Early nuclear alterations and immunohistochemical expression of Ki-67, Erb-B2, vascular endothelial growth factor (VEGF), transforming growth factor (TGF-BETA 1) and integrine-linked kinase (ILK) two days after tamoxifen in breast carcinoma

A.M.L. MORENA[1], C.T.F. OSHIMA[2], L.H. GEBRIM[4], M.I. EGAMI[5], M.R.R. SILVA[3], R.A. SEGRETO[1], O. GIANNOTTI FILHO[3], V.P.C. TEIXEIRA[3], H.R.C. SEGRETO[1*]

[1]Radiotherapy Division of the Department of Medicine, e-mail: hrcs.dmed@epm.br, Universidade Federal de São Paulo UNIFESP, São Paulo, Brazil; [2]Department of Pathology – UNIFESP and Oncocenter Foundation of São Paulo, [3]Department of Pathology - UNIFESP, São Paulo, [4]Discipline of Mastology of the Department of Tocogynecology, UNIFESP, São Paulo, and [5]Department of Morphology UNIFESP, São Paulo, Brazil

Received March 23, 2004

The purpose of the present study was to evaluate breast carcinoma samples before and two days after treatment with tamoxifen in order to analyse early histopathological alterations – particulary nuclear alterations – as well as immunohistochemical expression of Ki-67, Erb-B2, VEGF, TGf-beta1 and ILK proteins. Twenty one cases of invasive ductal and lobular breast carcinoma were studied. Patients were submitted to biopsy of the lesion and, after confirmation of the diagnosis, they received 20 mg of tamoxifen a day, beginning two days before surgery. The samples obtained during biopsy and after surgery were stained with HE for histopathological diagnosis. Estrogen receptor was positive in 18 cases and negative in 3. The immunohistochemical method was applied for the detection of Ki-67, Erb-B2, protein, vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-beta1) and integrin linked kinase (ILK).

Two days after tamoxifen treatment, the following results were observed: 1) decrease in the cell volume, chomatine condensation, nucleoli less evident and clearly defined nuclear limits; 2) significant reduction in the expression of Erb-B2 protein and significant increase in the expression of TGF-beta1 protein; 3) expression of others proteins (Ki-67, VEGF and ILK) was not altered during the indicated time frame.

Our results suggest that analyzing nuclear alterations and expression of Erb-B2 and TGF-beta1 proteins would be useful to assess the initial response to tamoxifen.

Key words: breast cancer, tamoxifen, immunohistochemistry, tumor markers, Erb-B2, TGF-beta 1

Breast cancer is a complex and heterogeneous disease. It results from several genetic alterations, in which specific genes or groups of genes take part in the induction and maintenance of the disease, leading to a progressive disarrangement in the several stages of the homeostatic mechanism that controls cell growth [29].

Tamoxifen is a drug largely used in hormonal treatment for breast cancer and it is considered prophylactic for women with risk factor for tumor [3]. This drug is a partial agonist of the estrogenic activity. It interacts with steroid receptors and competes with the hormone decreasing its activity in the breasts [30].

One of many tamoxifen mechanisms of action is related to the increase of the apoptosis index observed in cell cultures of breast carcinoma, evidenced by condensation and marginalization of the chromatin around the nuclear cover 24 hours after addition of the drug to the medium [6, 10].

In regard to protein expression, a decrease in Ki-67 expression occurs after the use of this drug, predominantly in estrogen positive tumors [7]. This specific nuclear antigen is expressed in cells throughout the active cell cycle, except for

*Corresponding author

G0 phase and at the beginning of G1 serving as a marker of cell proliferation [8]. In breast tumors, its expression is related to the histopathological degree, mitotic activity and absence of estrogen receptors [8, 23].

The *c-erbB2* protooncogene codifies a protein homologous to the epidermal growth factor (EGF) receptor. It is located in the 17q21 chromosome and codifies a transmembrane glycoprotein receptor denominated p185 neu/p28 erbB-2, which possesses tyrosine kinase activity and is involved in growth and cell differentiation [31]. The superexpression of this protein increases the metastatic potential of the breast neoplasic cells, augments resistance to therapy and decreases survival [9]. Tumors that do not express the Erb-B2- oncoprotein exhibit a high response to tamoxifen; nonetheless, a decrease in the Erb-B2 protein expression is not always observed after treatment with the drug, mainly in the metastatic disease [19].

The vascular endothelial growth factor (VEGF) represents one of the major angiogenic cytokines related to breast cancer and survival of the neoplasic cells, since its superexpression facilitates angiogenesis [13]. Evidence suggests that tamoxifen has an antiangiogenic action in breast tumors and that VEGF levels in breast cancer are relevant to predict the response to tamoxifen treatment [20]. When high levels of VEGF occur, there is a poor response to the drug, whereas low levels indicate a good response and, consequently, a better prognosis [12].

The transforming growth factor beta (TGF-beta) regulates the growth of several types of cells inhibiting or stimulating their growth [21, 26]. Regarding neoplasias, it is known that TGF-beta plays a role in the inhibition of cell proliferation at the beginning of tumoral growth. However, during the course of the disease, tumoral cells frequently become resistant to the inhibiting effects of TGF-beta and begin producing great amounts of this cytokine favoring angiogenesis, invasion and metastasization [26]. Tamoxifen regulates TGF-beta protein expression in breast carcinoma by interacting with estrogen receptor and increasing TGF-beta secretion, thus regulating the proliferation of hormone-dependent carcinomas in an autocrine fashion [15].

Another protein recently found in tumors which seems to play an important role in the invasion and tumoral metastases is integrin-linked kinase (ILK) [2, 28]. This protein is involved in extra cellular matrix-cell interactions. It plays an important role in cell survival and difficulty in these interactions may result in apoptosis [32]. In addition, overexpression of ILK in breast tumor bestows resistance to apoptosis and increases the capacity of tumoral invasion [28, 32]. To the best of our knowledge there are no report in the literature on ILK expression after tamoxifen treatment.

Thus, the present study was carried out with the purpose to examine the early response to tamoxifen before and two days after the treatment, in samples of breast carcinoma, investigating histopathological alterations, specially nuclear alterations and the immunohistochemical protein expression of Ki-67, Erb-B2, VEGF, TGF-beta and ILK.

Patients and methods

Twenty-one ductal and lobular breast carcinoma samples from the files of the Department of Pathology of the UNIFESP/EPM were studied. The patients were treated at the Division of Mastology of the Department of Tocogynecology and in the Radiotherapy Division of the Department of Medicine at the UNIFESP/EPM, from 2000 to 2001. The study was approved by the Ethical Committee of the Federal University of São Paulo.

Patients were submitted to biopsy of the lesion and after confirmation of the diagnosis they received tamoxifen 20 mg/day, two days prior to surgery. Patients with histological diagnosis of lobular and ductal breast carcinoma at clinical stage I and II, were included in this study. Pregnant women who had taken hormone at least 12 months prior to the study, patients who underwent previous treatment for breast carcinoma or patients with a history of thromboembolism or other circulatory disorders were excluded from the study.

Biopsy samples and surgical pieces were fixed in formaldehyde at 10% and embedded in paraffin. Sections 3 μm thick and a slide of each block were stained with HE for histopathological diagnosis, at the Department of Pathology of the UNIFESP/EPM. For the immunohistochemical study, the thin sections were adhered in silanized slides (3-aminopropiltrietoxisilane (APTS).

The immunohostochemical method was used to detect Ki-67, Erb-B2, VEGF, TGF-beta 1 and ILK proteins. The antibodies used were anti-Ki-67 monoclonal IgG (DAKO), anti Erb-B2 polyclonal IgG (A0485 DAKO), anti VEGF polyclonal IgG (A-20 Santa Cruz Biotechnology, Santa Cruz, CA), anti TGF-beta polyclonal IgG (Santa Cruz Biotechnology, Santa Cruz CA) and anti ILK polyclonal IgG (clone 65.1.9 Upstate Biotechnology). Positive controls for reactions were used and specificity controls were performed in the absence of a primary antibody.

The Ki-67 (Ki-67 LI) cell proliferation index was determined by the ratio between the total number of nuclei that showed positive reaction in, at least, 1,000 analyzed nuclei. Quantification was performed by means of an imaging digital analysis, using the IMAGE-PRO Plus Program, version 3.0. Images were generated in an Olympus microscope connected to an Adaptor Camera CMA-D2-Sony connected to the computer, by means of an image digitalized plate Image Capture Board. Reactions with nuclear positivity bellow 10% indicated poor proliferation index; between 10 and 25% moderate index, and above 25% high proliferation index.

For the Erb-B2 protein, the positivity pattern was that of the membrane, which allowed a uniform delineation of the cell edge. Cytoplasmic reactions were considered unspecific.

For VEGF, TGF-beta1 and ILK proteins, the positivity factor was cytoplasmic, being evaluated according to the criteria of intensity of expression in neoplasic cells.

The criteria used for evaluation of immunohistochemical data for Erb-B2, VEGF, TGF-beta1 and ILK were:

-(-) negative: absence of positivity in all neoplasic cells available in the sample or isolated positive cells.

-(+) presence of neoplasic cells with weak positivity

-(++) presence of neoplasic cells with moderate positivity

-(+++) presence of neoplasic cells with strong positivity

Analysis of pre and post-treatment values of Ki-67, Erb-B2, VEGF, TGF beta 1 and ILK variables was performed by the Wilcoxon's non-parametric test "signed rank sum". The level of rejection of significance was set at 0.05, where significant differences are signed by an asterisk, where as lack of significance is signed by the indication (NS) for non significant.

Results

The histopathological analysis of the samples harvested two days after treatment with Tamoxifen revealed an evident decrease in cell volume, marked chromatin condensation, less evident nucleoli, more defined nuclear edge and, apparently, a greater frequency of apoptosis in both histopathological types (Fig. 1).

Immunolocalization of the Ki-67 protein, before treatment with tamoxifen was detected in the cellular nucleus of tumoral tissue and, two days after treatment there was a change in the positivity pattern of the nucleus to the cytoplasm, although no alteration in protein expression was observed (p=0.296) in either histopathological types.

In pre-treatment samples, the Erb-B2 protein was observed in the membrane of tumoral tissue cells, and the cytoplasmic positivity was considered non-specific. A significant decrease (p=0.003) of the immunoexpression of this protein two days after tamoxifen therapy in both tumoral types (Fig. 2A, B; Fig. 3) was observed.

Before treatment, the VEGF protein was observed in the cytoplasm of tumoral cells and in the mammary stroma. Two days after tamoxifen treatment no alteration of immuno- positivity (p=0.108) was observed in both histopathological types.

Before treatment, the active TGF-beta1 protein was observed in the cytoplasm of the tumoral cells of the mammary stroma. A significant increase (p=0.001) in the immunopositivity was observed two days after tamoxifen therapy in both types of carcinoma (Fig, 2C, D; Fig. 4).

Immunolocalization of the ILK protein was detected in the cytoplasm of the tumoral cells and, in a few cases, in the mammary stroma before tamoxifen treatment. No alteration of immunoexpression of the ILK protein (p=0.228) occurred two days after tamoxifen treatment in both types of tumors.

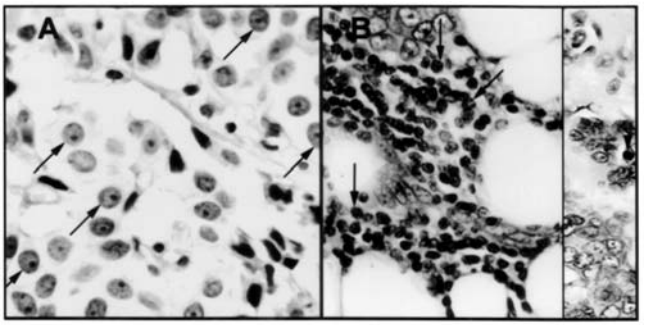

Figure 1. A – Photomicrography of ductal breast carcinoma before tamoxifen. Neoplastic cells with massive nuclei, evident nucleoli, thin granular heterochromatin with homogeneous distribution (arrows) (HE 630x). B – Predominance of neoplastic cells with heterochromatic nucleus and decrease in volume after tamoxifen (arrows). Apoptotic cell (arrow heads) (HE 630x).

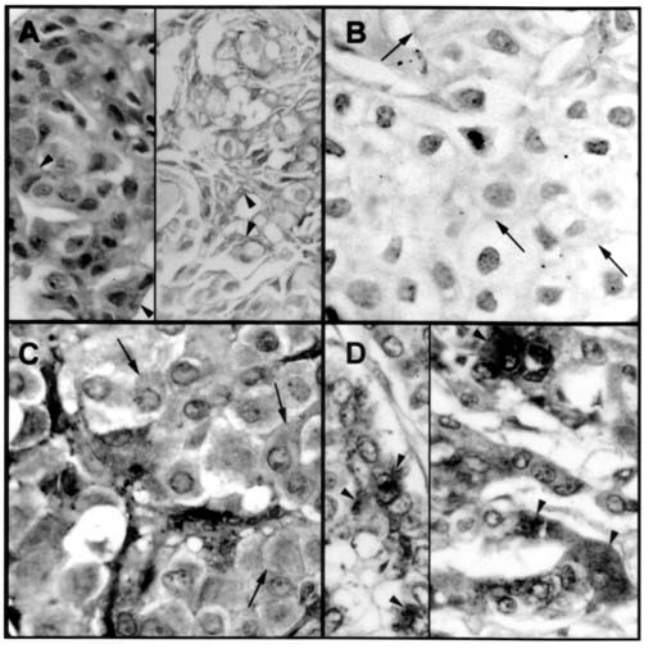

Figure 2. A – Photomicrography of breast ductal carcinoma. Neoplastic cells strongly immunopositive for the Erb-B2 protein in the membrane, before tamoxifen (arrow heads) (680x). B – Neoplastic cells moderately immunopositive for Erb-B2 in the membrane, after tamoxifen (arrows) (680x). Immunohistochemical for Erb-B2. Anti-Erb-B2 IgG polyclonal antibody (A0485 DAKO). C – Predominance of moderate and diffuse immunopositivity for TGF-beta1 protein in the cytoplasm of tumoral cells with homogenous distribution, before tamoxifen (arrows) (630x). D – Clear predominance of cells strongly immunopositive for the TGF-beta1 protein, with a granular pattern and heterogeneous distribution in the cytoplasm post-tamoxifen (arrows heads) (630x). Immunohistochemistry for TGF-beta1. Anti-TGF-beta1 IgG polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

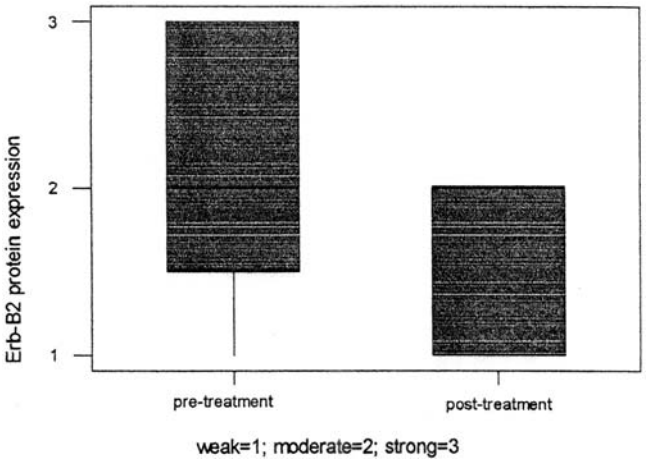

Figure 3. Box plot of the Erb-B2 protein expression in invasive ductal and lobular breast carcinoma before and after tamoxifen.

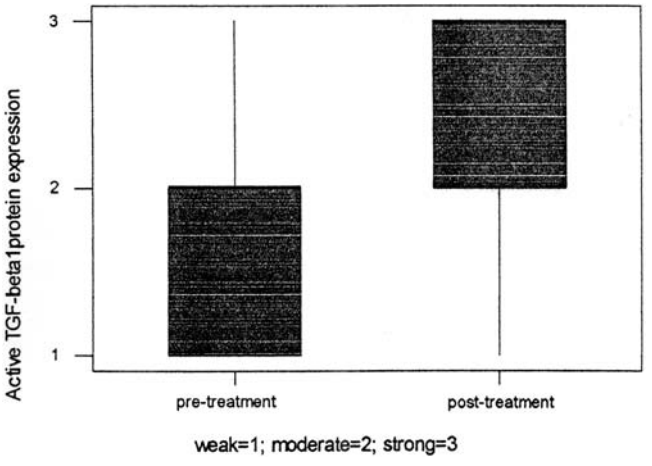

Figure 4. Box plot of the TGF-beta1 expression in invasive ductal and lobular breast carcinoma before and after tamoxifen.

Discussion

Due to the high incidence of breast cancer, there is a great interest to better understand the physiopathology of the disease, its clinical evolution and, mainly, its response to treatment, aiming at optimal results [22]. Among the available therapeutic strategies, tamoxifen is a synthetic antiestrogen, which has been largely used in breast cancer treatment, either in the initial or in the advanced stages of the disease, resulting in an early favorable response in tumors such as ductal and lobular mammary carcinoma [6, 11].

To evaluate the carcinoma response to this drug, some strategies have been used: determination of the tumor proliferation index, by assessing Ki-67 protein [23]; evaluation of the Erb-B2 oncoprotein, expressed in almost 30% of the mammary carcinomas 26; transforming growth factor beta (TGF-beta) [21, 24] and vascular endothelial growth factor [13, 34].

In the present study an initial response of the breast carcinoma to tamoxifen was evaluated by means of the nuclear histopathological alterations and expression of Ki-67, Erb-B2, TGF-beta1, VEGF and ILK proteins.

Histopathological results of the surgical specimens, two days after tamoxifen treatment, revealed an evident decrease of nuclear volume, marked chromatin condensation, less evident nucleoli and more defined nuclear edge evidencing an early response of the tumoral cells. Such results are well-established for tumor estrogen receptors positive [27]. They are in accordance with data observed with lineages of human (HeLa) and murine (BB-88) cervical carcinoma cells, and in cell lineages of human breast cancer, which include cellular shrinkage multinucleation and apoptosis after using the drug [4, 18].

Regarding the immunohistochemical study, there was a great amount of immunopositive nuclei for Ki-67 protein in the cells of the tumoral tissue before the treatment of cases of either ductal mammary carcinoma or invasive lobular cancer. After tamoxifen treatment, immunopositivity apparently decreased in the nucleus but this reduction did not reach statistical significance (p=0.269). Positivity was also observed in the cytoplasm of neoplasic cells after using the drug. This finding may likely be a sign of an initial response and a sign of cell proliferation halt, although two days were not sufficient for this process to occur, as indicated by the lack of decrease in the marker expression. Some authors report a decrease of the Ki-67 expression only in longer periods of treatment such as 7, 10, 14, 21 days and 3 months [6, 7, 8].

Immunolocalization of the Erb-B2 protein before tamoxifen treatment was observed in the membrane of cells of the tumoral tissue and a significant decrease of the immunoexpression in both histopathological types occurred after treatment, (p=0.003), indicating an early response of this marker in the samples studied. Tumors expressing Erb-B2 protein are tamoxifen-resistant and many times do not reveal alteration of immunoexpression after treatment, indicating a poor prognosis and contributing for the metastatic potential of the tumor [17]. However, some studies report a decrease in the Erb-B2 protein immunoexpression between 7 and 10 days after tamoxifen treatment in samples harvested by fine-needle aspiration (FNA) [11, 16].

Regarding TGF-beta1 immunoexpression, we observed a significant increase (p=0.001) after tamoxifen treatment. It is known that the use of this drug induces an increase in the latent and active TGF-beta immunoexpression in cell lineages of hormone-dependent mammary cancer, MCF-7, resulting in growth inhibition and induction of apoptosis [25, 33]. This action may be an attempt to control cell proliferation [24]. In the present study, increase in the TGF-beta1 protein expression was detected *in vivo*, two days

after the use of tamoxifen, suggesting that this initial response may later modulate cell proliferation.

Regarding VEGF, no significant alteration of its immunoexpression was observed two days after tamoxifen treatment (p=0.108), although its immunopositivity was seemingly more intensive. There is a report in the literature on an early increase (24 hours) of the VEGF protein in the serum of patients treated with the drug, although the authors do not comment on the meaning of this finding [1]. Some authors report VEGF increase *in situ* after tamoxifen treatment, that lasts for 3 years, and suggest that the drug is ineffective [5, 12].

In the present study perhaps the apparent and non-significant initial increase is a reaction to the increase of TGF-beta1 expression [3, 27].

Regarding integrin-linked kinase (ILK), its immunolocalization was detected in the cytoplasm of invasive lobular and ductal carcinoma cells before and two days after the use of tamoxifen. After treatment, the immuno-expression of this protein apparently was more intensive, although not significant (p=0.225). In some cell lineages, such as melanoma, there is an increase in ILK immunoreactivity, induced by the TGF-beta1 [14]. In the present study performed *in vivo*, two days after treatment were not enough to detect a statistical alteration of the ILK expression in the breast tumor samples. Possibly this apparent increase could be a reaction to the increase in the TGF-beta1 expression or yet, the beginning of a response, which would be later defined.

The results obtained in this study reveal premature nuclear alterations in the carcinoma cells of invasive lobular and ductal breast carcinoma, two days after tamoxifen treatment with a significant increase in the TGF-beta immunoexpression and significant decrease in the Erb-B2 protein expression. Our results suggest that these parameters could be used to evaluate the early and initial response to tamoxifen, therefore corroborating with the therapeutic optimization of the breast cancer treatment.

References

[1] ADAMS J, CARDER PJ, DOWNEY S, FORBES MA, BANKS E. Vascular endothelial growth factor (VEGF) in breast cancer: Comparison of plasma, serum and tissue VEGF microvessel density and effects of tamoxifen. Cancer Res 2000; 60: 2898–2905.

[2] ATTWELL S, ROSKELLEY C, DEDHAR S. The integrin-linked kinase (ILK) suppresses anoikis. Oncogene 2000; 33: 3811–3815.

[3] BREIER G, BLUM S, PELI J, GROOT M, WILD C et al. Transforming growth factor-beta and Ras regulate the VEGF/VEGF-receptor system during tumor angiogenesis. Int J Cancer 2002; 2: 142–148.

[4] BURSCH W, HOCHEGGER K, TÖRÖK L, MARIAN B, ELLINGER A et al. Autophagic and apoptotic types of programmed cell death exhibit different fates of cytoskeletal filaments. J Cell Science 2000; 113: 1189–1198.

[5] BUTTA A, MACLENNAN K, FLANDERS KC, SPORN MB, BAUM M et al. Induction of transforming growth factor beta in human breast cancer in vivo following tamoxifen treatment. Cancer Res 1992; 52: 4261–4264.

[6] CAMERON DA, RITCHIE AA, MILLER WR. The relative importance of proliferation and cell death in breast cancer growth and response to tamoxifen. Eur J Cancer 2001; 37: 1545–1533.

[7] CHANG J, POWLES TJ, ALLRED DC, ASHLEY SE, MAKRIS A et al. Prediction of clinical outcome from primary tamoxifen by expression of biologic markers in breast cancer patients. Clin Cancer Res 2000; 6: 616–621.

[8] CLARKE RB, LAIDLAW IJ, JONES LJ, HOWELL A, ANDERSON E. Effect of tamoxifen on Ki67 labelling index in human breast tumours and its relationship to oestrogen and progesterone receptor status. Br J Cancer 1993; 67: 606–611.

[9] DIHUA Y, MIEN-CHIE H. Overexpression of ErbB2 in cancer and ErbB2-targeting strategies. Oncogene 2000; 19: 6115–6121.

[10] DOWSETT M., ARCHER C, ASSERSOHN L, GREGORY RK, ELLIS PA et al. Clinical studies of apoptosis and proliferation in breast cancer. Endocrine-Related Cancer 1999; 6: 25–28.

[11] FARCZÁDI E, KASZÁS I, BAKI M. Changes in apoptosis, mitosis, Her-2, p53 and Bcl2 expression in breast carcinomas after short-term tamoxifen treatment. Neoplasma 2002; 49: 101–103.

[12] FOEKENS JA, PETERS HA, GREBENCHTCHIKOV N, LOOK MP, MEIJERVANGELDER ME et al. High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. Cancer Res 2001; 14: 5407–5414.

[13] HEER K, KUMAR H, READ JR, FOX JN, MONSON JR, KERIN MJ. Serum vascular endothelial growth factor in breast cancer: its relation with cancer type and estrogen receptor status. Clin Cancer Res 2001; 11: 3491–3494.

[14] JANJI B, MELCHIOR C, VALLAR L, KIEFFER N. Cloning of a isoform of integrin-linked kinase (ILK) that is upregulated in HT-144 melanoma cells following TGF-beta1 stimulation. Oncogene 2000; 19: 3069–3077.

[15] JORDAN VC. Molecular mechanisms of antiestrogen action in breast cancer. Breast Cancer Res Treat 1994; 31: 41–52.

[16] KUMAR R, YARMAND-BAGHERI R. The role of HER2 in angiogenesis. Semin Oncol 2001; 28 Suppl 16: 27–32.

[17] LAKHANI SR, VAN DE VIJVER MJ, JACQUEMIER J, ANDERSON TJ, OSIN PP et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterona receptor, HER-2, and p53 in patients with mutations in *BRCA1* and *BRCA2*. J Clin Oncol 2002; 20: 2310–2318.

[18] MAJUMDAR SK, VALDELLON JA, BROWN KA. *In vitro* investigation on the toxicity and cell death induced by tamoxifen on two non-breast cancer cell types. J Biomed Biotechnol 2001; 1(3): 99–107.

[19] MAKRIS A, POWLES TJ, DOWSETT M, OSBORNE CK, TROTT PA et al. Prediction of response to neoadjuvant chemoendocrine therapy in primary breast carcinoma. Clin Cancer Res 1977; 3: 593–600.

[20] MARSON LP, KURIAN KM, MILLER WR, DIXON JM. The effect of tamoxifen on breast tumour vascularity. Breast Cancer Res Treat 2001; (1): 9–15.

21] MASSAGUÉ J, BLAIN SW, LO RS. TGF beta signaling in growth control, cancer, and heritable disorders. Cell 2000; 103: 295–309.

[22] MICHAELSON JS, SILVERSTEIN M, WYATT J, WEBWE G, MOORE R et al. Predicting the survival of patients with breast carcinoma using tumor size. Cancer 2002; 95: 713–723.

[23] MICHELS JJ, DUIGOU F, MARNAY J, HENRY-AMAR M, DELOZIER T et al. Flow cytometry and quantitative immunohistochemical study of cell cycle regulation proteins in invasive breast carcinoma. Cancer 2003; 97: 1376–1386.

[24] MIYAZONO K, HELLMAN U, WERNSTEDT C, HELDIN CH. Latent high molecular weight complex of transforming growth factor beta1. J Biol Chem 1988; 263: 6407–6415.

[25] PERRY RR, KANG Y, GREAVES B. Effects of tamoxifen on growth and apoptosis of estrogen-dependent and independent human breast cancer cells. Ann Surg Oncol 1995; 2(3): 238–245.

[26] REISS M. TGF-beta and cancer. Microbes Infect 1999; (1): 1327–1347.

[27] SAITO H, TSUJITANI S, OKA S, KONDO A, IKEGUCHI M et al. The expression of transforming growth factor beta1 is significantly correlated with the expression of vascular endothelial growth factor and poor prognosis of patients with advanced gastric carcinoma. Cancer 1999; 86: 1455–1462.

[28] SHAO-CHUN W, KEISHI M, WEIYA X, JEONG SK, SEOCK-AH I et al. Doc-2/hDab-2 inhibits ILK activity and induces anoikis in breast cancer cells through na Akt-independent pathway. Oncogene 2001; 20(47): 6960–6964.

[29] SLAMON DJ, DEKERNION JB, VERMA IM. Expression of cellular oncogenes in human malignancies. Science 1984; 224(4646): 256–262.

[30] SLEDGE GW. Implications of the new biology for therapy in beast cancer. Semion Oncol 1996; 23 Suppl 2: 76–81.

[31] VAN DE VIJVER MJ. Assessment of the need and appropriate method for testing for the human epidermal growth factor receptor-2 (HER2). Eur J Cancer 2001; 37 Suppl 1: 11–17.

[32] WHITE DE, CARDIFF RD, DEDHAR S, MULLER WJ. Mammary epithelial-specific expression of the integrin linked kinase (ILK) results in the induction of mammary gland hyperplasias and tumor in transgenic mice. Oncogene 2001; 20(48): 7064–72.

[33] WIESER R. The transforming growth factor-beta signaling pathway in tumorogenesis. Curr Opin Oncol 2001; 13(1): 70–77.

[34] WU Y, SALDANA L, CHILLAR R. Plasma endotelial growth factor is useful in assessing progression of breast cancer post surgery and during adjuvant treatment. Int J Oncol 2002; 20(3): 509–516.