Histol Histopathol (2008) 23: 187-196

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Thymidine phoshorylase expression in breast cancer: the prognostic significance and its association with other angiogenesis related proteins and extracellular matrix components

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Summary. Thymidine phosphorylase (TP)/platelet-derived endothelial cell growth factor, stimulates chemataxis of endothelial cells and is involved in the angiogenesis of human solid tumours. In this study we investigated tissue sections from 93 breast carcinomas for the immunohistochemical expression of thymidine phosphorylase protein and in relationship to several clinicopathological parameters. The possible relationship to tumour neovascularization, VEGF expression, extracellular matrix components (tenascin, fibronectin, collagen type IV and laminin) and cathepsin D was also estimated. Nuclear and/or cytoplasmic TP expression was observed in tumour cells. Immunoreactivity was also often present in the stroma, endothelium and tumour-associated macrophages. High cytoplasmic TP expression, was observed in 35.5%, moderate in 30.1%, mild in 18.3%, while 16.1% of the cases were negative for TP expression. Moderate and high nuclear TP expression was observed in 30.1% of the tumours, low in 43%, while 26.9% did not show nuclear TP expression. High tumour stroma TP expression was expressed in 23.7% of the cases, moderate in 21.5%, mild in 45.2%, while 9.7% did not show stromal TP expression.

TP expression did not correlate with the conventional clinicopathological features as well as with the microvessel density and the VEGF expression. Patients with high levels of tumour cell TP expression were significantly associated with a favorable outcome in univariate method of analysis. A positive correlation of TP expression with Cathepsin D expression was noticed. In addition, tumour cell TP expression was correlated with the extracellular matrix component tenascin, while stromal cell TP expression was correlated with the growth fraction of the tumour.

Our data suggests that TP expression does not seem to affect directly the neovasculatur of breast carcinoma, although it seems to be implicated in the remodeling of breast cancer tissue, through the interaction with other extracellular matrix components or proteolytic enzymes. In addition, tumour cell TP expression could be considered as a prognostic indicator of breast cancer patients.

Key words: Thymidine phosphorylase, Cathepsin D, Extracellular matrix, Immunohistochemistry, Breast cancer

Introduction

Angiogenesis is a complex multistep dynamic process involving extravasation of plasma proteins, degradation of extracellular matrix, endothelial cell migration and proliferation and capillary tube formation. It is now well established that angiogenesis is orchestrated by a variety of activators and inhibitors that coordinate sequentially the complex series of new vessel growth. Recent advances in vascular biology have identified some of the key factors that control vascular growth including the intracellular enzyme thymidine phosphorylase. Several authors have demonstrated that TP is identical to platelet-derived endothelial cell growth factor (PD-ECGF) (Ishikawa et al., 1989). PD-ECGF

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Abbreviations. TP: Thymidine phosphorylase. TPtc: cytoplasmic tumour cells TP expression. TPtn: nuclear tumour cells TP expression. TPstr: stromal TP expression. MVD: microvessel density. VEGF: vascular endothelial growth factor. CD: cathepsin D. ECM : extracellular matrix. TN: tenascin. FN: fibronectin. Coll : collagen type IV. LN: laminin. PCNA : proliferative cell nuclear antigen. CA 15-3 : Carbohydrate antigen 15-3

thymidine phosphorylase was initially isolated from platelets as a non-heparin-binding angiogenic growth factor (Miyazono et al., 1987; Moghaddam et al., 1995). TP is capable of stimulating new vessel formation because the main metabolite of thymidine, 2-deoxyribose-1 phosphatase has chemotactic activity in vitro and angiogenic activity in vivo (Moghaddam et al., 1995). The mechanism by which PD-ECGF/TP induces angiogenesis is not fully elicited. Compared with adjacent normal tissues, TP activity has been reported to be increased in a variety of malignant tumours (Yoshimura et al., 1990; Takebayashi et al., 1996) including breast carcinoma, where it is expressed by both epithelial and stromal cells (Toi et al., 1995; Fox et al., 1996; Engels et al., 1997; Nagaoka et al., 1998; Yonenaga et al., 1998; Yang et al., 2002). Several studies have produced conflicting results as regards the angiogenic role of TP expression. Some studies have suggested that TP is angiogenic in human solid carcinomas (Takebayashi et al., 1996a,b; Imazano et al., 1997; Fukuiwa et al., 1999) including breast carcinoma (Toi et al., 1995, Nagaoka et al., 1998), while others have found that TP has little effect on tumour angiogenesis of invasive breast carcinoma (Engels et al., 1997; Lee et al., 1999; Yang et al., 2002). Stromal components also play a critical and often under appreciated role in the formation of vascular stroma through the regulation of functions such as cell adhesion, migration and gene expression, by controlling the availability of growth factors (Iozzo, 1995).

The objective of the present study was to demonstrate immunohistochemically the patterns of tumour cell TP expression (cytoplasmic-TPtc and nuclear-TPtn expression) and stromal TP expression (TPstr) in breast cancer tissues to define its prognostic significance, and to establish any relationship with clinicopathological features such as: tumour type, histological grade, lymph node involvement, hormone receptor status, proliferative activity and p53 protein expression. The alterations identified have been further correlated with tissue neovascularization (microvessel density-MVD), assessed by CD34, vascular endothelial growth factor (VEGF) expression, as well as with the expression of the extracellular matrix (ECM) components (tenascin-TN, fibronectin-FN, collagen type IV-Coll, laminin-LN), tumour and stromal cathepsin D (CD) in order to elucidate their interrelationships and the possible role of this protein in tumour stroma remodeling and angiogenesis.

Materials and methods

Patients and study design

A group of 93 patients with primary invasive breast carcinoma treated by surgical resection were investigated. For 67 patients we had complete follow up data and this number of patients was included in survival analysis. All the patients gave a verbal informed consent for surgical therapy consisting of mastectomy with axillary lymph node dissection performed as indicated and were followed up regularly at the Medical Oncology Department of University Hospital of Ioannina. Detailed clinical data was available for 90 patients: 22 had stage I disease, 50 patients stage II disease (pT1N1M0, pT2N0M0 and pT2N1M0) and 21 patients stage III (pT2N2M0, pT3N1M0 and pT3N2M0 or pT4N1M0). They were clinically disease free and had baseline CA15-3 serum levels below 30U/ml at the initiation of adjuvant therapy. Adjuvant therapies were administered according to standard guidelines and consisted of tamoxifen (36 patients), chemotherapy followed by tamoxifen (29 patients) and conventional chemotherapy, including 5-FU (25 patients). After a median follow-up of 4 years (range 6-132 months) in 41.8% of the patients the disease had progressed, and 32 of them had developed distant metastases.

Archived material was used from formalin fixed and paraffin embedded breast carcinoma tissue, including adjacent non neoplastic tissue or fibrocystic disease. Each specimen was examined histologically on hematoxyline-eosin (H&E) stained slides. Tumour size varied from 1 to 17 cm (mean=3.95cm). Tumour histiotype, lymph node status and patient age was recorded for each patient. Tumour grade was assessed on H&E stained sections by personnel blinded to the results of immunohistochemistry. Tubule formation, nuclear morphology and mitotic rate were evaluated and scored in the neoplastic cells according to the Elston and Ellis modification of the Bloom and Richardson system: grade 1, grade 2 and grade 3 corresponding to well, moderately and poorly differentiated invasive carcinoma of the breast (Elston and Ellis, 1991). Some patients characteristics are summarized in Table 1. Data for Ki-67, proliferating cell nuclear antigen (PCNA), p53, CD and extracellular matrix components were used from our other published studies (Ioachim et al., 1998, 1999, 2002).

Immunohistochemistry

Immunostainings were performed on formalin-fixed, paraffin-embedded tissue sections by the labelled streptavidin avidin biotin (LSAB) method. Tissue sections were cut (2-4 µm thick) placed on poly-L-lysine-coated glass slides. In brief, tissue sections were deparaffinized in xylene and dehydrated. For the detection of thymidine phosphorylase, VEGF, p53 and Ki-67 slides were immersed in citrate buffer (0.1m, pH 0.6) in plastic Coplin jars and subjected to microwave irradiation twice for 15 minutes. For the detection of TN we used the isoform TN2 (Table 1). TN , FN, Coll and LN, slides were pretreated with 1 µl/ml pronase (DAKO) for 10 minutes at room temperature. Subsequently, all sections were treated for 30 min with 0.3% hydrogen peroxide in methanol to quench endogenous peroxidase activity and then incubated with primary antibodies. Mouse primary monoclonal antibodies were incubated on tissue sections overnight at 4°C, then extensively washed in 0.05 M Tris-buffered

saline (pH 7.6), before the addition of biotinylated secondary biotinylated secondary Abs (goat anti-mouse). Sections were again washed and incubated with horseradish peroxidase-conjugated streptavidin (Dako, 1:100) and the immunoreactivity was revealed by diaminobenzidine (DAB) substrate. The slides were counterstained in Harris' haematoxylin, dehydrated and mounted. To assess the specificity of the reaction, negative controls were included, where tumour sections were not incubated with the primary antibody. The antibody sources and dilutions are shown in Table 2.

Immunohistochemical evaluation

The evaluation of immunostaining TP and CD was performed separately in both the parenchyma and stromal cells. To evaluate the expression of tumour cells thymidine phosphorylase, VEGF and cathepsin D proteins, we established a combined score, based on a previous study (Soini et al., 2001) corresponding to the sum of both (a) staining intensity (0: negative, 1: low, 2: intermediate, 3: strong, 4: very strong staining) and (b) the staining extensive, percentage of positive cells (0: 0%, 1: 1-25%, 2: 26-50%, 3: 50-75%, 4: >75%). The sum of both qualitative and quantitative immunostaining reached a maximum score of 8. The combined scores were then divided into 4 main groups: (-), no immunostaining, score 0; (1), low immunostaining, scores 1-2; (2), moderate immunostaining, scores 3-4; (3), strong immunostaining, scores 5-8.

As regards the evaluation of extracellular matrix components, the tumours were classified as `positive' when there was unequivocal immunostaining of the matrix components in at least one representative area of the tumour. The positive tumours were semi-quantitatively scored as +, ++, and +++ corresponding to weak, moderate and extensive immunoreactivity respectively. The cut-off points for immunohistochemical evaluation for each protein studied were based on the reports in literature (Poller et al., 1993) and on our own experience. The immunostaining was assessed from numerically coded slides without any knowledge of survival or other clinical data.

Table 1. Histopathalogical characteristics of breast cancer.

Type	
ductal	88%
lobular	12%
Grade	
G1	13.3%
G2	49.1%
G3	37.6%
Size	
<2 cm	15.8%
2-5 cm	60.8%
>5 cm	23.4%
Lymph node involvement	
negative	22.7%
positive	77.3%

Microvessel count

The criteria that we used for microvessel recognition were the same as used in previous studies. Briefly, as microvessels we considered individual or clusters of cells with or without lumens, positively stained by anti-CD34. The lumen diameter had to be smaller than approximately eight red blood cells. Areas of fibrosis, necrosis and inflammation, and vessels with muscle wall were excluded from counting. In each tumour, the five areas with the highest vascularization ("hot spot") were selected. Individual microvessel counts were then made on a 400x field (40x objective and 10x ocular, corresponding to an area of 0.63 mm^2) by two independent observers. The average count from the two observers was used as the final score. Microvessel density was labeled using anti-CD34 antibody by immunostaining according to a recent study (Zheng et al., 2007).

Statistics

Superior Performance Software System (SPSS) software 10.0 for windows (SPSS Inc., 1989-1999) was used by the authors to compare morphological features and protein expression data. Significant differences between the expression of the target proteins with regard to clinicopathological parameters were computed by the t-test for paired or no paired values or ANOVA test. Correlation between the other cell cycle related proteins was computed using the Pearson's or the Kendall's Tau rank correlation coefficient. Survival curves were displayed by the Kaplan-Meier method, and the effect of covariates on lifetime and recurrence was tested by Cox PH regression analysis. P-values <0.05 were considered statistically significant.

Results

Thymidine phosphorylase expression was detected in the nucleus and/or in the cytoplasm of tumour cells (Fig. 1-4). Immunoreactivity was also often present in

Table 2. Antibodies used.

Antibodies	Supplier	Dilution	Incubation time
PD-ECGF/TP(P-GF.44C)*	Neomarker	1:800	Overnight
VEGF(JH121)*	Neomarker	1:50	1 hour
CD34 (QBEnd/10)	Novo Castra	1:50	1 hour
Tenascin (TN2)#	Dako	1:50	1 hour
Fibronectin (clone, 568)#	Novo Castra	1:100	1 hour
Collagen IV (clone, CIV22)#	Dako	1:50	1 hour
Laminin (An No 078P)#	Menarin	1:1000	1 hour
Cathepsin D (013A)	Dako	1:300	1 hour
p53 (DO-7)*	Dako	1:50	1 hour
Ki-67*	Dako	1:10	1 hour
ER (M7047)	Dako	1:50	1 hour
PgR (M3569)	Dako	1:75	1 hour

*: with microwave oven antigen retrieval; #: incubation with pronase.

stromal, inflammatory and endothelial cell elements. We assessed the TP expression separately in cancer cells and stromal cells.

High cytoplasmic TP expression, was observed in 33/93 (35.5%), moderate in 28/93 (30.1%), mild in 17/93(18.3%), while 15/93 (16.1%) of the cases were negative for TP expression. Moderate and high nuclear TP expression was observed in 28/93 (30.1%) of the tumours, low in 40/93 (43%) while 25/93 (26.9%) did not show nuclear TP expression. High tumour stroma TP expression was expressed in 22/93 (23.7%) of the cases, moderate in 20/93 (21,5%), mild in 42/93 (45.2%), while

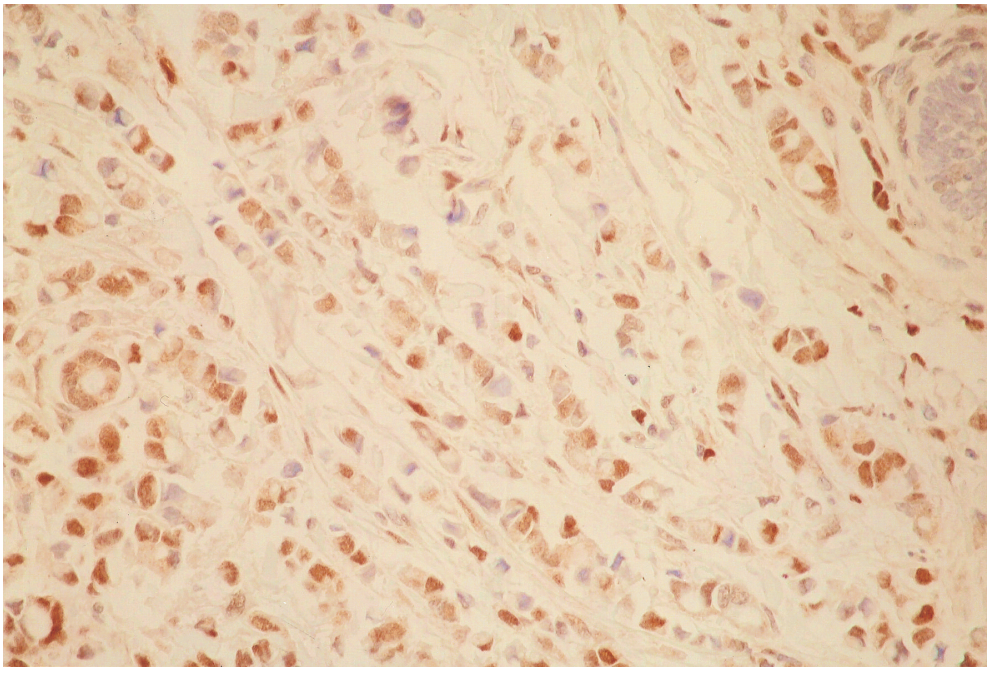

Fig. 1. Nuclear and cytoplasmic TP expression in a case of infiltrating lobular breast carcinoma. (Original magnification LSAB x 200)

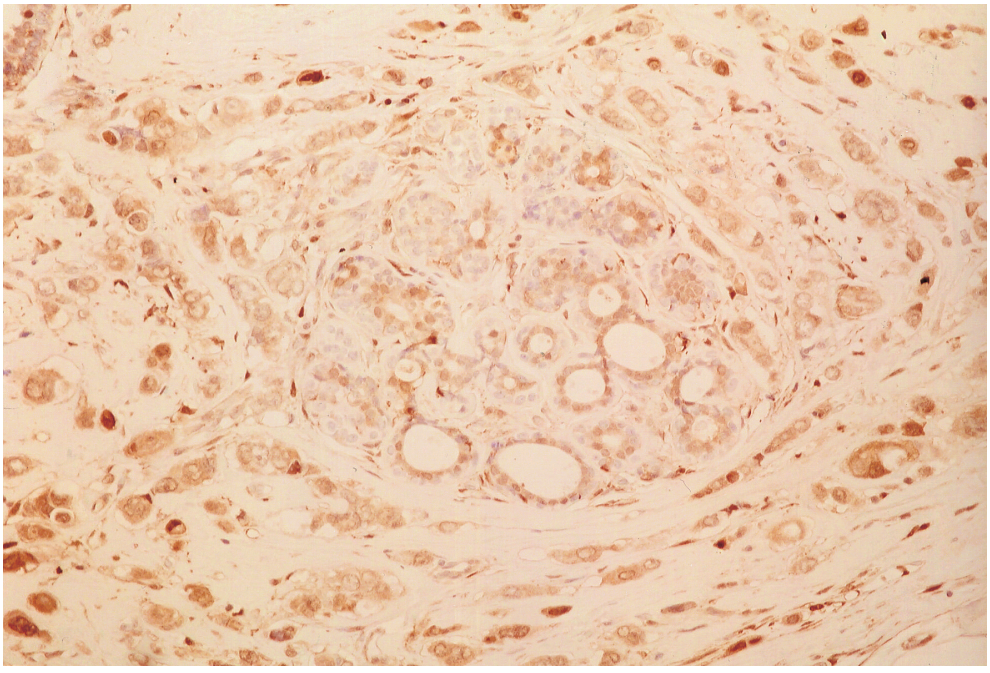

Fig. 2. Nuclear and cytoplasmic TP expression in a case of infiltrating ductal breast carcinoma. (Original magnification LSAB x 200)

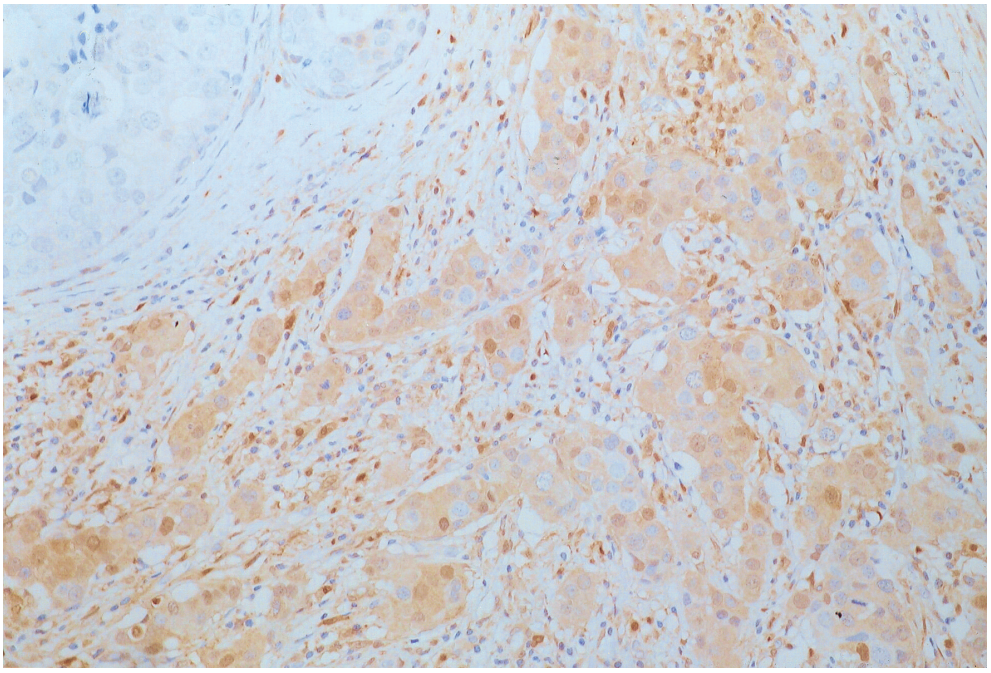

Fig. 3. Nuclear and cytoplasmic TP expression in a case of breast cancer. Stromal cells are also positive. The in situ component is negative for TP expression, upper corner left (Original magnification LSAB x 200).

Table 3. TN expression in correlation with clinicopathological data in breast cancer.

	TP expression						
	TPtc		TPtn		TPstr		
	(-),1	2,3	(-),1	2,3	(-),+	++	p value
Type							
ductal	21	39	24	36	29	31	
lobular	6	8	4	10	10	4	NS, NS, NS
mixed	5	11	6	10	9	7	
Size							
<2cm	1	10	4	7	5	6	
2-5cm	22	32	20	34	30	24	NS, NS, NS
>5cm	8	13	7	14	11	10	
Grade							
1	4	3	1	6	6	1	
2	13	28	18	23	21	20	NS, NS, NS
3	13	25	14	24	18	20	
LN involvement							
negative	10	15	8	17	15	10	NS, NS, NS
positive	18	34	20	32	23	29	
ER							
negative	10	14	10	13	11	13	NS, NS, NS
positive	15	37	16	36	31	31	
PgR							
negative	11	14	13	15	14	14	NS, NS, NS
positive	11	35	13	33	25	21	
Ki-67							
<10%	20	43	21	42	38	24	NS, NS, p=0.044
>10%	12	17	13	16	11	18	
PCNA							
<50%	15	22	11	25	25	12	NS, NS, p=0.014
>50%	17	38	22	32	25	30	
P53							
<5%	16	29	16	29	22	23	NS, NS, NS
>5%	15	31	18	28	27	19	

9/93 (9.7%) did not show stromal TP expression. TP expression did not correlated with hormone receptor status (ER, PgR), p53 expression, MVD and VEGF expression. TP expression did not correlate with the in situ or infiltrating component of the tumour. Stromal cell (macrophages) TP expression was positively correlated

Table 4. Correlation of TP expression with angiogenesis related markers, Cathepsin D and extracellular matrix components in breast cancer tissue.

	TP expression						
	TPtc		TPtn		TPstr		
	(-),1	2,3	(-),1	2,3	(-),+	++	p value
MVD							
low	21	35	24	32	31	25	NS, NS, NS
high	7	13	4	16	9	11	
VEGF							
<50%	2	4	3	3	3	3	NS, NS, NS
>50%	26	52	27	51	41	37	
CDtumour							
<10%	1	4	2	3	2	3	NS, p=0.041, NS
>10%	23	50	20	53	38	35	
CDstromal							
low	17	38	11	44	32	23	NS, p<0.0001,NS
high	7	16	11	11	8	15	
Tenascin							
-,+	9	28	13	24	21	16	P=0.012, NS, NS
++, +++	13	19	13	19	14	18	
Fibronectin							
-,+	14	26	14	26	19	21	NS, NS, NS
++, +++	9	20	12	17	16	13	
Collagen IV							
-,+	15	35	19	31	25	25	NS, NS., NS
++,+++	10	17	10	17	15	12	
Laminin							
-,+	16	43	22	37	33	26	NS, NS, NS
++, +++	8	12	6	14	8	12	

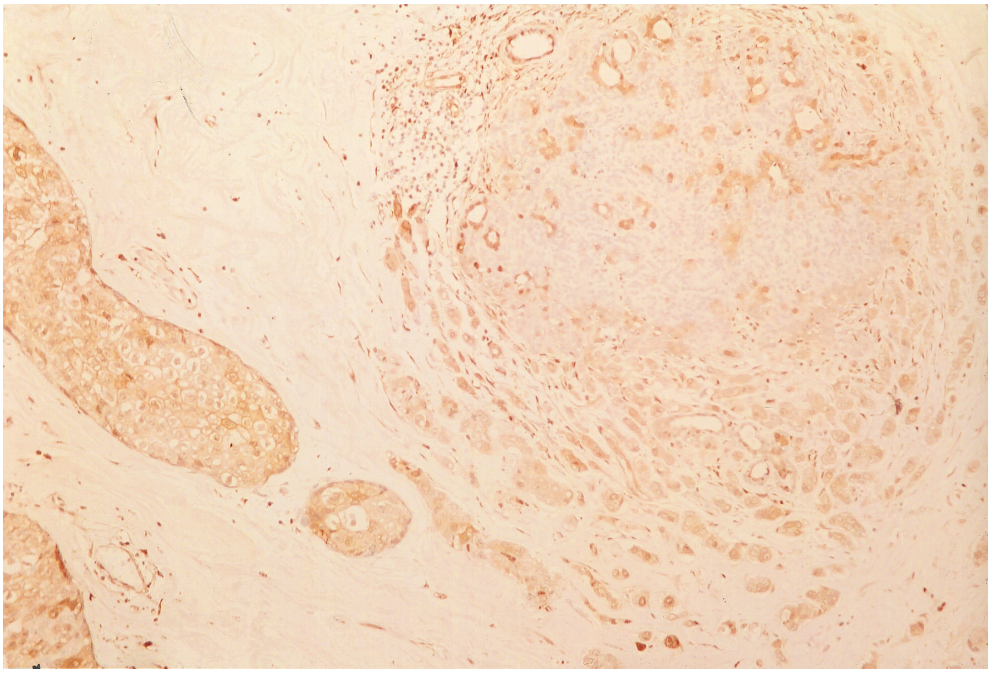

Fig. 4. TP positive cells in infiltrate and in situ component (Original magnification LSAB x 100).

Table 5. Interrelationship of TPtn, TPtn and TPstr in breast cancer tissue.

	TP expression			
	TPtc		TPtn	
TPtn				
0	15	10		
1	12	28		
2,3	5	23		
TPstr				
0	3	6	5	4
+	12	30	23	19
++	7	13	17	3
+++	10	12	20	2

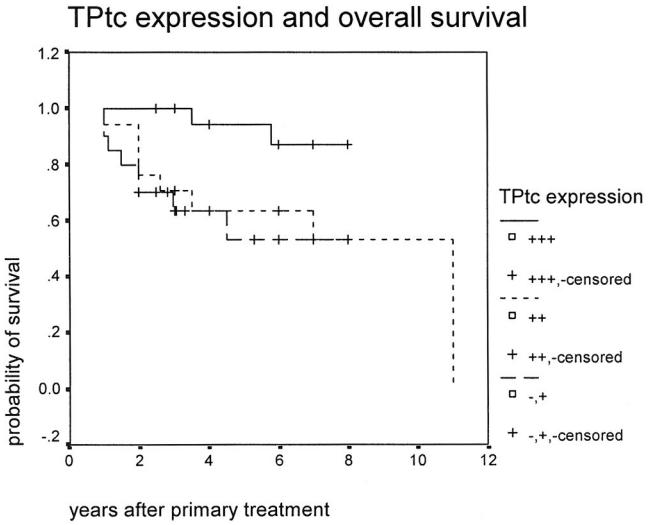

Fig. 5. Kaplan-Meier curves according to TP staining. Patients with higher tumour cells TP expression show a favorable overall survival than patients without or with low tumour cells TP expression (p=0.017).

with both proliferative associated indices (Table 3) Ki-67 and PCNA (p: 0.044 and p: 0.014 respectively). In addition, tumour cell TP expression was inversely correlated with the extracellular matrix component (Table 4) tenascin (p: 0.012) while no correlation with the other extracellular matrix components was found. Patients with higher TP expression had a favorable overall survival (Fig. 5) than patients without or with low tumour cells TP expression in univariate method of analysis (p: 0.017). Cytoplasmic tumour cell TP expression was positively correlated with nuclear tumour cell TP expression (p: 0.003), while stromal TP expression was correlated with nuclear tumour cell TP expression ($p<0.0001$) (Table 5). A strong positive relationship of stromal TP expression with stromal cathepsin D expression (Table 3) was noticed ($p<0.0001$). Nuclear tumour cell TP expression was also positively correlated with tumour cell CD expression (p: 0.041). In addition, VEGF expression did not correlate with microvessel density.

Discussion

The development of new blood vessels within a tumour depends upon the local balance between angiogenic and anti-angiogenic factors. These factors may be produced by the tumour cells themselves or by associated stromal and inflammatory cells. It has been found that thymidine phosphorylase is produced by macrophages and is present in mitochondria of neutrophils and cytoplasmic granules of breast, colorectal and gastric cells. In addition, it is more concentrated in human cancer tissues than in adjacent normal tissue (Kobayashi et al., 2004, 2005). The exact role of its overexpression is not fully elucidated. There are reports that correlate TP expression with some clinicopathological features while others did not find such a correlation according to our results. In particular, it has been shown that tumour cell TP expression was correlated with tumour size (Fox et al., 1996; Yonenaga 1998, Kubiak et al., 1999), histological grade (Fox et al., 1996; Yang et al., 2000, 2002), with lymph node involvement (Kubiak et al., 1999) estrogene receptor status (Relf et al., 1997; Yonenaga et al., 1998) and p53 protein expression (Yang et al., 2002). However, other investigators did not find any correlation of TP expression with tumour size (Toi et al., 1995; Yang et al., 2002, Li et al., 2004), grade of differentiation (Li et al 2004) lymph node involvement (Toi et al., 1995; Yang et al., 2002; Li et al., 2004), hormone receptor status (Toi et al., 1995; Yang et al., 2002) as well as p53 protein expression (Toi et al., 1995). Conflicting data also reported with regard to the prognostic value of TP expression in breast cancer patients. There are studies that show no correlation of TP expression with disease free survival and overall survival in both univariate and multivariate analysis (Engels et al., 1997; Relf et al., 1997; Li et al., 2004). Some investigators found that TP was of prognostic value in univariate analysis for disease free survival or overall survival or both (Fox et al., 1996, 1997; Seymour and Bezwoda, 1994; Engels et al., 1997; Nagaoka et al., 1998; Tominaga et al., 2002). In the present study, we found high levels of tumour cell TP expression to be correlated with a favorable patient outcome in univariate analysis. It has also been found that TP interact with 5-FU pathways, indicating their predictor role for chemotherapeutic response (Lehman 2002; Mizutani et al., 2002; Rose et al., 2002; Anan et al., 2003).

In invasive breast cancer, the relationship of TP expression with vascularity is controversial: Some authors report that no relationship was found between vascularity and TP expression in either carcinoma or the stromal cells in breast cancer (Engels et al., 1997; Kubiak et al., 1999; Lee et al., 1999; Yang et al., 2002, Teo et al., 2003), according to our results, whereas others reported the opposite (Toi et al., 1995; Nagaoka et

al., 1998). In addition, the expression mRNA level of the thymidine phosphorylase gene was correlated with angiogenesis, detected by immunostaining endothelial cells (Kanzaki et al., 2002). These conflincting data may reflect the complexity of the angiogenic process and may also suggest that in breast carcinogenesis TP is important in initial remodeling of the preexisting vascular network but that other factors are needed for extensive induction of vessel growth (Engels et al., 1997). Angiogenesis is mediated by several negative and positive factors, so it is unlikely that a single surrogate marker of angiogenic activity may be representative of all the steps leading to neovascularization of a human neoplasia (Gasparini 1996). Moreover, experimental studies suggest that a synergistic effect among different endothelial growth factors may contribute in promoting angiogenesis (Goto et al., 1993; Koolwijk et al., 1996) and that some angiogenesis inhibitors such as thrombospondin-1, may be regulated by tumour suppressor genes such as p53 (Boock 1996). In the current study we found no association of TP expression with VEGF expression according to the findings of other investigators (Toi et al., 1997) who evaluated TP cytosolic levels using immunometric methods and in contrast to the findings of other studies (Toi et al., 1995; Engels et al., 1997). In addition, we found no association of TP expression with the p53 protein expression in line with the findings of other investigators (Toi et al., 1995). However, other investigators found high TP expression to be correlated with low p53 expression (Yang et al.,2002) in breast cancer. Finally, we found no correlation of TP expression with hormone receptor status according to the findings of other studies (Toi et al., 1995; Yang et al., 2002) in breast cancer. These findings are in contrast to the findings of other investigators who found that TP expression was significantly higher in tumours negative for estrogene receptor status (Nagaoka et al., 1998; Yonenaga et al., 1998).

It is known that TP is chemotactic and non-mitogenic for endothelial cells (Haraguchi et al., 1994; Miyazono et al 1991) and induces angiogenesis in vivo and in wound healing (Moghaddam et al., 1995). In the current study, we found a positive relationship of stromal TP expression with growth fraction of the tumour as estimated with two proliferation associated indices. So, the stromal TP expression seems to have a different role from the tumour cell TP expression according to the findings of other investigators (Nagaoka et al., 1998).

The composition of the ECM may be modified in vivo in several ways and angiogenic factors are known to modulate the synthesis of matrix proteins (Madri et al., 1988; Sutton et al., 1991). Migration of endothelial cells and development of new capillary tubule structure is dependent not only on the cells and cytokines present, but also on the production and organization of ECM components, including fibronectin, collagen, vitronectin, tenascin and laminin. The ECM is critical for normal vessel growth and maintenance, acting as both scaffold support, through which endothelial cells may migrate, and reservoir and modulator for growth factors (Ruoslahti and Yamaguchi, 1991; Feng et al., 1999). Cell culture experiments suggest that TN promotes cell growth by augmenting the mitogenic effect of fibroblast growth factor that is a prerequisite for epidermal growth factor-induced proliferation (Swindle et al., 2001). The role of tenascin in angiogenesis has also investigated. It has been found that TN promotes microvascular migration and phosphorylation of focal adhesion kinase (Zagzag et al., 2002). In addition, perivascular TN showed strong correlation with microvascular density and vascular endothelial growth factor (VEGF) expression in glioblastoma (Behrem et al., 2005). Other investigators found that TN regulates angiogenesis in tumour through the regulation of vascular endothelial growth factor expression Tanaka et al., 2004). In breast cancer, it has been found that TN expression may be associated with endothelial cell activation and may play an important role in tumour angiogenesis (Tokes et al., 1999). In the current study we demonstrated, for the first time to our knowledge, that the TP expression was correlated with the ECM components TN. The results of the present study showed that TP does not correlated with microvessel density or VEGF expression suggesting that it has not a directly affect on breast cancer tissue angiogenesis. On the other hand TP correlated with the extracellular matrix component TN that has been found to play a role in breast tissue angiogenesis. So, TP could be contribute in tumour angiogenesis or in stromal remondeling through the interaction with TN.

Cathepsin D is an aspartyl lysosomal protease which is widely expressed in all cells throughout the body, which has the ability to digest extracellular matrix, including basement membrane components (Briozzo et al., 1988). It also has mitogenic properties (Briozzo et al., 1988) and affects multiple tumour progression steps in vivo: proliferation, angiogenesis and apoptosis (Berchem et al., 2002). There has also been showed to be a statistically significant association between CD expression of host stromal cells and higher vessel count (Gonzalez-Vela et al., 1999) in invasive breast carcinomas. In the present study a positive relationship of TP expression with Cathepsin-D expression was found, confirming the angiogenic affect of Cathepsin-D in breast cancer. It is likely that multiple angiogenic factors are involved in neovascularization of human breast carcinoma and that they may act concurrently (Goto et al., 1993) or sequentially at different times during tumour progression (Gasparini, 1996). In addition, it is presumed that the angiogenic activity of each single tumour may change in time, and may depend upon the net balance of positive and negative regulators (Hanahan and Folkman, 1996).

In conclusion, in the current study, TP expression did not correlate with the conventional clinico-pathological parameters in breast cancer. In addition, our data suggest that TP expression does not seem to affect directly the neovasculatur, although it seems to be implicated in the remodeling of breast cancer tissue through the interaction with extracellular matrix

components or proteolytic enzymes. Furthermore, tumour cell TP expression could be considered as a prognostic favorable indicator of breast cancer patients.

Acknowledments. We offer our thanks to Mrs A Christodoulou for her technical assistance

References

Anan K., Mitsuyama S., Tamae K., Suehara N., Nishihara K., Ogawa Y., Abe Y., Iwashita T. and Toyoshima S. (2003). Increased dihydropyrimidine dehydrogenase activity in breast cancer. J. Surg. Oncol. 82, 174-179.

Behrem S., Zarkovic K., Eskinja N. and Jonjic N. (2005). Distribution pattern of tenascin-C in glioblastoma: correlation with angiogenesis and tumor cell proliferation. Pathol. Oncol. Res. 11, 229-235.

Berchem G., Glondu M., Gleizes M., Brouillet J.P., Vignon F., Garcia M. and Liaudet-Coopman E. (2002). Cathepsin-D affects multiple tumour progression steps in vivo: proliferation, angiogenesis and apoptosis. Ongogene 29, 5951-5955.

Elston C.W. and Ellis I.O. (1991). Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer: experience from a large study with long term follow up. Histopathology 19, 403-410.

Engels K., Fox S.B., Whitehouse R.M., Gatter K.C. and Harris A.L. (1997). Up-regulation of thymidine phosphorylase expression is associated with a discrete pattern of angiogenesis in ductal carcinomas in situ of the breast. J. Pathol. 182, 414-420.

Feng X., Clark R.A., Galanakis D. and Tonnesen M.G. (1999). Fibrin and collagen differentially regulate human dermal microvascular endothelial cell integrins: stabilization of alphav/beta3mRNA by fibrin. J. Invest. Dermatol. 113, 913-919.

Fox S.B., Westwood M., Moghaddam A., Comley M., Turley H., Whitehouse R.M., Bicknell R., Gatter K.C. and Harris A.L. (1996). The angiogenic factor platelet-derived endothelial cell growth factor/thymidine phosphorylase is up-regulated in breast cancer epithelium and endothelium. Br. J. Cancer 73, 275-280.

Fox S.B., Engels K., Compley M., Whitehouse R.M. Turley H., Gatter K.C. and Harris A.L. (1997). Relationship of elevated tumour thymidine phosphorylase in node-positive breast carcinomas to the effects of adjuvant CMF. Ann. Oncol. 8, 271-275.

Fukuiwa T., Takebayashi Y., Akiba S., Matsuzaki T., Hanamure Y., Miyadera K., Yamada Y. and Akiyama S. (1999). Expression of thymidine phosphorylase and vascular endothelial cell growth factor in human head and neck squamous cell carcinoma and their different characteristics. Cancer 85, 960-969.

Gasparini G. (1996). Angiogenesis research up to 1996. A commentary on the state of art and suggestions for future studies. Eur. J. Cancer 32A, 2379-2385.

Gonzalez-Vela M.C., Garijo M.F., Fernandez F., Buelta L. and Val-Bernal J.F. (1999). Cathepsin D in host stromal cells is associated with more highly vascular and aggressive invasive breast carcinoma. Histopathology 34, 35-42.

Goto F., Goto K., Weindel K. and Folkman J. (1993). Synergistic effects of vascular endothelial growth factor and basic fibroblast growth factor on the proliferation and cord formation of bovine capillary endothelial cells within collagen gels. Lab. Invest. 69, 508-517.

Hanahan D. and Folkman J. (1996). Patterns of emerging mechanisms of the angiogenic switch guring tumorigenesis. Cell 86, 353-364.

Haraguchi M., Miyadera K., Uemura K., Sumizawa T., Furukawa T., Yamada K., Akiyama S. and Yamada Y. (1994). Angiogenic activity of enzymes. Nature 17, 368, 198.

Ioachim E., Skopelitou A., Kamina S., Nonni A. and Agnantis N.J. (1998). p53 Protein Expression in Human Breast cancer: An Immunohistochemical Study Including Correlations with Steroid Receptors' Status, Proliferation Markers, Collagen Type IV, Laminin, C-erbB-2 Oncoprotein and Cathepsin D Expression. Breast 7, 42-48.

Ioachim E., Sebasti K., Demou A., Kontostolis M., Lolis D. and Agnantis N.J. (1999) Immunohistochemical localization of Metallothionein in human breast cancer in correlation with Cathepsin D, stromelysin-1, CD44, extracellular matrix components, P53, Rb, C-erbB-2, EGFR, steroid receptor content and proliferation indices. Anticancer Res. 19, 2133-2139.

Ioachim E., Charchanti A., Briasoulis E., Karavasilis V., Tsanou H., Arvanitis D.L., Agnantis N.J. and Pavlidis N. (2002). Immunohistochemical expression of extracellular matrix components tenascin, fibronectin, collagen type IV and laminin in breast cancer: the prognostic value and role in tumour invasion and progression. Eur. J. Cancer 38, 2362-2370.

Imazano Y., Takebayashi Y., Nishiyama K., Akiba S., Miyadera K., Yamada Y, Akiyama S. and Ohi Y. (1997). Correlation between thymidine phosphorylase expression and prognosis in human renal cell carcinoma. J. Clin. Oncol. 15, 2570-2578.

Iozzo R.V. (1995). Tumour stroma as a regulator of neoplastic behavior. Lab. Invest. 73, 157-160.

Ishikawa F., Miyazono K., Hellman U., Drexler H., Wernstedt C., Hagiwara K., Usuki K., Takaku F., Risau W. and Heldin C.H. (1989). Identification of angiogenic activity and the cloning and expression of the platelet-derived endothelial cell growth factor. Nature 338, 557-562.

Kanzaki A., Takebayashi Y., Bando H., Eliason J.F., Watanabe S-I., Miyashita H., Fukumoto M., Toi M. and Uchida T. (2002). Expression of uridine and thymidine phosphorylase genes in human breast carcinoma. Int. J. Cancer 97, 631-635.

Kobayashi M., Okamoto K., Akimori T., Tochika N., Yoshimoto T., Okabayashi T., Sugimoto T. and Araki K. (2004). Localization of Thymidine phosphorylase in advanced gastric and colorectal cancer. J. Mol. Histol. 35, 69-74.

Kobayashi M., Sugimoto T., Okabayashi T., Okamoto K., Namikawa T., Tochika N., Moriki T. and Araki K. (2005). Localization of thymidine phosphorylase in breast cancer tissue. Med. Mol. Morphol. 38, 112-117.

Kubiak R., Miszczak-Zaborska E., Jesionek-Kupnicka D. and Greger J. (1999). The activity of thymidine phosphorylase correlates with tumor size and lymph nodes status in breast carcinoma. Z. Naturforsch. 54, 1096-1102.

Lehman N.L. (2002). Future potential of thymidylate synthase inhibitors in cancer therapy. Exp. Opin. Investig. Drugs 11, 1775-1787.

Lee A.H., Dublin E.A. and Bobrow L.G. (1999). Angiogenesis and expression of thymidine phosphorylase by inflammatory and carcinoma cells in ductal carcinoma in situ of the breast. J. Pathol. 187, 285-290.

Li H, Suo Z., Zhang Y., Risberg B., Karlsson M.G., Villman K. and Nesland J.M. (2004). The prognostic significance of thymidine phosphorylase, thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expressions in breast carcinomas. Histol. Histopathol. 19, 129-136.

Madri J.A., Pratt B.M. and Tucker A.M. (1988). Phenotypic modulation

of endothelial cell by transforming growth factor-b depends upon the composition of the extracellular matrix. J. Cell Biol. 106, 1375-1384.

Miyazono K., Okabe T., Urabe A., Takaku F. and Heldin C.H. (1987). Purification and properties of an endothelial growth factor from human platelets. J. Biol. Chem. 262, 4098-4103.

Miyazono K., Usuki K. and Heldin C.H. (1991). Platelet-derived endothelial cell growth factor . Prog. Growth Factor Res. 3, 207-217.

Mizutani Y., Wada H., Yoshida O., Fukushima M., Bonavida B., Kawauchi A. and Miki T. (2002). Prognostic significance of a combination of thymidylate synthase and dihydropyrimidine dehydrogenase activities in grades 1 and 2 superficial bladder cancer. Oncol. Rep. 9, 289-292.

Moghaddam A., Zhang H.T., Fan T.P., Hu D.E. Lees V.C., Turley H., Fox S.B., Gatter K.C., Harris A.L. and Bicknell R. (1995). Thymidine phosphorylase is angiogenic and promotes tumor growth. Proc. Natl. Acad. Sci. USA 92, 988-1002.

Nagaoka H., Iino Y., Takei H. and Morishita Y. (1998). Platelet-derived endothelial cell growth factor/thymidine phosphorylase expression in macrophages correlates with tumour angiogenesis and prognosis in invasive breast cancer. Int. J. Oncol. 13, 449-454.

Poller D.N., Roberts E.C., Bell J.A., Elston C.W., Blamey R.W. and Ellis I.O. (1993) p53 protein expression in mammary ductal carcinoma in situ: relationship to immunohistochemical expression of estrogen recdeptor and c-erbB-2 protein. Hum. Pathol. 24, 463-468.

Relf M., LeJeune S., Scott P.A.E., Fox S., Smith K., Leek R., Moghaddam A. Whitehouse R., Bicknell R. and Harris A.L. (1997). Expression of the angiogenic factor vascular endothelial cell growth factor, acidic and basic fibroblast growth factors, tumour growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res. 57, 963-969.

Rose M.G. Farrell M.P. and Schmitz J.C. (2002). Thymidylate synthase: a critical target for cancer chemotherapy. Clin. Colorectal Cancer 1, 220-229.

Ruoslahti E. and Yamaguchi Y. (1991). Proteoglycans as modulators of grwth factor activities. Cell 64, 867-869.

Seymour L. and Bezwoda W.R. (1994). Positive immunostaining for platelet derived growth factor (PDGF) is an adverse prognostic factor in patients with advanced breast cancer. Breast Cancer Res. Treat. 32, 229-233.

Soini Y., Vakkala M., Kahlos K., Paakko P. and Kimmula V. (2001). MnSOD expression is less frequent in tumour cells of invasive breast carcinomas than in in situ carcinomas or non-neoplastic breast epithelial cells. J. Pathol. 195, 156-162.

Sutton A.B., Canfield A.E., Schor S.L., Grant M.E. and Schor A.M. (1991). The response of endothelial cells to TGFß-1 is dependent upon cell shape, proliferative state and the nature of the substratum. J. Cell Sci. 99, 777-787.

Swindle C.S., Tran K.T., Johnson T.D., Banerjee P., Mayer A.M., Griffith L. and Wells A. (2001). Epidermal growth factor (EGF)-like repeats of human tenascin-C as ligands for EGF receptor. J. Cell Biol. 23, 459-468.

Takebayashi Y., Akiyama S., Akiba S., Yamada K., Miyadera K., Sumizawa T., Yamada Y., Murata F. and Aikou T. (1996a). Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinomas. J. Natl. Cancer Inst. 88, 1110-1117.

Takebayashi Y., Miyadera K., Akiyama S., Hokita S., Yamada K., Akiba S., Yamada Y., Sumizawa T. and Aikou T. (1996b). Expression of thymidine phosphorylase in human gastic carcinoma. Jpn. J. Cancer Res. 87, 288-295.

Takebayashi Y., Yamada K., Miyadera K., Sumizawa T., Furukawa T., Kinoshita F., Aoki D., Okumura H., Yamada Y., Akiyama S. and Aikou T. (1996). The activity and expression of thymidine phosphorylase in human solid tumors. Eur. J. Cancer 32, 1227-1232.

Takebayashi Y., Natsugoe S., Baba M., Akiba S., Fukumoto T., Miyadera K., Yamada Y., Takao S., Akiyama S. and Aikou T. (1999). Thymidine phosphorylase in human esophageal squamous cell carcinoma. Cancer 85, 282-289.

Tanaka K., Hiraiwa N., Hashimoto H., Yamazaki Y. and Kusakabe M. (2004). Tenascin-C regulates angiogenesis in tumour through the regulation of vascular endothelial growth factor expression. Int. J. Cancer 108, 31-40

Teo N.B., Shoker B.S., Jarvis C., Sloane J.P. and Holcombe C. (2003). Thymidine phosphorylase expression and stromal vascularity in ductal carcinoma in situ of the breast. J. Clin. Pathol. 56, 919-923.

Tokes A.M., Hortovanyi E., Kulka J., Jackel M., kerenyi T., Kadar A. (1999). Tenascin expression and angiogenesis in breast cancers. Pathol. Res. Pract. 195, 821-828.

Toi M., Hoshina S., Taniguchi T., Yamamoto Y., Ishistuka H. and Tominaga T. (1995). Expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast cancer. Int. J. Cancer 64, 79-82.

Toi M., Gion M., Biganzoli E., Dittadi R., Boracchi P., Miceli R., Meli S., Mori K., Tominaga T. and Gasparinin G. (1997). Co-determination of the angiogenic factors thymidine phosphorylase and vascular endothelial growth factor in node-negative breast cancer: prognostic implications. Angiogenesis 1, 71-83.

Tominaga T., Toi M., Ohashi Y. and Abe O. (2002). Prognostic and predictive value of thymidine phosphorylase activity in early-stage breast cancer patients. Clin. Breast Cancer 3, 55-64.

Yang Q., Sakurai T., Shan L., Yoshimura G., Yu Z., Suzuma T., Tamaki T., Umemura T., Nakamura Y., Nakamura M., Utsunomiya H., Mori I. and Kakudo K. (2000). Thymidine phosphorylase expression correlates with tumor differentiation and Bcl-2 in invasive breast cancer. Breast Cancer 7, 210-214.

Yang Q., Barbareschi M., Mori I., Mauri F., Muscara M., Nakamura M., Nakamura Y., Yoshimura G., Sakurai T., Caffo O., Galligioni E., Dalla Palma P. and Kakudo K. (2002). Prognostic value of thymidine phosphorylase expression in breast carcinoma. Int. J. Cancer 97, 512-517.

Yonenaga F., Takasaki T., Ohi Y., Sagara Y., Akiba S., Yoshinaka H., Aikou T., Miyadera K., Akiyama S. and Yoshida H. (1998). The expression of thymidine phosphorylase/platelet-derived endothelial cell growth factor is correlated to angiogenesis in breast cancer. Pathol. Int. 48, 850-856.

Yoshimura A., Kuwazuru Y., Furukawa T., Yoshida H., Yamada K. and Akiyama S. (1990). Purification and tissue distribution of human thymidine phosphorylase: high expression in lymphocytes, reticulocytes, and tumors. Biochim. Biophys. Acta 1034, 107-113.

Zagzag D., Shiff B., Jallo G.I., Greco M.A., Blanco C., Cohen H., Hukin J., Allen J.C. and Friedlander D.R. (2002). Tenascin-C promotes microvascular cell migration of focal adhesion kinase. 62, 1660-1668.

Zheng H., Tsuneyama K., Cheng C., Takahashi H., Cui Z., Murai Y., Nomoto K. and Takano Y. (2007). Maspin expression was involved in colorectal adenoma-adenocarcinoma sequence and liver metastasis of tumors. Anticancer Res. 27, 259-265.

Accepted August 14, 2007