteristics of cellular distribution of STAT3, HIF-1α, EPO and EPOR

STAT3 was detected in 38 of 76 cancers (50%). HIF-1α was found in 55 cases (72%). EPO positive tumors comprised 89% of all the cancers (68 cases). EPOR was also visualized in 55 cases (72%). Anti-STAT3 accumulated also in cytoplasm (Fig. 1a) and nuclei of breast cancer in diffuse and finely granular pattern (Fig. 1b). Anti-HIF-1α stained cytoplasm (Fig. 1c) and nuclei (Fig. 1d) of breast cancer cells in finely granular fashion and occasionally nuclear reaction was depicted in adjacent stromal cells. Strong membranous expression of EPO was found in most of cancer cells (Fig. 1e). EPO was also distributed focally in cytoplasm with varying degree of diffuse or finely granular staining. The pattern of expression for EPOR was mixed cytoplasmic and membranous granularity (Fig. 1f). Expressions of each protein alone failed to associate with any of clinicopathological features. The exceptions were linkage of increased expression of HIF-1α with lack of chemotherapy ($p<0.0242$) and EPOR with higher differentiated cancers (G2) ($p=0.0078$).

Comparison between STAT3 and the other studied proteins

There were significant associations between expression of STAT3 and HIF-1α in general ($r=0.4012$, $p<0.0001$) and in patients' subgroups of different features including age, sex, nodal involvement, chemotherapy and tumor size except for G2, G3 and T1 group with only trends toward significance (Table 1). STAT3 was significantly associated with expressions of EPO and EPOR in cancers of all patients ($r=0.2370$, $p=0.039$ and $r=0.3336$, $p=0.003$, respectively). STAT3 particularly correlated with EPOR in node negative cancers (N-) ($r=0.4544$, $p=0.010$), in pT2 tumors ($r=0.5897$, $p=0.013$) and chemotherapy spared patients ($r=0.3766$, $p=0.012$). STAT3 was not related with EPO and EPOR in other subgroups of various age, sex, node involvement, grading and staging (Table 1).

Comparison of HIF-1α to EPO and EPOR

HIF-1α was correlated with EPO and EPOR in most of analyzed groups with exceptions shown in Table 2. Similarly reactivity to EPO generally was associated with expression of EPOR ($r=0.3520$, $p=0.002$). There were also significant ties between EPO and EPOR in clinicopathological groups except for groups of chemotherapy spared and chemotherapy treated patients, node negative (N-) tumors, pT2 neoplasms, moderately (G2) and poorly (G3) differentiated cancers (Table 2).

Discussion

STAT3 has been expected by us to play important role in breast cancer development [23]. Considering few publications on STAT-3 significance in vivo human tumors, we are deeply convinced that gap of this literature is reduced by our comparisons of immunoreactivities to STAT-3 to its co-operating proteins in reference to different pathological advancement of breast cancer and clinical characteristics of the patients. Nevertheless, we are conscious of the fact that our present results implicate some limitations because we applied STAT-3 antibody that reacts both with phosphorylated (activated) and non phosphorylated (unactivated) STAT3. However, nuclear location of STAT3 is a certain evidence for the fact that STAT3 has been activated because only activation (phophorylation of 705 tyrosine residue) enables translocation of STAT3 from cytoplasm to nucleus [5,7]. HIF-1α was also detected in nuclei of cancer cells in our present study. This location marks its activity as nuclear transcriptional factor [30,31]. The evident immunoreactivity to HIF-1α that we depicted in spindle stromal cells (Fig. 1d), shows that they could express the same regulatory proteins of cancer growth as neoplastic cells. It is not surprising, if we consider that stromal cells could modulate dynamics of growth of breast cancers [26].

In our study expressions of STAT3 did not significantly varied among the groups of different cancer advancement, though (data not shown). Similarly, the expressions of other proteins did not associate with any clinicopathological trait with certain exceptions. It is easy to explain the higher expression of EPOR is in better differentiated cancers (G2) in comparison to poorer differentiated ones (G3). The better differentiation the more proteins expressed. Occurrence of HIF-1α was significantly increased in chemotherapy spared tumors compared to chemotherapy treated neoplasms because chemotherapy could destructively affect cancer cells via inhibition of protein expression.

Referring to our findings on relation between STAT3 and HIF-1 (Table 1), it is highly possible that STAT3 mediates its survival promoting stimuli on hypoxia-affected breast cancer cells in co-operation with HIF-1. In studies of Jung *et al.* activated STAT-3 was particularly reported to stabilize HIF-1α and support its further synthesis in hypoxic human renal cancer cells [16].

Table 1. Analysis of correlations between STAT-3, HIF-1α, EPO and EPOR expressions in primary tumors of the breast cancer. Spearman's correlation rank test.

Primary tumors	n*	STAT3 - HIF-1α		n*	STAT3 - EPO		n*	STAT3 - EPOR	
		r	p		r	p		r	p
All	76	0.4012	<0.0001	76	0.2370	0.039	76	0.3336	0.003
G2	33	0.2992	0.091	33	0.1642	0.361	33	0.4199	0.015
G3	11	0.5910	0.056	11	0.4944	0.122	11	0.1321	0.699
(N-)	31	0.3879	0.031	31	0.1642	0.378	31	0.4544	0.010
(N+)	45	0.3926	0.008	45	0.2908	0.053	45	0.1960	0.197
T1	27	0.3416	0.081	27	0.2345	0.239	27	0.2669	0.178
T2	17	0.3724	0.141	17	0.2257	0.384	17	0.5897	0.013
(Ch-)	44	0.3728	0.013	44	0.2392	0.118	44	0.3766	0.012
(Ch+)	32	0.4683	0.007	32	0.2681	0.138	32	0.2604	0.150

Table 2. Analysis of correlations between HIF-1α, EPO and EPOR expressions in primary tumors of the breast cancer. Spearman's correlation rank test.

Primary tumors	n*	HIF-1α -EPO		n*	HIF-1α - EPOR		n*	EPO - EPOR	
		r	p		r	p		r	p
All	76	0.3501	0.002	76	0. 4379	<0.0001	76	0.3520	0.002
G2	33	0.2311	0.196	33	0.2311	0.196	33	0.2072	0.247
G3	11	0.5996	0.003	11	0.6317	0.037	11	0.3166	0.343
(N-)	31	0.4356	0.014	31	0.5859	0.001	31	0.2948	0.107
(N+)	45	0.2784	0.064	45	0.2834	0.059	45	0.4225	0.004
T1	27	0.5538	0.003	27	0.4663	0.014	27	0.4582	0.016
T2	17	-0.0483	0.854	17	0.4340	0.082	17	0.1456	0.529
(Ch-)	44	0.3255	0.031	44	0.4397	0.003	44	0.3347	0.026
(Ch+)	32	0.4192	0.017	32	0.4191	0.017	32	0.3883	0.028

n - number of cases, G2 - moderately differentiated, G3 - poorly differentiated, (N+) - tumors metastasized to lymph nodes, (N-) - tumors which didn't metastasize to lymph nodes, (Ch+) - with chemotherapy, (Ch-) - without chemotherapy. *The numbers of investigated cancers varied and were reduced because G and T were not assessed in certain primary tumors because of cellular damage due to chemotherapy, that appeared to spare more nodal metastases than primary foci of cancers.

Correlations between STAT3 and EPO suggested their action in accord to support survival of breast cancer cells in human tumors in the same fashion as in cell lines [2,12]. However, correlations between STAT3 and EPO or EPOR didn't seem to be associated with progress of cancer in our evaluation. Node involvement (N) didn't appear to depend on participation of STAT3 in EPO- EPOR cell signaling if we considered our variable results. Particularly, comparison between STAT3 and EPO showed no significance in regard to N feature. Furthermore, STAT3 was significantly related to EPOR exclusively in node negative cancers. Chemotherapy was not able to break down strong statistically significant ties between STAT3 and HIF-1α or HIF-1α and its downstream protein - EPO or HIF-1α and EPOR or statistical relationship between EPO EPOR (Table 1 and 2).

HIF-1 is known for induction of transcription such survival proteins as EPO [28]. EPO, which was downstream protein of HIF-1, counteracted apoptosis of hypoxia experienced breast cancer cells [2]. This dependence was expressed by correlations between degrees of immunohistochemical expressions of HIF-1α and EPO in our studies (Table 2). Consequently, EPO expression correlated with its membranous receptor EPOR that confirmed their functional loop in breast cancer similarly to other reports [2,4,6].

Linkages among expressions of the proteins suggested functional dependences among STAT3, HIF-1α EPO and EPOR in cell to cell signaling in breast can-

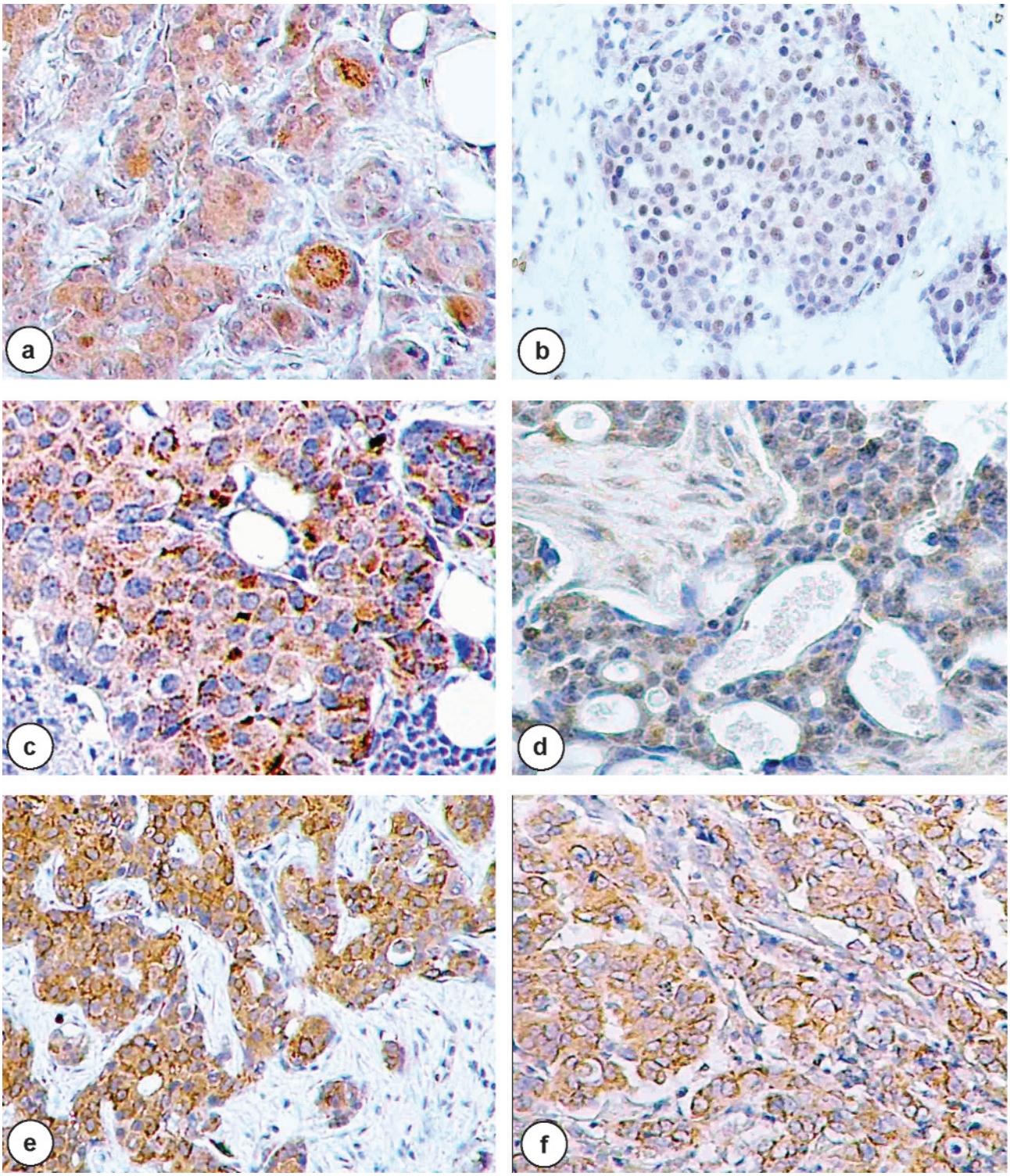

Fig. 1. a. Predominantly cytoplasmic, granular staining showing STAT3 expression in breast cancer cells (magnification ×200). **b**. Nuclear accumulation of STAT3 in breast cancer cells arranged in cribiform nests of tubule-like cancer structures (magnification ×200). **c**. Cancer cells with cytoplasmic, coarse granules and intense perinuclear rims of HIF-1α in vicinity of retracted necrotic conglomerate (magnification ×400). **d**. Nuclear immunoreactivity to HIF-1α of breast cancer cells and benign stromal cells. Note lack of staining selectivity for malignant cells (magnification ×400). **e**. The immunoreactivity to EPO presents as confluent granules of variable size and irregular sharp outlines, that constitute linear paranuclear or paramebranous accumulations in cancer cells (magnification ×200). **f**. Membrane and cytoplasm location of EPOR in linear and diffuse fashion. Primary breast cancer (magnification ×200).

cer. Our analysis showed these relationships weren't disconnected by chemotherapy and persisted in chemotherapy treated patients except relationships between STAT3 and EPOR.

References

[1] Acs G, Acs P, Beckwith SM, Pitts RL, Clements E, Wong K, Verma A. Erythropoietin and erythropoietin receptor expression in human cancer. Cancer Res, 2001; 61: 3561-5

[2] Acs G, Chen M, Xu X, Acs P, Verma A, Koch CJ. Autocrine erythropoietin signaling inhibits hypoxia-induced apoptosis in human breast carcinoma cells. Cancer Lett, 2004; 214: 243-51

[3] Acs G, Xu X, Chu C, Acs P, Verma A. Prognostic significance of erythropoietin expression in human endometrial carcinoma. Cancer, 2004; 100: 2376-86

[4] Acs G, Zhang PJ, Rebbeck TR, Acs P, Verma A. Immunohistochemical expression of erythropoietin and erythropoietin receptor in breast carcinoma. Cancer, 2002; 95: 969-81

[5] Akira S. Functional roles of STAT family proteins: lessons from knockout mice. Stem Cells, 1999; 17: 138-46

[6] Arcasoy MO, Amin K, Karayal AF, Chou SC, Raleigh JA, Varia MA, Haroon ZA. Functional significance of erythropoietin receptor expression in breast cancer. Lab Invest, 2002; 82: 911-8

[7] Bromberg J. Stat proteins and oncogenesis. J Clin Invest, 2002; 109: 1139-42

[8] Chapman RS, Lourenco PC, Tonner E, Flint DJ, Selbert S, Takeda K, Akira S, Clarke AR, Watson CJ. Suppression of epithelial apoptosis and delayed mammary gland involution in mice with a conditional knockout of Stat3. Genes Dev, 1999; 13: 2604-16

[9] Dales JP, Garcia S, Meunier-Carpentier S, Andrac-Meyer L, Haddad O, Lavaut MN, Allasia C, Bonnier P, Charpin C. Overexpression of hypoxia-inducible factor HIF-1alpha predicts early relapse in breast cancer: retrospective study in a series of 745 patients. Int J Cancer, 2005; 116: 734-9

[10] Garofalo C, Koda M, Cascio S, Sulkowska M, Kanczuga-Koda L, Golaszewska J, Russo A, Sulkowski S, Surmacz E. Increased expression of leptin and the leptin receptor as a marker of breast cancer progression: possible role of obesity-related stimuli. Clin Cancer Res, 2006; 12: 1447-53

[11] Garcia R, Bowman TL, Niu G, Yu H, Minton S, Muro-Cacho CA, Cox CE, Falcone R, Fairclough R, Parsons S, Laudano A, Gazit A, Levitzki A, Kraker A, Jove R. Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. Oncogene, 2001; 20: 2499-513

[12] Gritsko T, Williams A, Turkson J, Kaneko S, Bowman T, Huang M, Nam S, Eweis I, Diaz N, Sullivan D, Yoder S, Enkemann S, Eschrich S, Lee JH, Beam CA, Cheng J, Minton S, Muro-Cacho CA, Jove R. Persistent activation of stat3 signaling induces survivin gene expression and confers resistance to apoptosis in human breast cancer cells. Clin Cancer Res, 2006; 12: 11-9

[13] Haq R, Halupa A, Beattie BK, Mason JM, Zanke BW, Barber DL. Regulation of erythropoietin-induced STAT serine phosphorylation by distinct mitogen-activated protein kinases. J Biol Chem, 2002; 277: 17359-66

[14] Huang M, Page C, Reynolds RK, Lin J. Constitutive activation of stat 3 oncogene product in human ovarian carcinoma cells. Gynecol Oncol, 2000; 79: 67-73

[15] Hung W, Elliott B. Co-operative effect of c-Src tyrosine kinase and Stat3 in activation of hepatocyte growth factor expression in mammary carcinoma cells. J Biol Chem, 2001; 276: 12395-403

[16] Jung JE, Lee HG, Cho IH, Chung DH, Yoon SH, Yang YM, Lee JW, Choi S, Park JW, Ye SK, Chung MH. STAT3 is a potential modulator of HIF-1-mediated VEGF expression in human renal carcinoma cells. FASEB J, 2005; 19: 1296-8

[17] Kirito K, Nakajima K, Watanabe T, Uchida M, Tanaka M, Ozawa K, Komatsu N. Identification of the human erythropoietin receptor region required for Stat1 and Stat3 activation. Blood, 2002; 99: 102-10

[18] Kronblad A, Jirstrom K, Ryden L, Nordenskjold B, Landberg G. Hypoxia inducible factor-1alpha is a prognostic marker in premenopausal patients with intermediate to highly differentiated breast cancer but not a predictive marker for tamoxifen response. Int J Cancer, 2006; 118: 2609-16

[19] Laudański P, Koda M, Kozłowski L, Świątecka J, Wojtukiewicz M, Sulkowski S, Wołczyński S. Expression of glucose transporter GLUT-1 and estrogen receptors ER-alpha and ER-beta in human breast cancer. Neoplasma, 2004; 51: 164-8

[20] Leslie K, Lang C, Devgan G, Azare J, Berishaj M, Gerald W, Kim YB, Paz K, Darnell JE, Albanese C, Sakamaki T, Pestell R, Bromberg J. Cyclin D1 is transcriptionally regulated by and required for transformation by activated signal transducer and activator of transcription 3. Cancer Res, 2006; 66: 2544-52

[21] Okada K, Osaki M, Araki K, Ishiguro K, Ito H, Ohgi S. Expression of hypoxia-inducible factor (HIF-1alpha), VEGF-C and VEGF-D in non-invasive and invasive breast ductal carcinomas. Anticancer Res, 2005; 25: 3003-9

[22] Sartor CI, Dziubinki ML, Yu CL, Jove R, Ethier SP. Role of epidermal growth factor receptor and STAT-3 activation in autonomous proliferation of SUM-102PT human breast cancer cells. Cancer Res, 1997; 57: 978-87

[23] Sulkowska M, Gołaszewska J, Wincewicz A, Koda M, Baltaziak M, Sulkowski S. Leptin-from regulation of fat metabolism to stimulation of breast cancer growth. Pathol Oncol Res, 2006; 12: 69-72

[24] Sulkowska M, Wincewicz A, Chabowska A, Kanczuga-Koda L, Szymańska M, Koda M. To Give or Not to Give Recombinant EPO to Anemia Endangered Cancer Patients. Prague Med Rep, 2006; 107: 281-290

[25] Koda M, Sulkowska M, Wincewicz A, Kanczuga-Koda L, Musiatowicz B, Szymańska M Sulkowski S. Expression of Leptin, Leptin Receptor and Hypoxia-Inducible Factor 1α in human Endometrial Cancer. Ann N Y Acad Sci, 2006; 1091: 1-9, in press

[26] Surowiak P, Suchocki S, Gyorffy B, Gansukh T, Wojnar A, Maciejczyk A, Pudelko M, Zabel M. Stromal myofibroblasts in breast cancer: relations between their occurrence, tumor grade and expression of some tumour markers. Folia Histochem Cytobiol, 2006; 44: 111-6

[27] Tacchini L, De Ponti C, Matteucci E, Follis R, Desiderio MA. Hepatocyte growth factor-activated NF-kappaB regulates HIF-1 activity and ODC expression, implicated in survival, differently in different carcinoma cell lines. Carcinogenesis, 2004; 25: 2089-100

[28] Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci USA, 1993; 90: 4304-8

[29] Wincewicz A, Sulkowska M, Koda M, Kanczuga-Koda L, Witkowska E, Sulkowski S. Significant co-expression of GLUT-1, Bcl-xl and Bax in colorectal cancer. Ann N Y Acad Sci, 2006; 1091: 1-9, in press

[30] Wincewicz A, Sulkowska M, Koda M, Sulkowski S. Clinicopathological significance and linkage of HIF-1 α and GLUT-1 distributions in human primary colorectal cancers. Pathol Oncol Res, 2007; accepted for publication

[31] Wincewicz A, Sulkowska M, Koda M, Sulkowski S. Cumulative expression of HIF-1α, Bax and Bcl-xl in human colorectal cancer. Pathology, 2007; accepted for publication

[32] Xu Q, Briggs J, Park S, Niu G, Kortylewski M, Zhang S, Gritsko T, Turkson J, Kay H, Semenza GL, Cheng JQ, Jove R, Yu H. Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. Oncogene, 2005; 24: 5552-60

Received: 20 November, 2006
Accepted after revision: 12 December, 2006