

Predictive Value of Thymidine Phosphorylase in Gastric Carcinoma

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ABSTRACT

Background and purpose: Thymidine Phosphorylase/Platelet Derived - Endothelial Cell Growth Factor (TP/PD-ECGF) has an angiogenic and chemotherapeutic effects. The aim of this work was to evaluate TP/PD-ECGF expression in gastric adenocarcinoma and find its correlation with established clinicopathological parameters and patients' survival.

Materials and Methods: Samples studied consisted of fifty-two gastric specimens (27 cases of chronic gastritis and 25 cases of malignant adenocarcinoma). Immunohistochemical staining for TP/PD-ECGF was done and the tumor was considered positive when more than 5% of cells showed positive staining.

Results: TP/PD-ECGF expression was significantly higher in the malignant group when compared to the control group ($p = 0.001$). Tumor and stromal cell TP/PD-ECGF expression in the malignant group was significantly correlated with size of the tumor, mitotic count, stage grouping, depth of invasion, number of lymph nodes (LN) involved, perineural invasion, lymphoplasmacytic and tumor associated macrophages (TAMs) infiltration and vessel density (VD). Furthermore, stromal expression of TP/PD-ECGF was significantly correlated with postoperative chemotherapy and apoptotic count. Survival analysis revealed that proximal tumor, small size, early stage, negative LN metastasis, absence of perineural invasion, low VD and negative tumor and stromal TP/PD-ECGF expression correlated with better patients' survival.

Conclusions: TP/PD-ECGF might have an angiogenic function and role in tumor growth, invasion and metastasis. TP/PD-ECGF could enhance the effect of postoperative chemotherapy.

Key Words: *Thymidine phosphorylase/platelet derived - Endothelial cell growth factor (TP/PD-ECGF) - Gastric carcinoma.*

INTRODUCTION

Gastric carcinoma is the second most common cancer in the world after carcinoma of the lung. The highest incidence is in Japan (100

per 100.000) [1]. Its incidence is declining in the West. The median age is 55 years with male predominance. In Egyptian National Cancer Institute series, gastric carcinoma constituted about 1.64% of all total cancer during the period between 1990 to 1997 [2].

Thymidine Phosphorylase (TP) catalyses the reversible synthesis of thymidine and inorganic phosphate from thymine, using deoxy-1-phosphate as a co-substrate. It plays its role in regulation of plasma thymine level. TP also catalyses the conversion of the thymidine anti-metabolite 5-Fluorouracil (5 Fu) to 5-Fluro-2-deoxyuridine, the first step in the metabolic activation of this cancer chemotherapeutic agent [3].

Moreover, TP was found to be structurally similar to platelet derived-endothelial cell growth factor (PD-ECGF) and so it is now abbreviated as TP/PD-ECGF. It is also able to promote secretion of the stress-induced angiogenic factors e.g. vascular endothelial growth factors and Interlukin-8 as well as induces matrix metalloproteinase-1 [4].

Immunohistochemical study of TP/PD-ECGF in aesophagus, colo-rectum, pancreas and lung demonstrated that its expression in the malignant cells was significantly higher than adjacent non-neoplastic tissues and its proportion of positivity in advanced carcinoma was also significantly higher than that in early carcinoma [5-8]. In cancer bladder, TP/PD-ECGF expression showed significant correlation with histological grade, infiltration pattern, local invasion, lymph node metastasis and overall prognosis of the patients [9-12].

No reliable method is available for predicting the response to chemotherapy in patients with gastric carcinoma. However, recently the determination of expression of TP/PD-ECGF was reported to be useful for predicting the efficacy of post-operative chemotherapy with 5 - Fluorouracil to prevent recurrence in advanced gastric carcinoma patients who undergo curative gastrectomy [13-15].

In gastric cancer, few preliminary studies have been done on expression of TP/PD-ECGF and little is known about its clinicopathological significance [15,16].

The aim of this study was to evaluate thymidine phosphorylase expression in gastric cancer and correlate its expression with other classical clinicopathological parameters as well as patients' survival trying to estimate its predictive and prognostic value.

MATERIALS AND METHODS

Case selection:

This study included 52 gastric specimens of Egyptian patients taken during the period between 2000 & 2001 from the pathology department, Faculty of Medicine, Menoufeya University and the pathology department, National Cancer Institute (NCI), Cairo University. Clinicopathological data related to the selected cases were obtained from patients' records including age, sex and in the malignant cases tumor size, site, lymph node metastasis, recurrence, presence of distant metastasis and type of operation done. Adjuvant therapy by chemotherapy, radiotherapy or both and survival of patients were also collected from patients' medical records.

Types of specimens:

The control group included 27 cases; formed of 24 cases of chronic gastritis without dysplasia and three cases showing chronic gastritis with mild dysplasia. The malignant group included 25 cases; 16 showed intestinal-type gastric adenocarcinoma, 7 cases of diffuse-type gastric adenocarcinoma and two cases showed gastric adenocarcinoma with neuroendocrine differentiation. Four sections 5 μ m thick were cut from the available paraffin blocks. One was stained with routine Hx. & E., to confirm the diagnosis and evaluate the histopathological characteristics of the tumor. Two sections were put on poly L lysine coated slides and were subjected to im-

munostaining. The last section was stained by Giemsa stain to evaluate the presence of *H. pylori*.

Histopathological examination:

Grading of adenocarcinoma was performed according to the criteria of Dixon [17]. Staging was done according to TNM system [18]. Mitotic and apoptotic tumor cells were examined in Hx. & E. stained sections in ten high-power fields (x40 and x10 ocular) magnification (field size of 0.274 mm² and field diameter of 0.59 mm,) [19]. Vessel density determination was performed according to the method described by Weidner et al. [20]. Individual vessels were counted on a 200 x microscope high power field (10 x ocular lens x 20 x objective lens equal to 0.74 mm² per field) on an Olympus CH2 microscope.

Immunohistochemistry:

The improved streptavidin-biotin amplified system was applied. Primary antibody is monoclonal mouse anti thymidine phosphorylase (Lab vision, USA) (cat. # MS-499-R7). The detection kit was ultravision detection system anti-polyvalent, HRP/DAB (catalog # TP - 015-HD) (Lab vision, USA).

Sections were dewaxed in xylene and rehydrated in graded alcohol. Peroxidase block was done by using hydrogen peroxidase for 10 minutes at room temperature in a humidity chamber. Subsequently, the slides were incubated for 10 minutes with Ultra V Block to block non-specific background staining. Tissue sections were then incubated with primary antibody in a humidity chamber over night at room temperature. Incubation with biotinylated goat anti-polyvalent and streptavidine peroxidase for 10 minutes each was then done. The color reaction was developed with DAB substrate. Mayer's Haematoxyline was used as a counter stain.

Thymidine phosphorylase immunostaining evaluation:

The extent and intensity of a positive staining of TP/PD-ECGF in both the cytoplasm and nuclei of malignant cells, as well as in the stromal cells were graded on a scale 0-3+ [21]. For statistical purpose, evaluation of TP/PD-ECGF immunostaining was simplified as: negative expression < 5% positive cells and positive expression > 5% positive cells.

Statistical Analysis:

Data were collected and statistically analyzed using SPSS version 9 program and Stat View software. Chi-square test (χ^2), Fisher exact test, student's *t*-test and Mann Whitney U-test were used. For survival analysis, univariate analysis was based on Kaplan-Meier product-limit estimates and Log rank test was used to compare factors affecting survival. Value of $p < 0.05$ was considered statistically significant [22].

RESULTS

Clinicopathological results:

The mean age of the investigated cases was slightly higher for the malignant group (50y±12.1) than the control group (47.1y±15.9) and male/female ratio was 2.1:1 in the malignant group. Infection by *H. pylori* (Fig. 1) was significantly higher in the control group ($p < 0.05$). Vessel density was significantly higher in the malignant group (12.8±7.5um) than the control group (2.9±1.7) ($p = 0.001$). Diffuse-type adenocarcinoma was significantly higher in age than the intestinal type, however other clinicopathological data were not statistically different between intestinal and diffuse types.

Immunohistochemical results (Tables,1,2,3):

Expression of TP/PD-ECGF was visualized as intracytoplasmic brownish granules with or without nuclear staining, in glandular and malignant epithelial cells as well as in stromal lymphoplasma cells and tumor associated macrophages (TAMs) (Figs. 2-5). TP/PD-ECGF expression was significantly higher in the malignant group than the control group ($p = 0.001$) while no significant difference between the two groups regarding stromal expression was found. A significant correlation was found between tumor cell and stromal expression of TP/PD-ECGF ($p = 0.001$). In the malignant group, 46.7% of positive cases showed both nuclear and cytoplasmic expression, 40% showed nuclear expression and 13.3% showed cytoplasmic expression only.

Tumor cell TP/PD-ECGF expression in the malignant group correlated significantly with the size of tumor, mitotic count, stage grouping, depth of invasion, number of L.N involved, perineural invasion, lymphoplasmacytic and TAMs infiltration and VD.

Stromal TP/PD-ECGF expression significantly correlated with chemotherapy, mitotic and apoptotic counts, perineural invasion, VD and lymphoplasmacytic and TAMs infiltration.

TP/PD-ECGF expression significantly correlated with tumor size and mitotic count, LN metastasis, perineural invasion and VD in diffuse and intestinal type adenocarcinoma. While, TP/PD-ECGF expression significantly correlated with stage grouping, depth of invasion and lymphoplasmacytic and TAMs infiltration in the intestinal type only.

Survival analysis:

Survival data were available for 21 out of 25 (84%) patients of the malignant group. The time of follow up ranged from 6 to 36 months with a median of 21 months. Two cases died from the disease after 6 and 10 months; whereas other patients were lost to follow up at different intervals. Statistical analysis as regards survival was done according to months of follow up.

On doing the Log rank test and construct the Kaplan Meier survival plots, the following factors were associated statistically with better survival: proximal tumor site, small size, early stage, perineural invasion, low depth of invasion, low vessel density and negative tumor and stromal expression of TP/PD-ECGF (Figs. 6-8).

Table (1): TP/PD-ECGF expression in the control and the malignant groups.

	Control N=27	Malignant N=25	Total	<i>p</i> Value
<i>Glandular exp.*:</i>				
Negative	26 (72.2%)	10 (27.8%)	36	0.001
Positive	1 (6.2%)	15 (93.8%)	16	
<i>Stromal exp.*:</i>				
Negative	13 (61.9%)	8 (38.1%)	21	0.24
Positive	14 (45.2%)	17 (54.8%)	31	

* exp. = Expression.

Table (2): Relation between tumor expression of TP/PD-ECGF and stromal expression in the malignant group.

Stromal expression	Tumor expression		Total	<i>p</i> Value
	Negative	Positive		
Negative	8 (100%)	0-	8	0.001
Positive	2 (11.8%)	15 (88.2%)	17	

Table (3): Correlation between TP/PD-ECGF expression in tumor cells and clinicopathological data of malignant cases.

	TP/PD-ECGF expression		Total (N)	p Value
	Positive (n=15)	Negative (n=10)		
Age $\bar{X} \pm SD$	53.9 \pm 11.38	45.3 \pm 11.93		0.08
<i>Sex:</i>				
Male	11 (64.7%)	6 (35.3%)	17	0.67
Female	4 (50%)	4 (50%)	8	
<i>Recurrence:</i>				
-ve	14 (60.9%)	9 (39.1%)	23	1.00
+ve	1 (50%)	1 (50%)	2	
<i>Chemotherapy:</i>				
-ve	1 (20%)	4 (80%)	5	0.12
+ve	14 (70%)	6 (30%)	20	
<i>Site:</i>				
Cardiac + Fundal + L.C	4 (50%)	4 (50%)	8	0.67
Antral + Pyloric	11 (64.7%)	6 (35.3%)	11	
Size $\bar{X} \pm SD$ CC.	6.73 \pm 2.08	4.3 \pm 1.95		0.007*
<i>Grade:</i>				
Well& mod. D.	13 (61.9%)	8 (38.1%)	21	1.00
Poorly dif.	2 (50%)	2 (50%)	4	
Mean mitotic count \pm SD	7.13 \pm 3.58	3.8 \pm 2.1		0.01*
<i>Apoptotic count:</i>				
$\bar{X} \pm SD$	3.13 \pm 1.68	4.3 \pm 2.26		0.21
<i>Stage grouping:</i>				
I & II	0 –	5 (100%)	5	0.02*
III & IV	4 (57.1%)	3 (42.9%)	7	
<i>Depth of invasion:</i>				
Mucosa & submucosa	6 (42.9%)	8 (57.1%)	14	0.05*
Serosa & adjacent	9 (81.8%)	2 (18.2%)	11	
L.N. $\bar{X} \pm SD$	5.67 \pm 4.3	0.5 \pm 0.85		0.01*
<i>Vasc. Invasion:</i>				
Negative	13 (56.5%)	10 (43.5%)	23	0.5
Positive	2 (100%)	0	2	
<i>Perineural Inv.:</i>				
Negative	6 (37.5%)	10 (62.5%)	16	0.003*
Positive	9 (100%)	0 –	9	
<i>Lymphoplasma & TAM:</i>				
Mild & Moderate	10 (50%)	10 (50%)	20	0.05*
Severe	5 (100%)	0	5	
VD $\bar{X} \pm SD$ um.	17.4 \pm 5.90	5.9 \pm 2.73		0.001*
<i>H-pylori (18 cases):</i>				
Negative	8 (66.7%)	4 (33.3%)	12	1.00
Positive	4 (66.7%)	2 (33.3%)	6	
<i>Pathological Type:</i>				
Intestinal	10 (62.5%)	6 (37.5%)	16	
Diffuse	5 (71.4%)	2 (28.6%)	7	1.00
Other Variants	0	2 (100%)	2	
<i>Surgical Margin:</i>				
Negative	13 (56.5%)	10 (43.5%)	23	0.5
Positive	2 (100%)	0	2	

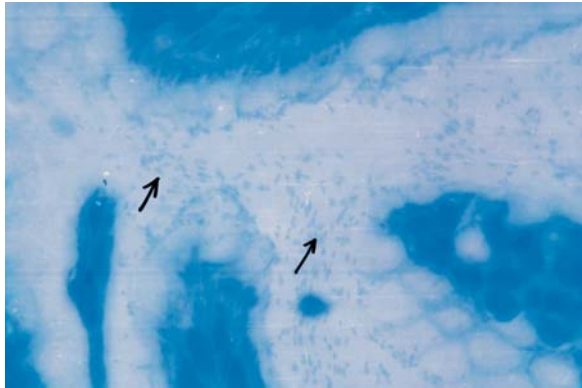


Fig. (1): Positive H pylori in well differentiated intestinal type adenocarcinoma. (Giemsa S X1000).

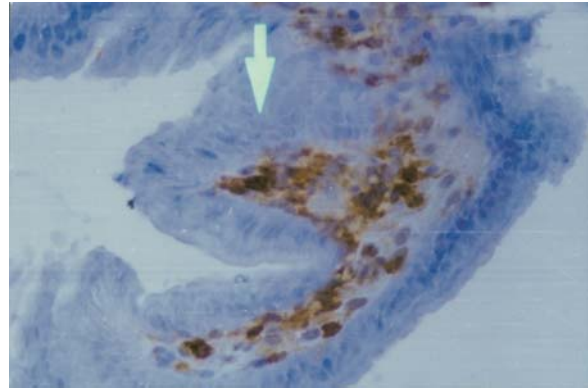


Fig. (2): Chronic gastritis showing stromal expression of TP/PD-ECGF. (IPS X400).

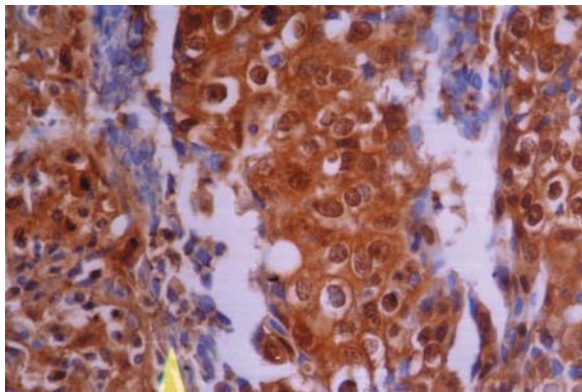


Fig. (3): Cribriform pattern of intestinal-type adenocarcinoma, showing strong positive nuclear and cytoplasmic expression of TP/PD-ECGF (IPS X400).

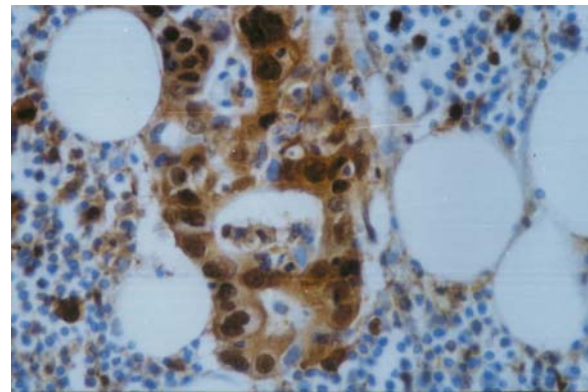


Fig. (4): Intestinal-type adenocarcinoma showing invasion of lymph node by malignant glands detected by strong nuclear and cytoplasmic expression of TP/PD-ECGF. (IPS X400).

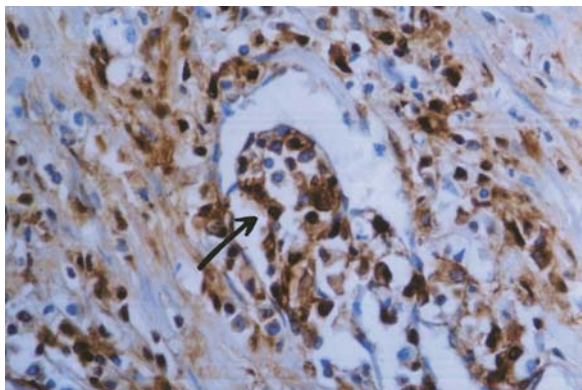


Fig. (5): Vascular invasion in intestinal-type adenocarcinoma, showing positive nuclear and cytoplasmic expression. (IPS X400).

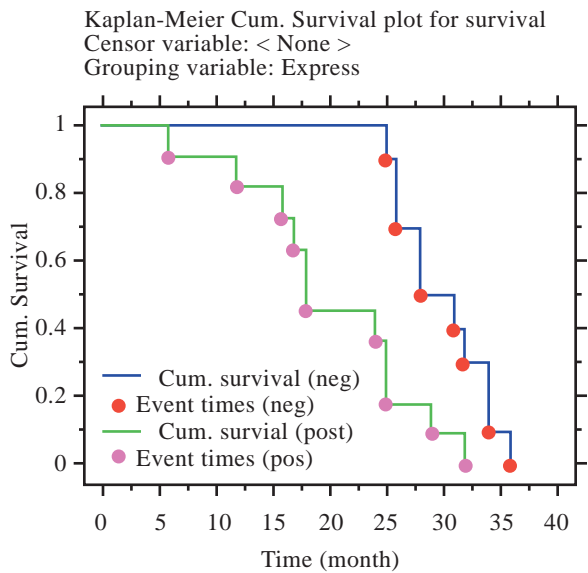


Fig. (6): Survival analysis according to tumor expression of TP/PD-ECGF.

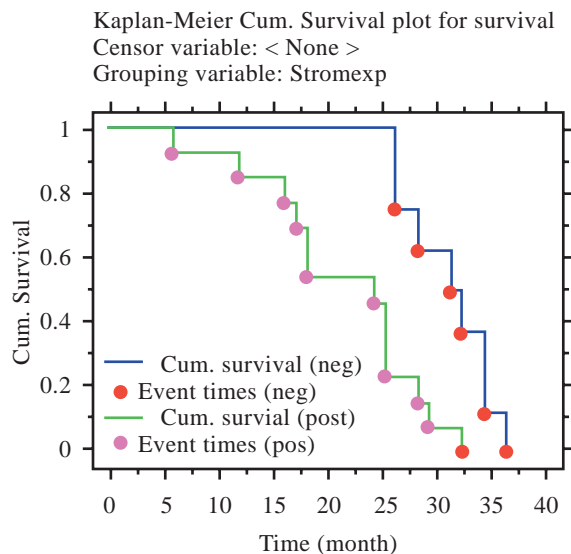


Fig. (7): Survival analysis according to stromal expression of TP/PD-ECGF.

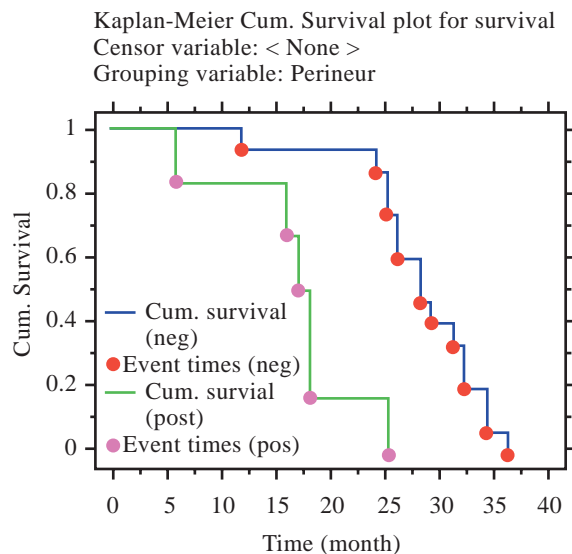


Fig. (8): Survival analysis according to perineural invasion.

DISCUSSION

Thymidine phosphorylase (TP) catalyses the reversible phosphorylation of thymidine to thymine and was found to be identical to the angiogenic factor platelet derived-endothelial cell growth factor (PD-ECGF) [23]. Among all identified angiogenic factors, TP/PD-ECGF was found to be the sole agent correlating with vascular density [24,25].

In the present study, 3.7% of cases of chronic gastritis (CG) showed positive staining in glandular epithelial cells and 56.8% regarding stromal expression for TP/PD- ECGF. These results may be due to increased infiltration by TP/PD-ECGF secreting cells such as leucocytes and macrophages [23]. Moreover Brown and Bicknell [24] found that TP/PDECGF was elevated in chronically inflamed tissues, in which angiogenesis is observed. *Helicobacter pylori* (*H. pylori*) infection seems not to influence the expression of TP/PD-ECGF, once gastric cancer has developed. However, *H. pylori* infection may increase the extension of expression of TP/PD-ECGF by recruiting inflammatory cells, which may help in creating a favorable environment for tumor development.

TP/PD- ECGF activity in several carcinomas was reported to be significantly higher than in the adjacent non-neoplastic tissues [26-30]. In our study the expression of TP/PD-ECGF was

significantly higher in the malignant than the control group (3.7%) and the expression was more in the stromal cells (68%) than tumor cells (60%). Yoshikawa et al. [31] reported 48.3% of their patients with more than two times the level of TP/PD-ECGF in tumor tissue than in normal tissue and these figures were higher for the stromal expression.

Expression of TP/PD-ECGF significantly correlated with vessel density in the infiltrating edge of the tumor. It is possible that TP/PD-ECGF acts through a second messenger system either by inducing the expression of other directing angiogenic factors or by contributing to angiogenesis through some type of enzymatic activity [31,32]. Brown et al. [4] suggested that thymidine catabolism by TP/PD-ECGF increases carcinoma cell secretion of interleukin-8, vascular endothelial cell growth factor and matrix metalloproteinase-1. Moreover, TP/PD-ECGF has been shown to stimulate chemotaxis of endothelial cells [30]. In contrast, TP/PD-ECGF inhibitors (TPI) were suggested to suppress the angiogenic as well as invasiveness effects of TP/PD-ECGF [33].

In the present study, expression of TP/PD-ECGF correlated with stage grouping of the malignant cases. This was detected by increased expression with the increase in depth of invasion. A similar result was obtained by Takebayashi et al. [28]. The insufficient perfusion to

areas of the tumor resulting in the existence of hypoxia, glucose depletion and low pH which might be involved in the higher amounts of TP/PD-ECGF [31].

In the current study, a significant correlation was found between tumor and stromal expression of TP/PD-ECGF. This finding suggests that stromal cell expression plays an important role in angiogenesis and progression of gastric carcinoma. Stromal expression of TP/PD-ECGF was observed in lymphocytes, plasma cells and tumor associated macrophages (TAMs) invading tumor stroma. Maeda et al. [34] supported the hypothesis that TP/PD-ECGF production in carcinoma cells might be induced by microenvironment [27]. Lenz et al. [16] reported that macrophage like other stromal cells as well as tumor cells express TP/PD-ECGF. Furthermore, TAMs produce several important angiogenic factors as well as tumor necrosis factor- α , interleukin-1 and interferon- γ which regulate TP/PD-ECGF expression in tumor cells [13].

Apoptotic count negatively correlated with stromal TP/PD-ECGF expression. Ueda et al. [36,37] found that intra-arterial infusion chemotherapy induced apoptotic cell death in locally advanced cervical carcinomas through the inhibition of tumor angiogenesis and TP/PD-ECGF expression of tumor cells. In contrast, positive significant correlation was found between mitotic count and TP/PD-ECGF expression in both tumor and stromal cells.

In our patients suffering from gastric carcinoma, better survival was associated with negative tumor and stromal TP/PD-ECGF expression, small size tumors, superficial tumors, negative lymph node involvement, low mitotic and high apoptotic counts, absence of perineural invasion and low vessel density. Similar results were recorded by Maeda et al. [34] and Takebayashi et al. [28]. However, these associations were not found in two other studies [15,34].

In the present study, TP/PD-ECGF expression significantly correlated with the response to postoperative chemotherapy by 5-fluorouracil derivatives (5-Furd). A similar result was obtained by Yoshikawa et al. [31] who reported that higher tumor-normal (T/N) ratios of TP/PD-ECGF activity are preferable in patients who are treated by 5-Furd, because of the probable high cytotoxicity against tumor tissue and the

low toxicity against normal tissue. So determination of tissue levels of TP/PD-ECGF would be useful for selecting patients for chemotherapy [31]. Moreover, a direct correlation between the enhanced effect of (5 Fu) chemotherapy in patients' survival and positive tumor TP/PD-ECGF expression was found. Similar results were reported by other authors [38-42]. Another useful approach would be a combination therapy with regulators of TP/PD-ECGF. Several cytokines, such as IL-1, TNF- α and TNF- γ regulate TP/PD-ECGF in cancer cells. Also the taxanes-anticancer agent- and X ray irradiation may increase TP/PD-ECGF indirectly through up-regulation of TNF- α [39]. Up-regulation of TP/PD-ECGF was found also to enhance the anti-proliferative function of 5-Furd [35]. Moreover, Yoshimura et al. [43] suggested that detection of plasma level of TP/PD-ECGF could be helpful for monitoring the efficacy of chemotherapy.

In conclusion, TP/PD-ECGF was significantly higher in the malignant group than in control-chronic gastritis- group and was significantly correlated with aggressive tumor parameters. TP/PD-ECGF might have an angiogenic function and important role in tumor growth, invasion and metastasis. Better patients' survival was associated with negative tumor and stromal expression of TP/PD-ECGF. Determination of TP/PD-ECGF expression might be useful for predicting the result of postoperative chemotherapy.

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