IMMUNOHISTOCHEMICAL EXPRESSION OF TISSUE INHIBITOR OF METALLOPROTEINASE-1 (TIMP-1) IN INVASIVE BREAST CARCINOMA

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Abstract

Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a natural inhibitor of matrix metalloproteinases (MMPs). Aim of this study was to assess the immunohistochemical expression of TIMP-1 in invasive breast carcinomas, and to examine its association with classical clinico-pathological parameters, oestrogen receptor, progesterone receptor and Her-2/neu protein expression. Immunohistochemistry was used to determine the expression of TIMP-1 on 38 paraffin-embedded breast tissue specimens - 18 with invasive ductal carcinoma, 10 with invasive lobular carcinoma, and 10 specimens from patients with fibrocystic breast disease. TIMP-1 protein was immunodetected in the carcinoma cells, fibroblasts and inflammatory cells of the stroma in 92,9%, 65,8%, and 65,8% of cases, respectively. TIMP-1 protein expression in carcinoma cells showed positive correlation with TIMP-1 protein expression in peritumoural fibroblasts (p=0,010). Positive peritumoural fibroblast TIMP-1 expression was associated with histological tumour type with higher frequency in ductal carcinomas (p=0,023). Negative association was found between TIMP-1 protein expression in carcinoma cells and HER-2/neu nuclear staining (p=0,005). TIMP-1 may be particularly useful as a predictive marker in breast carcinoma when evaluated along with HER-2/neu protein being a promising indicator of favourable prognosis in breast carcinoma.

KEY WORDS: breast carcinoma, tissue inhibitor of metalloproteinase-1, immunohistochemistry

Introduction

Matrix metalloproteinases (MMPs) belong to a family of zinc-dependent extracellular endopeptidase capable of degrading most components of the extracellular matrix (1). MMPs are proteolytic enzymes known to play an important role in tumour development, in tumour cell survival (2), and in cancer dissemination (3). Disruption of the balance between MMPs and TIMPs may influence invasion and metastasis of cancer and may thus modify patient outcome (4). Being an MMP inhibitor, TIMP-1 is capable of protecting against excessive degradation of the extracellular matrix consequently rendering the dissemination of cancer cells difficult (5). High levels of TIMP-1 mRNA, as well as, TIMP-1 protein have been demonstrated in several types of cancer, including breast cancer. This has been associated with a poor prognosis of the patients (6, 7). TIMP-1 is a multifunctional protein, which in addition to the MMP-inhibitory effect has distinct tumour-promoting functions. Proposed functions include growth promotion, anti-apoptotic effects, anti-angiogenic and pro-angiogenic functions (3). The aim of our study was to determine immunohistochemically the presence and cellular location, as well as, expression of TIMP-1 in 38 samples of breast tissue using serial sections.

Materials and Methods

Patients and tissue specimens

Complete data and tissue specimens were obtained from 28 breast carcinoma patients, who underwent surgery at University of Sarajevo Clinics Centre between 2004 and 2005 and were analysed at the Institute of Pathology, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina. All patients had a histological confirmed diagnosis of primary breast carcinoma. The patients were age from 37 to 72 years (median age being 55,5 years). None of them received radiation or chemotherapy preoperatively. Axillary lymph node dissection was performed on all of the patients. Patients with distant metastases at the time of diagnosis were excluded from the study. 10 breast tissue samples from patients with fibrocystic breast disease were also analysed. The clinico-pathological data of the patients are summarized in Table 4.

Immunohistochemistry

Immunohistochemical study was performed using monoclonal mouse anti-human TIMP-1 antibody (Clone VT7; DAKO, Denmark). Four-μm thick sections of 10% buffered neutral formalin-fixed, paraffin-embedded breast tissue were cut, mounted on capillary gap microscope slides (DAKO, REAL™, Denmark), and heated overnight at 60°C. The sections were deparaffinised in xylene (Semikem, Sarajevo, Bosnia and Herzegovina), and rehydrated in a decreasing series of Ethanol absolute (Kemika, Zagreb, Croatia). Endogenous peroxidase activity was blocked by incubating the sections with 0,3% hydrogen peroxide (Alcaloid, Skopje, Macedonia) in methanol (Kemika, Zagreb, Croatia) for 10 minutes at room temperature. For staining with anti-TIMP-1, sections were pre-treated with Tris-EDTA buffer (pH 9,0) at 800 W in a microwave oven for 10 min. After washing with phosphate-buffered saline (PBS) (pH 7,2), sections were incubated for 30 minutes with monoclonal antibody (DAKO/TIMP-1/Clone VT7; dilution 1:50; Dako, Denmark). The sections were then washed 3 times in PBS (2 minutes each). A two-step technique (EnVision; Dako, Glostrup, Denmark) was used for visualization, with diaminobenzidine (DAB) as a chromogen (DAB Chromogen, Dako, Glostrup, Denmark). Finally, after three washes in distilled water (5 minutes each) sections were counterstained with Mayer haematoxylin and mounted. A single block of normal human adrenal gland (positive staining of chromaffin cells) and pancreas tissue (positive staining of endocrine cells in the islet of Langerhans) was used as a positive control (8), and was included in each immunostaining run to provide quality control.

Evaluation

All samples were evaluated for immunoreactivity of TIMP-1 protein expression and scored semi-quantitatively by two independent pathologists (KS and RS), who were both blinded to patients clinico-pathological parameters. Immunohistochemical analysis of TIMP-1 expression has been examined in nucleus and cytoplasm of tumour cells and stromal cells, as well as in inflammatory cells. Both, the intensity and extent of IHC were assessed and quantified under light microscope Olympus BX40. In each case, 20 optical fields of high magnification (x400) were measured and 300 cells were counted. Staining intensity was rated in the following manner: 0 point - no staining; 1 point - weak intensity; 2 points - moderate intensity; 3 points - strong intensity. The percentage of positively stained cells was classified into groups: 0 points - 0% to 5%; 1 point - 6% to 25%; 2 points - 26% to 50%; 3 points - 51% to 75%; 4 points - 76% to 100%. The staining index (SI) is a product of the intensity and the percentage of positive staining. SI was used to define negative (SI 0-2; ≤ 2) and positive (SI 3-7; > 2) expression of TIMP-1, as well as to define

low (SI < 5) or high (SI ≥ 5) expression of TIMP-1 with cut-off point at 5. The criterion of Herceptest/Pathway system (Dako, Glostrup, Denmark) was followed to score HER-2/neu. Cases with strong complete membranous staining in more than 10% of the tumour cells were considered strongly positive (+3). Cases with weak to moderate complete membranous staining in more than 10% of the tumour cells were considered moderately positive (+2) and cases with little or no membranous staining were considered negative (0 or +1). ER and PR values were categorized using H Score test.

Statistical analysis

All statistical analyses were conducted using the statistical program SPSS 13.0 for windows (SPSS, Inc. Chicago, IL, USA). Relationships and correlations between TIMP-1 protein expression and clinico-pathological parameters were analysed using Pearson Chi-square (χ^2) test and Fisher's exact test. The same method was used to test the associations of TIMP-1 protein with ER, PR, and HER-2/neu, respectively. p values of less than 0,05 were considered statistically significant.

Results

We explored potential interaction of TIMP-1 expression in cytoplasm and in cell nucleus of the both invasive breast carcinoma and fibrocystic breast disease. Percentage of TIMP-1 protein positive cells was significantly higher in cancer cells comparing to fibrocystic breast disease cells (p=0,0001). Intensity of positive staining showed no significant association (p=0,084), with moderate and strong intensity in cancer cells (50,0% and 28,6% specimens, respectively), and moderate and weak intensity in fibrocystic breast disease cells (both 50%) (Data not shown). Evaluating TIMP-1 protein expression we found difference in dependence of quantification method. Significant association between breast carcinoma and fibrocystic breast disease was found when using cut-off value (≥5) with strong intensity of TIMP-1 staining in most of the cells (p=0,002) (Table 1.), although no association was observed using negative-positive TIMP-1 immunodetection (≤ 2, > 2, respectively) (Table 2.).

		N° (%) of patients				
		Carcinoma		Fibrocystic breast disease		*p*
		N°	%	N°	%	
TIMP-1 protein expression*	Low	9	32,1	9	90,0	0,002
	High	19	67,9	1	10,0	

*TIMP-1 score ≥5

TABLE 1. TIMP-1 protein expression in breast carcinoma and fibrocystic breast disease epithelium

		N° (%) of patients				
		Carcinoma		Fibrocystic breast disease		*p*
		N°	%	N°	%	
TIMP-1 protein expression*	Negative	2	7,1	1	10,0	0,774
	Positive	26	92,9	9	90,0	

*TIMP-1 score > 2

TABLE 2. TIMP-1 protein expression in breast carcinoma and fibrocystic breast disease epithelium

TIMP-1 protein was immunodetected in the carcinoma cells, fibroblasts and inflammatory cells of the stroma in 92,9%, 65,8%, and 65,8% of cases, respectively. Approximately equal TIMP-1 protein expression was observed in fibroblasts of invasive breast carcinoma and fibrocystic breast disease specimens (80% and 75%, respectively) (Data not shown).

		TIMP-1 protein expression in peritumoural stromal cells					
		Negative*		Positive†		*p*	*R*
		N°	%	N°	%		
TIMP-1 protein expression in carcinoma cells	Negative	2	28,6	0	0,0	0,010	0,480
	Positive	5	71,4	21	100,0		

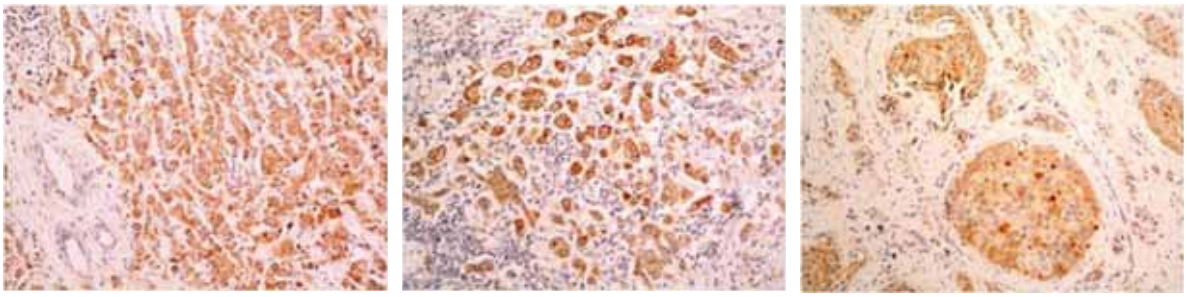
*TIMP-1 negative immunostaining ≤ 2
†TIMP-1 positive immunostaining > 2

TABLE 3. Correlation of TIMP-1 protein expression in carcinoma cells and peritumoural fibroblasts within invasive breast carcinoma specimens

TIMP-1 protein expression showed positive correlation between carcinoma cells and peritumoural fibroblasts (p=0,010) in invasive breast carcinoma specimens. TIMP-1 protein expression was detected in malignant cells and in peritumoural fibroblasts in 26 and 21 of cases, respectively. 21 out of 26 specimens with positive/high tumour expression of TIMP-1 demonstrated positive expression of TIMP-1 in peritumoural fibroblasts. Consistently, 2 out of 2 specimens with negative, relatively low levels of tumour expression of TIMP-1 were accompanied by peritumoural fibroblasts that expressed TIMP-1 at low levels (Table 3.).

TIMP-1 protein expression was detected mainly in cytoplasm of malignant cells (Figure 1.).

Negative association was found between TIMP-1 expression in carcinoma cells and HER-2/neu nuclear staining (p=0,005), as well as, positive association between peritumoural fibroblasts and histological tumour type (Table 4.).



a) b) c)

FIGURE 1. TIMP-1 protein expression in breast carcinoma. The micrographs show TIMP-1 in carinoma cells cytoplasm of lobular carcinoma (a, b), and ductal carcinoma (c). (Immunohistochemical staining, original staining, original magnification x 200)

	TIMP – 1 expression*							
	Carcinoma cells				Peritumoural fibroblasts			
Parameters	Total	N°	%	*p* value	Total	N°	%	*p* value
Age								
<50	6	4	15,4	0,005 (†)	6	4	19,0	0,595 (†)
>50	22	22	84,6	0,006 (‡)	22	17	81,0	0,602 (‡)
Tumour size (Pt)								
< 2 cm	13	12	42,6	0,916 (†)	13	8	38,1	0,126 (†)
2-5 cm	15	14	53,8	0,918 (‡)	15	13	61,9	0,133 (‡)
> 5 cm	0	0,0						
Histological tumour type								
Ductal	18	16	61,5	0,274 (†)	18	11	52,4	0,023 (†)
Lobular	10	10	38,5	0,283 (‡)	10	10	47.6	0,025 (‡)
Tumour grade								
Grade I	7	6	23,1	0,534 (†)	7	5	23,8	0,090 (†)
Grade II	12	11	42,3	0,274(‡)	12	7	33,3	0,155 (‡)
Grade III	9	9	34,6		9	9	42,9	
Lymphovascular tumour invasion								
Present	12	12	46,2	0,204 (†)	12	9	42,9	1,000 (†)
Absent	16	14	53,8	0,212 (‡)	16	12	57,1	1,000 (‡)
Axillary lymph node invasion								
Present	4	4	15,4	0,549 (†)	4	4	19,0	0,212 (†)
Absent	24	22	84,6	0,556 (‡)	24	17	81,0	0,221 (‡)
Oestrogen status								
Negative	7	6	23,1	0,642 (†)	7	5	23,8	0,987 (†)
Low positive	9	8	30,8	0,230 (‡)	9	7	33,3	1,000 (‡)
Moderate positive	5	5	19,2		5	4	19,0	
Strong positive	7	7	26,9		7	5	23,8	
Progesterone status								
Negative	9	7	26,9	0,208 (†)	9	7	33,3	0,829 (†)
Low positive	10	10	38,5	0,118 (‡)	10	7	33,3	0,926 (‡)
Moderate positive	2	2	7,7		2	2	9,5	
Strong positive	7	7	26,9		7	5	23,8	
HER-2 status								
Negative (0)	18	18	69,2	0,005 (†)	18	14	66,7	0,060 (†)
Negative (1+)	5	5	19,2	0,002 (‡)	5	5	23,8	0,089 (‡)
Moderate positive (2+)	1	1	3,8		1	1	4,8	
Strong positive (3+)	4	2	7,7		4	1	4,8	

* TIMP-1 score > 2
† p value (Pearson chi-square test)
‡ p value (Fisher's exact test)

TABLE 4. TIMP-1 protein expression according to clinico-pathological parameters of breast carcinoma

Discussion

Several studies have demonstrated that the expression of TIMP-1 is enhanced in breast carcinoma compared with benign or normal breast tissue (9, 6). McCarthy et al. (5) found that concentrations of TIMP-1 are significantly higher in breast carcinomas than in fibroadenomas. In accordance with these we observed that percentage of TIMP-1 protein expression significantly was higher in cancer cells than in fibrocystic breast disease cells (p=0,0001). However, the intensity of their positive staining showed no significant association (p=0,084). In our study there was no significant interaction between TIMP-1 expression in invasive breast carcinoma and fibrocystic breast disease using negative-positive TIMP-1 immunodetection (≤ 2; > 2) (Table 2.). We made an attempt of identifying a cut point (≥5) for dividing tumours into TIMP-1-low and TIMP-1 -high ones. Using a cut-off point to evaluate TIMP-1 protein expression in cytoplasm of invasive breast carcinoma and fibrocystic breast disease we noticed significant association (p=0,002), with strong intensity of TIMP-1 staining in most of the cells. TIMP-1 protein was immunodetected in the carcinoma cells, fibroblasts and inflammatory cells of the stroma. We also found positive correlation between TIMP-1 protein expression in the cytoplasm of carcinoma cells and peritumoural fibroblasts (p=0,010). A number of studies have demonstrated the expression of TIMPs in tumour stroma and tumour tissues (2, 10), with TIMP-1 protein immunostaining predominantly localised in tumour cells (11). It is known that in addition to their production by epithelial cells TIMPs gene expression may be induced in stromal fibroblasts and inflammatory cells that infiltrate tumour around them (6). Although tumour cells are the ones that pass through multiple tissue barriers to metastasize, increasing evidence demonstrates a critical role of stromal cells in tumour microenvironments (12). It has been suggested that the tumour stroma does not play a passive role in cancer progression, and it may in fact actively participate in the process of cancer invasion (7). There is convincing evidence that overexpression of TIMPs by cancer cells or by the host reduces the invasive and metastatic capacity of tumour cells (2).

Examining any possible statistical association between TIMP-1 expression and clinico-pathological parameters we found association between peritumoural fibroblasts and histological tumour type with TIMP-1 expression frequently observed in ductal carcinomas (p=0,023). A strong inverse correlation and association was observed between TIMP-1 protein expression and HER-2/neu expression in breast carcinoma samples (p=0,001, p=0,005, respectively). Most widely accepted prognostic biomarkers for breast carcinoma are hormone receptor (ER and/or PR) and oncoprotein HER-2/neu. Positive HER-2 protein expression usually is related with aggressive tumours having high histological grade and adverse outcome. In our study, majority of carcinomas with TIMP-1 protein positive expression were HER-2/neu protein negative. The findings suggest that the breast tumours with high TIMP-1 expression might show less malignant potential than those with low TIMP-1 expression. Therefore, it could be speculated that TIMP-1 protein expression is associated with favourable outcome. Currently, it is not known whether TIMP-1 is regulated by HER-2/neu. To this end, it could be suggested that overexpression of TIMP-1 may be particularly useful as a predictive marker in breast carcinoma when evaluated along with HER-2/neu protein being a promising indicator of favourable prognosis in breast carcinoma. However, to use this information in a clinical setting, a large confirmatory study must be executed. Also several aspects of methodological and statistical importance should be addressed, including identification of an appropriate cut-off value for separation of patients (7).

Conclusion

Positive correlation between TIMP-1 protein expression in carcinoma cells and peritumoural fibroblasts in breast carcinoma suggests an active role in process of cancer invasion. TIMP-1 protein expression in carcinoma cells inversely correlate with expression of HER-2/neu protein, suggesting that favourable prognosis in breast carcinoma may be associated with overexpression of TIMP-1.

List of Abbreviations

TIMP-1	-	tissue inhibitor of matrix metalloproteinase-1
MMPs	-	matrix metalloproteinases
ER	-	oestrogen receptor
PR	-	progesterone receptor
IHC	-	immunohistochemistry
SI	-	staining index

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