Infiltration of thymidine phosphorylase-positive macrophages is closely associated with tumor angiogenesis and survival in intestinal type gastric cancer

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Abstract. Thymidine phosphorylase (TP), an enzyme catalyzing the reversible phospholysis of thymidine, deoxyuridine and their analogs at their respective bases and 2-deoxyribose-1-phosphate, thus promoting angiogenesis, is often expressed in macrophages present in tumor stroma. In this study, we investigated whether infiltration of TP-positive macrophages as well as tumor-associated macrophages affected tumor angiogenesis. TP was expressed in human macrophage-like cells, but not in gastric cancer cells in culture. The expression level of TP, the number of infiltrating CD68^{+} and CD163^{+} macrophages, and microvessel density (MVD) in the tumor were further analyzed by immunohistochemistry in 111 patients with gastric cancer. Biostatistical analysis of digitized data obtained by image analysis showed that TP expression was significantly correlated with the number of infiltrating macrophages and MVD in intestinal type gastric cancer ($p < 0.05$). The number of infiltrating macrophages was also correlated with MVD in both the intestinal and diffuse types ($p < 0.05$). An increased number of CD68^{+} macrophages was significantly associated with poor outcome in patients with intestinal type ($p < 0.001$), but not diffuse type cancer. TP could be a specific marker enzyme that is expressed in tumor-infiltrating macrophages, being associated with tumor angiogenesis and poor prognosis in patients with intestinal-type gastric cancer.

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Introduction

Thymidine phosphorylase (TP) is an enzyme catalyzing the reversible phospholysis of thymidine, deoxyuridine and their analogs at their respective bases, and 2-deoxyribose-1-phosphate, thus playing a role in angiogenesis (1). The angiogenic activity of TP is due in part to production of reactive oxygen species by 2-deoxy-D-ribose, leading to up-regulation of angiogenic factors such as vascular endothelial growth factor and interleukin-8/CXCL8 through induction of oxidative stress and inflammatory signaling (2-4). TP is more highly expressed in differentiated than in undifferentiated gastric adenocarcinoma and patients whose tumors are TP-positive have a poorer prognosis than those with TP-negative tumors (5). Tumor expression of TP is significantly correlated with angiogenesis and invasion/metastasis in cancers of the colon and rectum, stomach, pancreas and bladder (6-9). We have previously observed a marked increase of TP expression in human macrophages in culture through transcriptional activation in response to inflammatory cytokines (10). We have also observed that in melanoma, the number of infiltrating macrophages increases depending upon invasion depth, in close association with angiogenesis (11), and that these macrophages are positive for TP expression (12). Shimaoka *et al* have further reported that TP expression in macrophages is correlated with the development of tumor microvessels, and also that TP plays an important role in tumor invasiveness and progression in differentiated gastric adenocarcinoma (8).

On the other hand, infiltration of macrophages in the tumor stroma often promotes the malignant progression of cancer through interaction with cancer cells (13,14). Among such macrophages, those activated by bacterial products and

interferon γ, known as inflammatory M1 type macrophages, have an IL-2^{high}, IN-23^{high}, IL-10^{low} phenotype and produce NO, IL-1ß, TNF-α and IL-6. By contrast, anti-inflammatory and activated macrophages, known as M2 type macrophages, have an IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype and produce pro-angiogenic factors and various receptors such as scavenger receptors and mannose receptors (15,16). These tumor-supporting macrophages are known as tumor-associated macrophages (TAMs), and support the processes of invasion, metastasis and angiogenesis by the production of inflammatory cytokines, chemokines, proteases, prostanoids, growth factors and angiogenic factors (13,14), suggesting that TAMs are rather more closely linked to the M2 type than to the M1 type. Clinical studies have demonstrated a close association between the abundance of TAMs and poor prognosis or tumor angiogenesis in colon cancer and glioma (17,18) as well as various other types of solid tumor including cancers of the breast, bladder and cervix (19). Various cytokines and growth factors are instrumental in the recruitment and accumulation of macrophages in the tumor stroma, and these macrophages then play a key role in the angiogenesis and malignant transformation (20). Depletion of these macrophages/TAMs by macrophage-targeting bisphosphonate encapsulated in liposomes markedly inhibits tumor growth, angiogenesis, and bone metastasis (21-23), thus corroborating the involvement of macrophages in these cancer-related activities (14). Tumor-supporting macrophages such as TAMs can often be differentially characterized from tumor-suppressive macrophages. Tumor-supportive macrophages are active in extracellular matrix remodeling, tissue repair and angiogenesis, whereas tumor-suppressive macrophages are active in antimicrobial and antitumor activities through immunostimulatory functions (24-26). In patients with gliomas we have previously reported that the number of macrophages in stage IV glioblastoma is higher than that in grade II or III glioma, and is also closely correlated with microvessel density in the tumors (27).

Concerning the role of macrophages/TAMs in gastric cancer, infiltrating macrophages have been associated with a good prognosis, suggesting a beneficial effect of TAM aggregation in advanced gastric cancer (28). However, it has not been determined whether the effect of TAMs is dependent on the histological type of gastric cancer. Migita *et al* have reported that the number of macrophages along the invasive margin is significantly greater in patients without, than in those with, liver metastasis (29). Although no significant correlation has been observed between the number of macrophages and the depth of cancer invasion or lymph node metastasis, a significant association between macrophage number and the survival of patients with intestinal type gastric cancer has been reported (29). With regard to the classification of gastric cancer (30), it has been reported that the intestinal type tends to metastasize through blood vessels, whereas the diffuse type tends to cause peritoneal dissemination and/or lymph node metastasis (31). However, it still remains unknown whether tumor-supporting macrophages such as TAMs play a role in the malignant progression of gastric cancer in relation to histological type.

In the present study, we screened the expression of TP in human cancer cell lines and macrophage-like cell lines by Western blot analysis. Our aim was to determine whether TP could be a specific biomarker of infiltrating macrophages in gastric cancer, and also whether such macrophages could affect angiogenesis, lymph node metastasis and peritoneal dissemination, as well as patient survival. We also discuss the possible relationship of TP-expressing macrophages with tumor angiogenesis and other malignant characteristics.

Materials and methods

Cell culture. The human gastric cancer cell lines MKN-28, MKN-45, MKN-74, AZ-521, NUGC-3 and KATO-III were cultured with RPMI supplemented with 10% fetal bovine serum (FBS) and incubated in humidified atmosphere of 5% CO_2 at 37˚C. The macrophage cell lines THP-1 and U937 were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in RPMI supplemented with 10% FBS and incubated in humidified atmosphere of 5% CO_2 at 37˚C. We also used KB/TP expressing high amount of TP (3,4).

Western blot analysis. Cells were rinsed with ice-cold PBS and lysed in buffer containing 50 mmol/l Tris-HCl, 350 mmol/l NaCl, 0.1% NP40, 5 mmol/l EDTA, 50 mmol/l NaF, 1 mmol/l phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 1 mmol/l Na_3VO_4. Cell lysates were subjected to SDS-PAGE and blotted onto Immobilon membranes (Millipore Corp., Bedford, MA) as described previously. After transfer, the membrane was incubated with blocking solution followed by primary antibody. Antibody detection was done using an enhanced chemiluminescence system (Amersham Biosciences Corp., Piscataway, NJ). The intensity of the luminescence was quantified using a CCD camera combined with an image analysis system (LAS-1000; Fuji Film, Japan).

Patients and tumor samples. We examined 111 patients with advanced gastric cancer whose tumors had been surgically removed in the Department of Surgery of Kurume University, between 2001 and 2004. The age of the gastric cancer patients ranged from 33 to 86 years (median, 69), 77 were men and 34 were women. Histological types were performed according to the criteria of Lauren classification (21), and tumor stage was performed according to the TNM classification. Patient characteristics are summarized in Table I. Cancer stages were 18.0% Stage I (IA+IB), 14.4% Stage II, 22.5% Stage III (IIIA+IIIB) and 45.0% Stage IV. At the time of surgery, 73 (65.8%), 17 (15.3%) and 26 (23.4%) patients had lymph node metastasis, liver metastasis and peritoneal dissemination, respectively. No patients had been administered drugs before surgery, and the standard chemotherapy was performed after surgery: Stage II or III patients were administered by TS-1, and Stage IV patients were administered by a combination of TS-1 and cisplatin.

Immunohistochemistry (IHC) analysis. Paraffin-embedded tissue samples were cut at 4 μm and examined on a coated slide glass and labeled with the following antibodies using the BenchMark XT (Ventana Automated Systems, Inc., Tucson, AZ, USA) and ChemMate Envision methods

Table I. Clinicopathological characteristics in gastric cancer

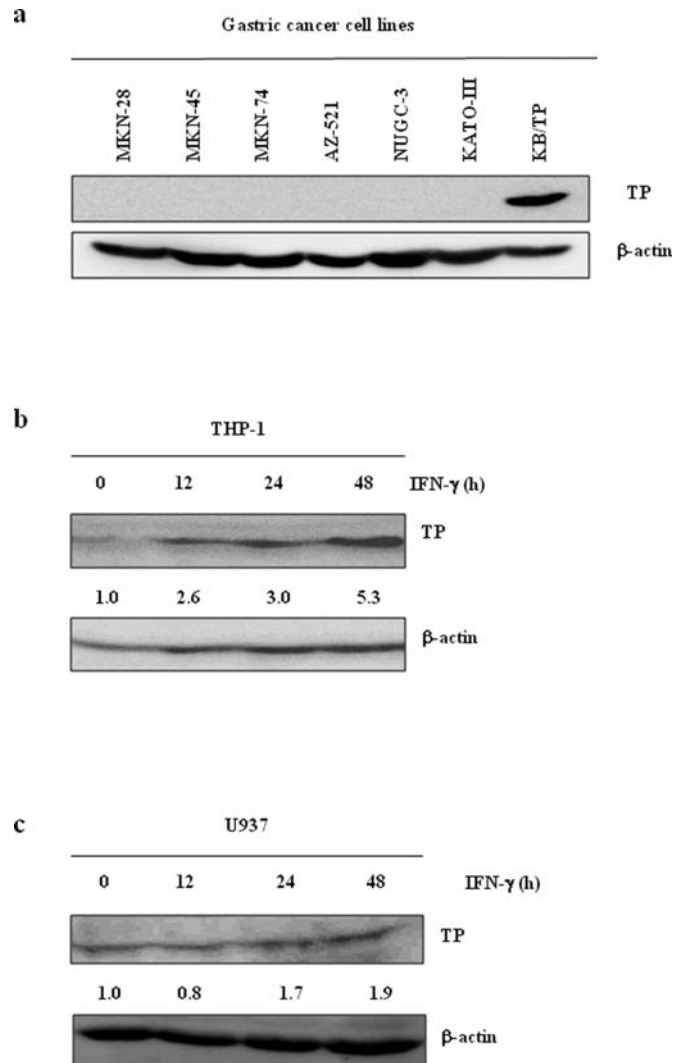|	Total (%) (n=111)	Intestinal (n=57)	Diffuse (n=54)
		Histological type	
Age			
Median (min-max)	69 (33-86)	67.5 (33-86)	69 (39-85)
Gender			
Male	77 (69.4)	47	30
Female	34 (30.6)	10	24
Stage			
I	20 (18.0)	11	9
II	16 (14.4)	9	7
III	25 (22.5)	13	12
IV	50 (45.0)	24	26
Lymph node metastasis			
Present	73 (65.8)	37	36
Absent	38 (34.2)	20	18
Liver metastasis			
Present	17 (15.3)	14	3
Absent	94 (84.7)	43	51
Peritoneal dissemination			
Present	26 (23.4)	7	19
Absent	85 (76.6)	50	35

Figure 1. Expression levels of TP in 6 human gastric cancer cell lines (a) and 2 human macrophage-like cell lines (b, c) in culture. Macrophages were treated with 50 U/ml of IFNγ. Western blot analysis of TP by specific antibody against TP was performed. Protein (100 μg) of total cell lysates was loaded. Quantitative analysis for TP expression is presented when TP level in macrophages treated with INFγ for 0 h is normalized as 1.0.

(DakoCytomation, Glostrup, Denmark), TP (x2000, antibodies supplied by Taiho Pharmaceutical Co., Ltd., Tokyo Japan), CD68 (x1200, KP-1, DakoCytomation), CD163 (x100, Novocastra, Newcastle, UK) and CD34 (x200, Novocastra). The ChemMate Envision method was used for TP, CD68 and CD34. Endogenous peroxidase activity was inhibited by incubating the slides in 3% H_2O_2 for 5 min. CD68 and CD34 antigen retrieval was performed by treating with proteinase K for 5 min. Each slide was incubated for 30 min with the antibody at room temperature. For staining detection, the ChemMate Envision method was used with DAB as chromogen. For CD163, BenchMark XT was used. This automated system used the streptavidin biotin complex method with DAB as chromogen (Ventana iView DAB Detection kit).

IHC expression area analysis for TP, infiltration macrophages and MVD assessment. We extracted the digital data of the expression using the following image analysis systems. TP, CD68, CD163 and CD34-stained specimens were examined to identify the areas with the high density. Images of the expression area were selected for clarity from 6 fields at x200 for each IHC specimen, using a CCD digital camera (Nikon, DXM1200). Expression analysis was performed to measure the expression area of TP, number of macrophages

and MVD in all cases, using 'Win ROOF' (version 5.7; Mitani Corp., Osaka, Japan) computer software. The digitized data of the expression area were measured and averaged. The expression of TP often showed not only cancer cells, but also cancer stroma cells, such as macrophages, fibroblasts or vessels. The expression of TP was investigated, including both cancer cells and stroma cells.

Determination of co-expression of TP and CD68. We extracted the digital data of the expression levels using the following image analysis systems in all IHC specimens. Co-expression of TP and CD68 was revealed by the Nuance System (Cambridge Research and Instrumentation Inc., Woburn, MA, USA) (32). Double-stained slides for TP and CD68 antibodies were imaged at x200 through a liquid crystal tunable filter. TP and CD68 were labeled with a very intensely purple (VIP) substrate kit (Vector Laboratories, Burlingame, CA, USA) and
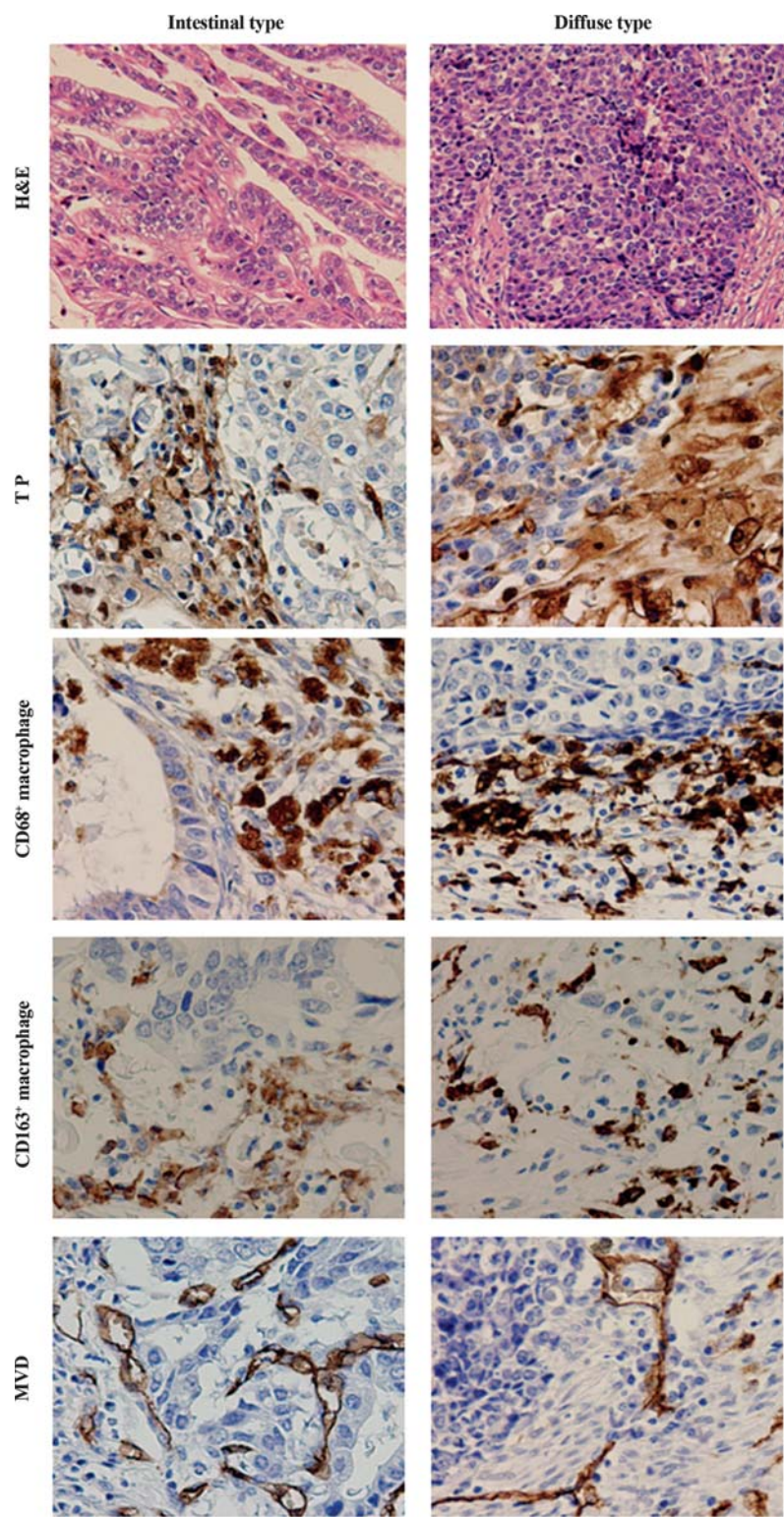
Figure 2. Representative images of immunohistochemical analysis of gastric cancer patients with intestinal and diffuse type (x200). Expression of TP, CD68, CD163 and CD34 is observed in cancer stroma in both histological types.

DAB as chromogen, respectively. Counterstaining was with hematoxylin. Multispectral image data were acquired from 420 to 720 nm at x200 in 3 fields from double-stained slides of 10 patients selected at random. Spectral unmixing was accomplished using Nuance software v2.8 and pure spectral libraries of individual chromogens. Expression areas of TP, CD68 and TP-CD68 co-expression were evaluated using Nuance software.

Statistical analysis. Associations among TP, CD68, CD163 and CD34 were evaluated using Spearman's rank correlation coefficients. The overall survival was defined as days from surgery until death due to any cause. Log-rank tests and Kaplan-Meier methods were applied to examine the effect of TP (CD68, CD163 and CD34) on overall survival classifying patients into negative and positive according to their TP (CD68, CD163 and CD34) were less than median or equal
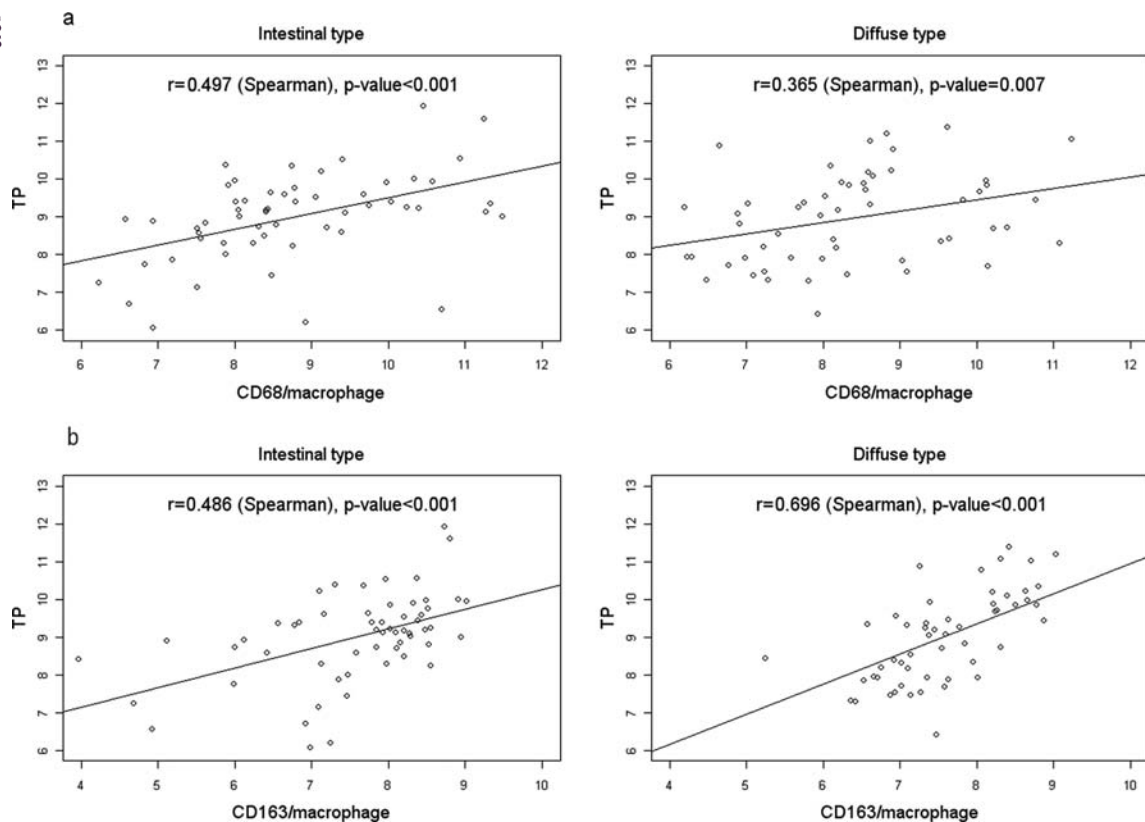
Figure 3. Correlation with TP and infiltrating macrophages (CD68 and CD163) in intestinal type (a) and diffused type (b) of gastric cancer.

to or more than median respectively. In addition, the Cox proportional hazards models were applied to examine the effect of TP (CD68, CD163 and CD34) on overall survival adjusting for possible confounding factors. The effects of TP (CD68, CD163 and CD34) on lymph node metastasis, liver metastasis and peritoneal dissemination were examined by logistic regression. In logistic regression analysis and Cox regression analysis, TP, CD68, CD163 and CD34 were not categorized into two categories of negative and positive and were log-transformed since they were highly skewed. When adjusting for possible confounding factors, Stage was not adjusted for since it might be an intermediate variable between TP (CD68, CD163 and CD34) and overall survival (33). Statistical analysis was performed by SAS version 9.1 (SAS Institute Inc., Cary, NC), StatXact (Cytel Inc., Cambridge, MA) and R version 2.8.1.

Results

Expression of TP in macrophage-like cell lines. TP is known to be expressed in both cancer cells and macrophages in the tumor stroma of gastric cancer (4). We first examined the expression of TP in human gastric cancer cell lines and macrophage-like cells in culture. Western blot analysis demonstrated no apparent expression of TP in any of the gastric cancer cell lines (Fig. 1a). By contrast, macrophage-like THP-1 and U937 cells expressed TP. Expression of TP was further stimulated by the inflammatory cytokine interferon γ (Fig. 1b and c). TP was expressed rather specifically in macrophages relative to its expression in gastric cancer cells, consistent with the results of our previous study (10).

Expression of TP in infiltrating macrophages in relation to microvessel density in intestinal and diffuse type gastric cancer. To determine whether TP could be a specific marker enzyme of macrophages, we examined the expression of TP in tumor stromal macrophages as well as cancer cells in clinical specimens of gastric cancer using IHC. Fig. 2 shows representative IHC images for TP, CD68^{+} and CD163^{+} macrophages and MVD in gastric cancers. TP was expressed mainly in cancer stromal cells. The numbers of infiltrating macrophages and MVD were found to be higher in cancerous than in non-cancerous regions. Box plots based on quantitative determination of the expression levels of various biomarkers by IHC analysis showed that the expression levels of TP, CD68^{+} macrophages, CD163^{+} macrophages and MVD were all similar between intestinal-type and diffuse-type gastric cancers (data not shown).

Close association of TP with infiltrating macrophages and TAMs in gastric cancer. CD68 is a known marker by which all monocytes/macrophages can be recognized. By contrast, CD163 is monocyte/macrophage-specific membrane protein that is closely associated with the anti-inflammatory M2 macrophage phenotype, and is a useful marker for distinguishing M2 macrophages that are linked to TAMs from pro-inflammatory M1 macrophages (18). Fig. 3 shows cross-plots between TP and infiltrating CD68^{+} and CD163^{+} macrophages with Spearman's rank correlation coefficents in patients with both intestinal and diffuse type gastric cancer. There was a positive correlation between TP and CD68 in both intestinal type (Spearman's rank correlation r=0.497, p<0.001) and diffuse type (r=0.365, p=0.007) cancer. Furthermore, there
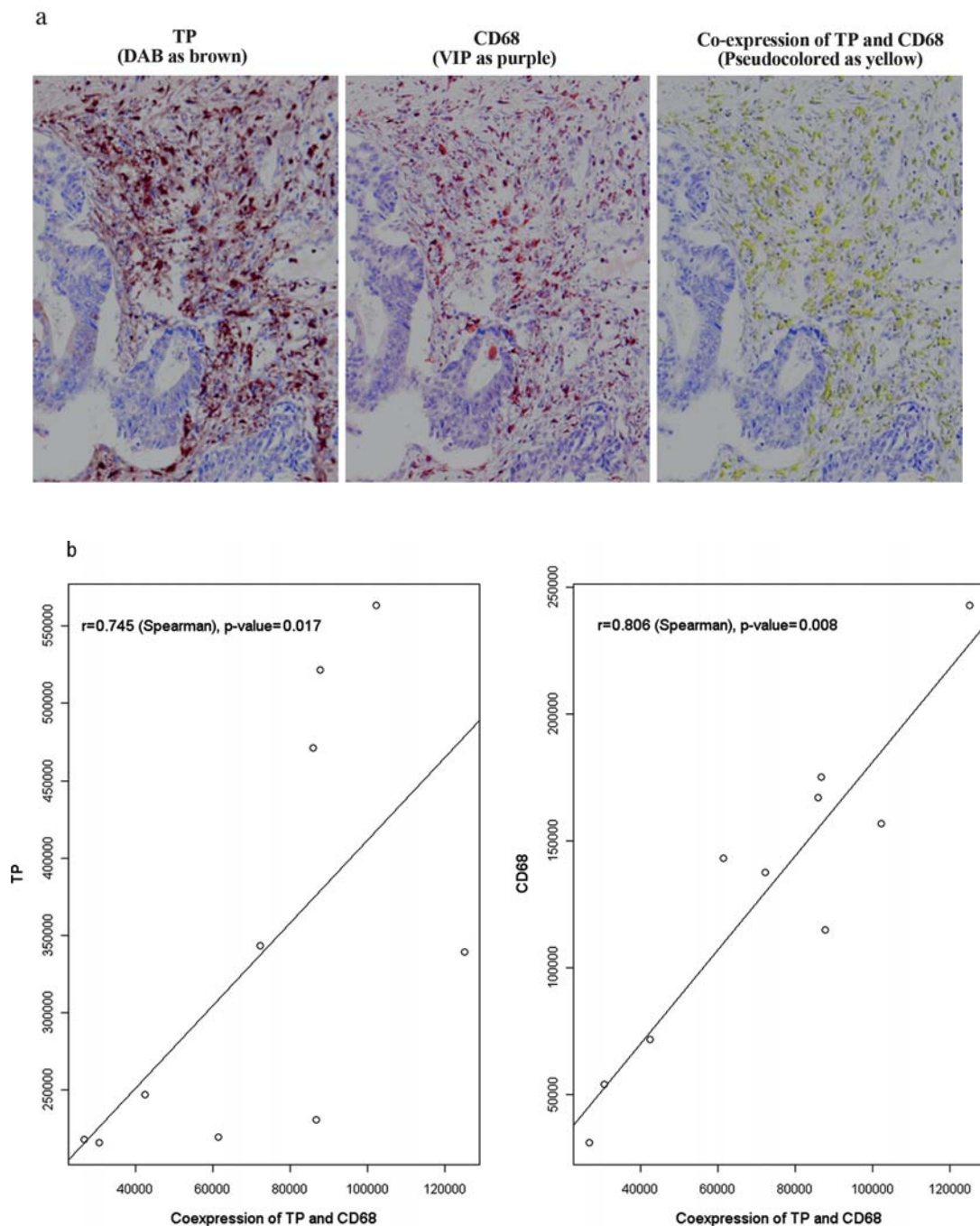
Figure 4. The quantitative analysis of close association of TP expression and CD68+ macrophage in IHC images of gastric cancer. CD68+ macrophage was labeled with VIP (purple) and TP was labeled with DAB (brown), and the co-expression of CD68 and TP was pseudocolored as yellow (a). Panel b is a cross plot of the average co-expression area of CD68 and TP (y-axis) over the average expression area of CD68 (x-axis) was presented for 10 randomly selected patients. The average expression area was calculated from three points for each specimen (b).

was a positive correlation between TP and CD163 in both intestinal-type (r=0.486, p<0.001) and diffuse type (r=0.696, p<0.001) cancer. Thus in gastric cancers there was a positive correlation between TP and infiltrating macrophages/TAMs.

Fig. 4a shows the expression of TP in macrophages in representative IHC images. TP was found to be expressed in macrophages that infiltrated gastric cancers. TP was labeled brown with DAB, and CD68+ macrophages were labeled purple with VIP. Co-expression of TP and CD68 was pseudo-colored in yellow, indicating that most macrophages expressed TP (TP-positive macrophages). Quantitative analysis of speci-

mens from 10 patients indicated that the area of co-expression of CD68 and TP showed a statistically significant positive correlation with the area of expression of DAB-labeled TP (r=0.745, p=0.017) and VIP-labeled CD68 (r=0.806, p=0.008) (Fig. 4b).

TP and infiltrating macrophages affect tumor angiogenesis in gastric cancers. We then investigated the relationship between TP, infiltrating macrophages and MVD in gastric cancers. Fig. 5 shows cross-plots among TP, infiltrating macro-phages and MVD with Spearman's rank correlation co-

Correlation of TP, MVD and infiltrating macrophage with lymph node metastasis, liver metastasis and peritoneal dissemination in intestinal type.

	Intestinal type of gastric cancer		
	P-value	OR	95% CI
Lymph node metastasis			
TP	0.040	1.898	(1.029, 3.502)
CD68+ macrophage	0.093	2.091	(0.885, 4.943)
CD163+ macrophage	0.003	4.307	(1.657, 11.197)
MVD	0.431	1.531	(0.530, 4.417)
Liver metastasis			
TP	0.326	1.426	(0.702, 2.894)
CD68+ macrophage	0.103	2.166	(0.856, 5.481)
CD163+ macrophage	0.856	0.932	(0.436, 1.991)
MVD	0.059	3.029	(0.959, 9.564)
Peritoneal dissemination			
TP	0.612	0.818	(0.377, 1.777)
CD68+ macrophage	0.149	2.299	(0.743, 7.115)
CD163+ macrophage	0.749	1.166	(0.456, 2.979)
MVD	0.660	0.714	(0.158, 3.216)

Odds ratio (OR) and its 95% CI are those of patients with 75% quantile of TP, macrophage number and MVD to those with 25% quantile. OR is calculated by fitting logistic regression model with TP, macrophage number and MVD, age and gender, where TP, macrophage and MVD were log-transformed.

efficients in intestinal and diffuse type gastric cancers. There was a significant positive correlation between TP and MVD in intestinal type (r=0.338, p=0.010) gastric cancer, but not diffuse type (r=0.209, p=0.129) gastric cancer (Fig. 5a). There was a positive correlation between CD68+ macrophages and MVD in both intestinal (r=0.750, p<0.001) and diffuse type (r=0.553, p<0.001) gastric cancer (Fig. 5b). Similarly, there was a positive correlation between CD163+ macrophages and MVD in both intestinal (r=0.307, p=0.021) and diffuse type (r=0.272, p=0.046) cancer (Fig. 5c). Thus, infiltrating CD68+ and CD163+ macrophages are positively associated with MVD in both intestinal and diffuse type gastric cancer.

TP and infiltrating macrophages are associated with metastasis and/or survival of cancer patients. We examined whether lymph node metastasis, liver metastasis and peritoneal dissemination were associated with TP, infiltrating macrophages and MVD (Table II). In intestinal type cancer, TP (p=0.040) and CD163+ macrophages (p=0.003) were significantly associated with the occurrence of lymph node metastasis. By contrast, TP, infiltrating CD68+ and CD163+ macrophages and MVD were unassociated with metastases in diffuse type cancer (data not shown).

We also examined whether the expression of TP, infiltrating macrophages and MVD was associated with the overall survival of gastric cancer patients. P-values of the log-rank tests for TP, infiltrating macrophages and MVD are summarized in Table III. In intestinal type cancer, the association of CD68+ macrophages was statistically significant (p<0.001), and that of MVD was nearly significantly (p=0.060). Table III also gives the p-values and hazard ratio estimates with Cox regression adjustment for age and gender as possible confounding factors. The number of CD68+ macrophages had a significant effect on overall survival (p=0.010), and the p-value for MVD was close to statistical significance (p=0.060) even after adjusting for possible confounding factors.

Kaplan-Meier estimates of overall survival for patients with intestinal type and diffuse type cancer are presented in Fig. 6 in relation to CD68+ macrophage status. Patients with the intestinal type and an increased number of CD68+ macrophages showed poor overall survival (Fig. 6a), whereas for patients with the diffuse type there was no apparent association of CD68+ macrophages with overall survival (Fig. 6b).

Table III. Effects of TP, infiltrating macrophage number and MVD on overall survival by log-rank test and Cox regression in gastric cancers.

	Intestinal type				Diffuse type			
	Log-rank test	Cox regression			Log-rank test	Cox regression		
	P-value	P-value	HR	95% CI	P-value	P-value	HR	95% CI
TP	0.476	0.845	0.965	(0.678, 1.375)	0.380	0.817	0.935	(0.531, 1.647)
CD68+ macrophage	<0.001	0.010	2.045	(1.183, 3.535)	0.487	0.587	0.847	(0.467, 1.539)
CD163+ macrophage	10.089	0.242	1.300	(0.838, 2.017)	0.918	0.752	0.926	(0.574, 1.493)
MVD	0.060	0.060	1.938	(0.973, 3.860)	0.089	0.249	0.727	(0.423, 1.250)

Hazard ratio (HR) and its 95% CI are those of patients with 75% quantile of TP, macrophage number and MVD to those with 25% quantile. HR is calculated by fitting Cox regression model with TP, macrophage and MVD, age and gender where TP, macrophage and MVD were log-transformed.
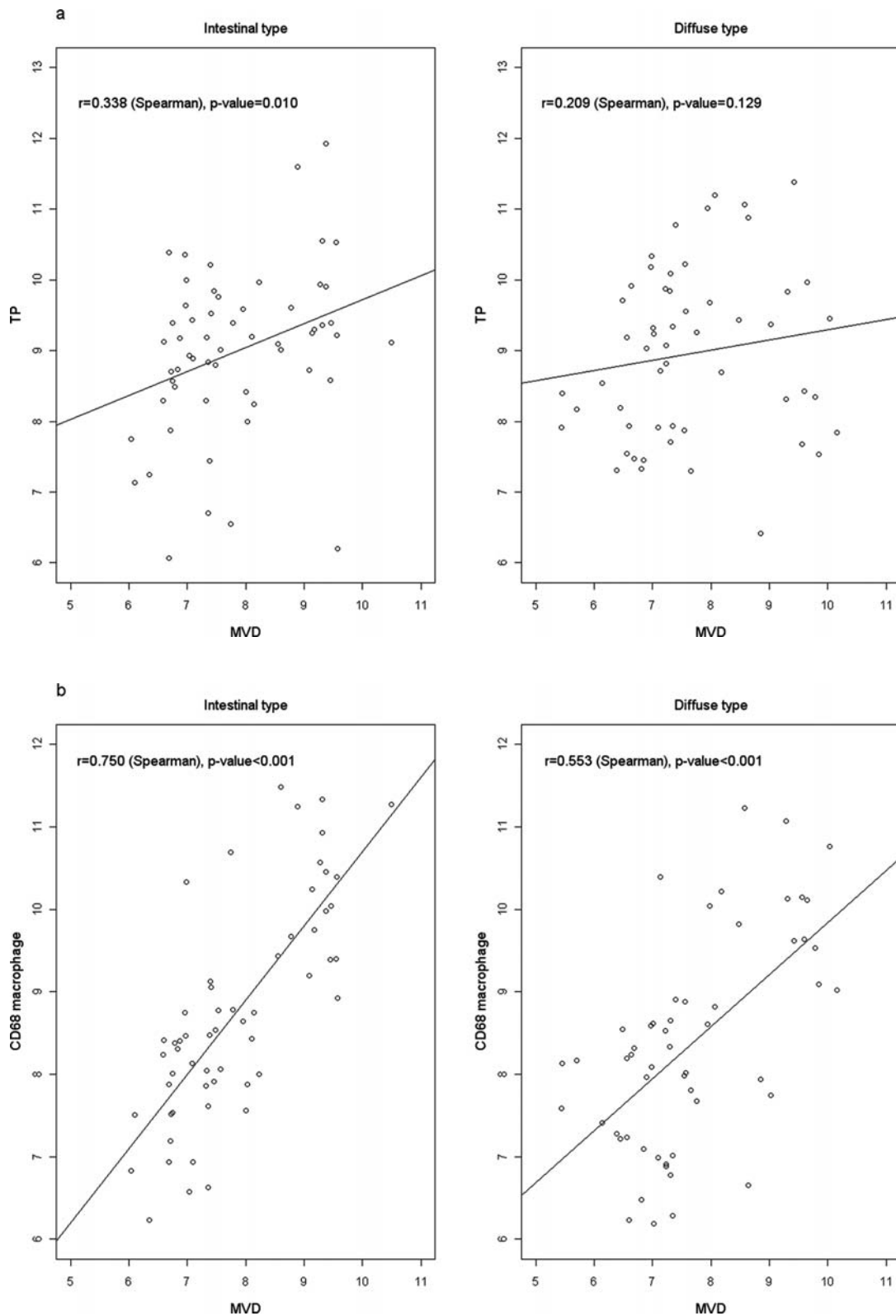
Figure 5. a and b. Correlation with MVD and TP (a) or CD68+ macrophage (b).

Discussion

In our present study, we demonstrated that expression of TP in macrophages may affect tumor angiogenesis and prognosis in patients with intestinal type gastric cancer. i) Expression of TP was observed in macrophages infiltrating both intestinal and diffuse type gastric cancer; ii) Expression of TP and the number of infiltrating macrophages were significantly associated with tumor angiogenesis in intestinal-type gastric cancer; iii) The number of infiltrating macrophages was significantly associated with overall survival in patients with intestinal-type gastric cancer.

Infiltrating macrophages were closely correlated with TP expression and tumor angiogenesis. Our present findings
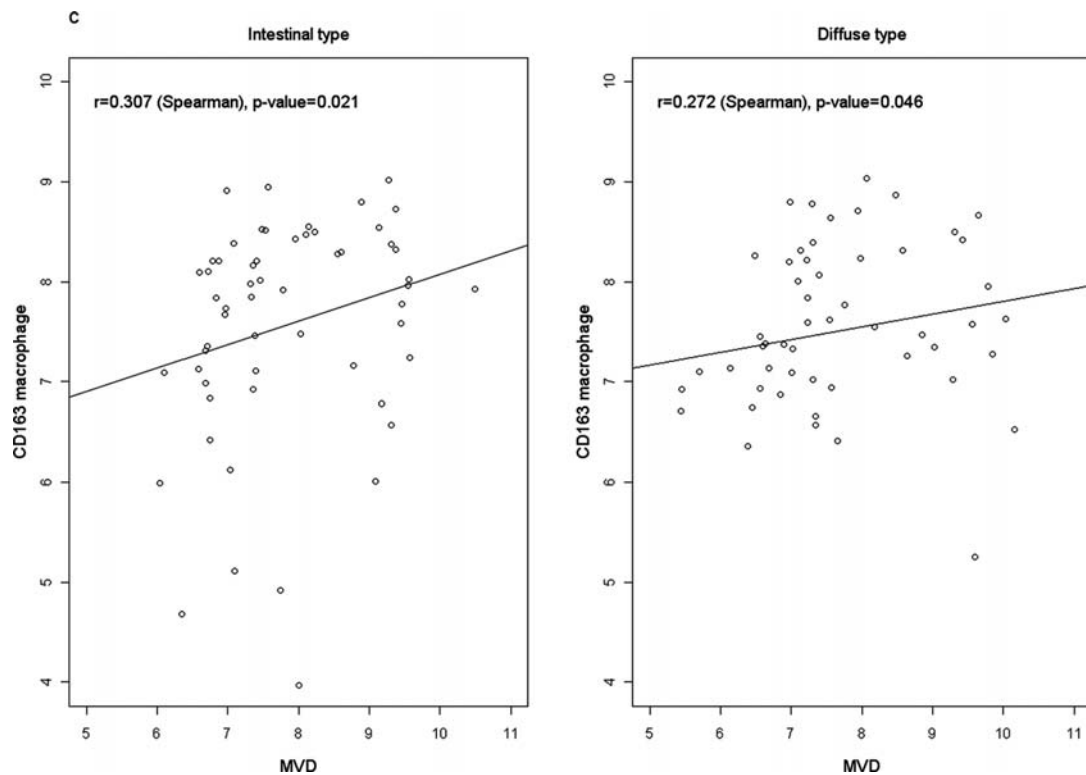
Figure 5. Correlation with MVD and TP (a) or CD68+ macrophage (b) or CD163+ macrophage (c) in intestinal type (left) and diffuse type (right) of gastric cancer.
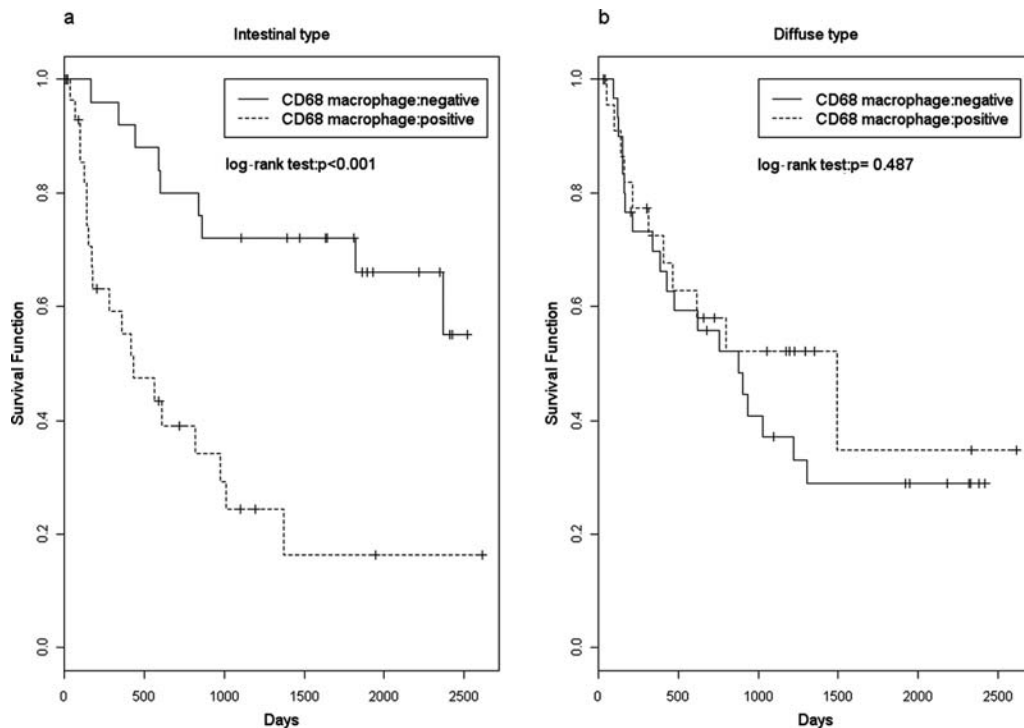

Figure 6. Kaplan-Meier estimators for overall survival in intestinal type (a) and diffuse type (b) of gastric cancer according to higher (---) and lower (—) infiltration of macrophage status with p-value by the log-rank test. In patients with higher number of infiltrating macrophages, increased number of macrophages have statistically significantly poor prognosis for overall survival.

suggest that the expression of TP in infiltrating macrophages and TAMs affects tumor angiogenesis in intestinal-type gastric cancer. The presence of extensive macrophage/TAM infiltration in tumors has often been shown to correlate with poor prognosis in cancers of the breast, cervix and bladder, and also in melanoma (19). However, the possible role of

infiltrating macrophages/TAMs in the progression of gastric cancer remains unknown. Concerning possible predictive markers of macrophages or TAMs in gastric cancer, Migita *et al* have reported that the number of CD68+ macrophages tends to be associated with better patient survival, but not to a significant degree (29). Furthermore, Ohno *et al* have reported that the degree of CD68+ macrophage infiltration in cancer cell nests is a significant predictor of better prognosis in patients with gastric cancer (28). At present, it remains unclear why infiltration of CD68+ macrophages tends to be a marker of favorable prognosis only in gastric cancer, whereas it is a marker of poor prognosis in other human malignancies (see Introduction) (19). In the present study, however, the number of CD68+ macrophages was found to be significantly correlated with poor prognosis in patients with intestinal type cancer, but not in patients with diffuse type cancer, suggesting that infiltration of tumor-associated macrophages could affect malignant progression and prognosis according to the histological type of gastric cancer. Furthermore, tumor angiogenesis was also closely associated with infiltration of CD68+ or CD163+ macrophages (Fig. 5). Based on these findings, we consider that infiltration of macrophages/TAMs affects the prognosis of intestinal type gastric cancer. However, a more detailed study will be necessary to clarify how tumor-associated macrophages differentially modulate prognosis or malignant progression in intestinal-type and diffuse-type gastric cancer.

Akiyama and colleagues have previously reported rather specific expression of TP in macrophages relative to cancer cells in differentiated gastric adenocarcinoma (4,8), and patients with TP-positive carcinomas have a poorer prognosis than those with TP-negative differentiated adenocarcinomas. Our present study also demonstrated that tumor-infiltrating macrophages are a potent prognostic marker in intestinal-type, but not diffuse-type, gastric cancer. Inflammatory stimuli markedly enhance the expression of TP in macrophages through transcriptional activation (Fig. 1) (10). Expression of TP results in enhanced production of reactive oxygen species by 2-deoxy-D-ribose, a downstream mediator of TP, indicating that TP induces oxygen stress (2,4,34). TP-positive macrophages are thus expected to be highly active, not only in the production of reactive oxygen, but also various angiogenesis factors, growth factors, chemokines and proteases, indicating that such macrophages are highly angiogenic. In fact, through cross-talk with cancer cells, macrophages are induced to undergo transformation into tumor-supporting macrophages that actively stimulate angiogenesis and cancer cell invasion (13,14,20). Thus, the expression of TP in macro-phages might play a key role in angiogenesis, tumor growth and anti-apoptosis in malignant cancers in the stomach as well as those of the colon and other organs, possibly through the enhanced production of angiogenesis factors by macrophages (2,4). In both intestinal and diffuse type gastric cancer, the number of macrophages was significantly correlated with tumor angiogenesis, and TP expression was also positively correlated with the presence of both CD68+ and CD163+ macrophages. By contrast, TP expression was significantly associated with angiogenesis only in intestinal type gastric cancer, and not in the diffuse type. A further study will be needed to clarify why TP expression thus differentially affects angiogenesis in gastric cancer depending on its histological type.

In conclusion, our present study demonstrates a close association of TP expression in tumor-infiltrating macrophages with angiogenesis in gastric cancer, lending support to the idea that TP-positive macrophages promote angiogenesis and metastasis in this cancer. TP expression in macrophages could be a useful and novel biomarker for diagnosis of gastric cancer and the development of new therapeutic strategies.

Acknowledgements

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