Review Article

The role of thymidine phosphorylase, an angiogenic enzyme, in tumor progression

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(Received August 12, 2004/Revised August 27, 2004/Accepted August 27, 2004)

Thymidine phosphorylase (TP), an enzyme involved in pyrimidine metabolism, is identical with an angiogenic factor, platelet-derived endothelial cell growth factor (PD-ECGF). TP is overexpressed in various tumors and plays an important role in angiogenesis, tumor growth, invasion and metastasis. The enzymatic activity of TP is required for the angiogenic effect of TP. A novel, specific TP inhibitor, TPI, inhibits angiogenesis induced by overexpression of TP in KB/TP cells (human KB epidermoid carcinoma cells transfected with TP cDNA), as well as the growth and metastasis of KB/TP cells *in vivo*. 2-Deoxy-D-ribose, the degradation product of thymidine generated by TP activity, has both angiogenic and chemotactic activity. Both 2-deoxy-D-ribose and TP inhibit a hypoxia-induced apoptotic pathway. These findings suggest that 2-deoxy-D-ribose is a downstream mediator of TP function. 2-Deoxy-L-ribose, a stereoisomer of 2-deoxy-D-ribose, inhibits the promotion of angiogenesis, tumor growth and metastasis by TP. Although the mechanism of the action of 2-deoxy-D-ribose is still unknown, 2-deoxy-L-ribose may inhibit the physiological activities of 2-deoxy-D-ribose, and consequently those of TP. Inhibition of TP activity and function appears to be a promising approach for the chemotherapy of various tumors. (Cancer Sci 2004; 95: 851–857)

Thymidine phosphorylase (TP; EC 2.4.2.4) catalyzes the reversible phosphorolysis of thymidine, deoxyuridine and their analogs to their respective bases and 2-deoxyribose-1-phosphate.[1–3] It also catalyzes deoxyribosyl transfer from one deoxynucleoside to another base to form a second nucleoside.[4–6] The enzyme consists of two identical subunits, the molecular weight of each being about 55,000 daltons in mammals.[7] TP is identical with platelet-derived endothelial cell growth factor (PD-ECGF),[4] and the enzymatic activity of TP is required for angiogenesis.[9] Plasma TP activity has been shown to be elevated in cancer patients and tumor-bearing animals, and its expression in various solid tumors is higher than that in the adjacent normal tissues.[10]

TP stimulates chemotaxis of endothelial cells *in vitro* and angiogenic activity *in vivo*.[11–13] Among the degradation products of thymidine generated by TP, 2-deoxy-D-ribose (D-dRib), a dephosphorylation product derived from 2-deoxy-D-ribose-1-phosphate, also has chemotactic activity *in vitro* and angiogenic activity *in vivo*.[11] These findings suggest that the enzymatic product of TP may stimulate the chemotaxis of endothelial cells and possibly other cells, causing angiogenesis.

Angiogenesis is controlled by various pro-angiogenic and anti-angiogenic molecules. It is believed that changes in the angiogenic balance mediate the angiogenic switch.[14] Tumors produce a variety of factors that cause an angiogenic switch, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and transforming growth factor (TGF)-β, besides TP. Many direct angiogenesis inhibitors, such as vitaxin, angiostatin and other 'statins,' that prevent vascular endothelial cells from proliferating and/or migrating have been investigated. Some indirect angiogenesis inhibitors, such as ZD1839 (Iressa), Herceptin and interferon (IFN)-α, that suppress the expression of or inhibit the activity of pro-angiogenic tumor proteins, are already used for the treatment of cancer patients.[15] A more detailed understanding of the molecular basis for angiogenesis will aid the development of improved anti-angiogenic agents.

We therefore investigated the role of TP and D-dRib in the progression of tumors. We have developed agents that suppress the function of TP and D-dRib, and which may therefore be useful for anti-tumor therapy.

1. TP and Gliostatin are identical with PD-ECGF

PD-ECGF stimulates chemotaxis of endothelial cells *in vitro* and angiogenesis *in vivo*.[12, 13] Although it is found extracellularly, it lacks a hydrophobic signal sequence and is not a classical secretory protein. Two other factors, TP and gliostatin, that have previously been described in connection with other biological activities, have been identified as being identical with PD-ECGF.

Several lines of evidence indicate that human TP and PD-ECGF are identical. The amino acid sequence deduced from the nucleotide sequence of a partial human TP cDNA is identical with the sequence of PD-ECGF (residues 14–244). The amino acid sequence of all four lysyl endopeptidase fragments of TP can be aligned with the amino acid sequence of PD-ECGF (residues 125–139, 140–157, 158–178 and 236–244, respectively). Our data indicate that residues 125–244 of PD-ECGF are identical with the sequence of TP.[8] The N-terminal amino acid sequence of TP is identical with the amino acid sequence deduced from PD-ECGF cDNA, except for deletion of the first methionine and acetylation of the second alanine.[16] Conversely, PD-ECGF has been shown to exhibit TP activity. Lysates of COS cells transfected with PD-ECGF cDNA exhibited a high TP activity.[17] Recombinant PD-ECGF also displayed a TP activity whose specific activity was similar to that of TP.[17] Usuki *et al*. reported that human PD-ECGF has TP activity.[18] The accumulated data thus indicate that human TP is identical with PD-ECGF.

Gliostatin, a polypeptide growth inhibitor with a ho-

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modimeric structure comprising two 50-kDa subunits, acts on both astrocytes and astrocytoma cells. The amino acid sequences of 13 tryptic peptides of gliostatin, including the amino terminus, are identical with those of PD-ECGF. Gliostatin and PD-ECGF, purified from human placenta, both caused growth inhibition of glial cells and growth promotion of endothelial cells, and exhibited similar values of half-maximal dose for these activities.[19] The immunochemical identity of both factors was confirmed by a two-site enzyme immunoassay.[20] These results suggested that gliostatin is identical with PD-ECGF, and consequently, with TP.

2. The enzymatic activity of TP is required for TP-induced angiogenic activity

We have confirmed that TP does have an angiogenic activity using chorioallantoic membrane (CAM), gelatin sponge, rat corneal and mouse dorsal air sac assays. Our investigations have also shown that the enzymatic activity of TP is indispensable for the angiogenic activity. Thus, whereas wild-type TP can confer angiogenic activity on lysates of TP-transfected COS-7 cells, three different mutants of TP that lack enzymatic activity [K115E (Lys-115→Glu), L148R (Leu-148→Arg) and R202S (Arg-202→Ser)] could not. The requirement for TP activity for angiogenesis was further confirmed by the fact that an inhibitor of TP, 6-amino-5-chlorouracil, inhibited the angiogenic activity of purified TP.[9] Moghaddam *et al*. have also shown that TP is strongly angiogenic in a rat sponge and freeze-injured skin graft model, and have confirmed that the enzyme activity of TP is a condition for its angiogenic activity using neutralizing antibodies and site-directed mutagenesis.[21]

We have explored the mechanism by which TP activity might induce angiogenesis and have shown that an enzymatic product of TP is angiogenic. D-dRib, generated by TP-degradation of thymidine, has both angiogenic and chemotactic activity.[11] These results may explain why the enzyme activity of TP is indispensable to the angiogenic activity of TP. They also provide a basis for the design of inhibitors of TP activity that could suppress angiogenesis and consequently tumor progression in TP-expressing tumors.

3. Development of TP inhibitors

In collaboration with Taiho Pharmaceutical Co., Ltd., we have developed a novel selective inhibitor of TP, TPI [5-chloro-6-[1-(2-iminopyrrolidinyl)methyl] uracil hydrochloride (K_i=2×10^{-8} *M*)], that has several advantages over previously described inhibitors of TP (Fig. 1). TPI has a 1000-fold higher inhibitory activity than 6-amino-5-chlorouracil and does not inhibit uridine phosphorylase (up), another enzyme involved in pyrimidine nucleoside metabolism.[22] TPI partially suppressed the growth of TP-expressing tumors and completely suppressed angiogenesis induced by KB/TP in the mouse dorsal air sac assay model.[23]

Purine derivatives have also been shown to be useful for the inhibition of TP-induced angiogenesis. The purine derivative, 7-deazaxanthine (7DX) inhibited TP enzymatic activity in a concentration-dependent manner and also had a marked inhibitory effect on angiogenesis. 7DX was the first purine derivative shown to be a potent inhibitor of purified TP and angiogenesis (Fig. 1).[24] From 7DX as a lead compound, a novel type of inhibitor of TP, KIN59 (5′-*O*-tritylinosine), has been developed. KIN59 suppresses TP-triggered angiogenesis via a noncompetitive mechanism.[25] An orally administrable combination of α,α,α-trifluorothymidine (FTD) and TPI [a 1:0.5 mixture on a molar basis], named TAS-102, is currently undergoing clinical trials as a new anti-tumor drug preparation (Fig. 1). TAS-102 can inhibit liver metastasis. A particularly useful attribute of TAS-102 is that it shows a similar efficacy towards both 5-fluorouracil (5-FU)-resistant and parent cells in a FU-resistant xenograft model. 5-FU is widely used in the treatment of solid tumors, but the inherent or acquired resistance of certain tumors to 5-FU therapy is a major clinical problem. These findings suggest that TAS-102 is a promising candidate for clinical use.[26]

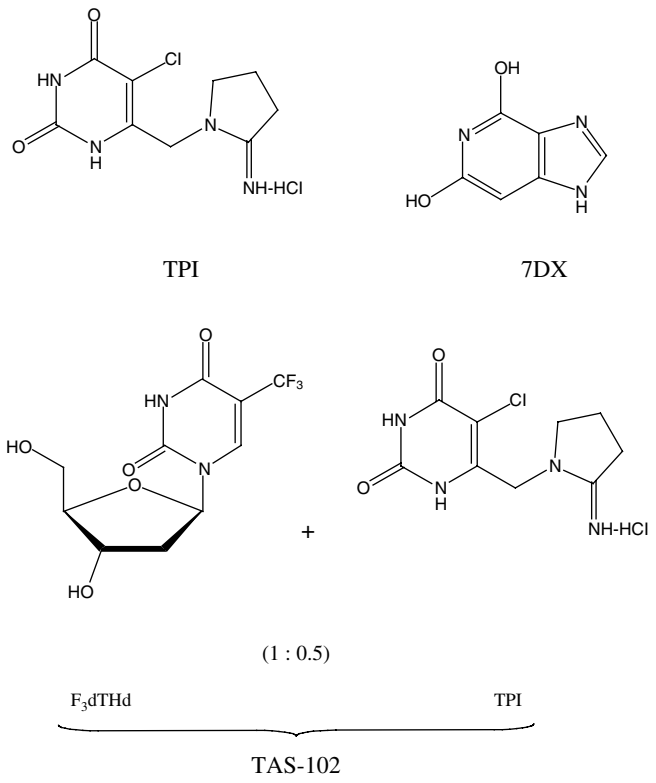

Fig. 1. Chemical structures of TPI, 7DX and TAS-102. TPI, an analog of uracil, has about a 1000-fold higher inhibitor activity than 6-amino-5-chlorouracil, one of the most potent TP inhibitors. 7DX is the first purine derivative shown to be a potent inhibitor of TP. TAS-102 is a new anti-tumor drug preparation composed of α,α,α-trifluorothymidine (FTD; 1 *M*) and a thymidine phosphorylase inhibitor (TPI; 0.5 *M*).

Recently the crystal structure of human TP complexed with TPI in an active (closed) confirmation has been reported.[27] The human TP protomer has a similar folding to that reported for *E. coli* TP (EcTP) and *B. stearothemophilus* pyrimidine nucleoside phosphorylase (BsPYNP), and comprises an α domain and a mixed α/β domain connected by three polypeptide loops. The α domain consists of six helices (1–4 and 8–9), and the α/β domain consists of a central mixed sheet (1–5, 13) surrounded by helices (5–7 and 10–16) and small antiparallel sheets (consisting of strands 6, 7, 9 and 11, and 8, 10 and 12 respectively) flanked by two helices (17 and 18). The three loops act as a hinge allowing the two domains to move between the open (inactive) and closed (active) conformations, bringing together the active site residues. The main structural differences between humanTP, EcTP and BsPYNP are in the α/β domain where humanTP has an additional helix, an extra turn in helix 16, and a more extended C-terminal region. The small anti-parallel sheet (8, 10 and 12) is present in both humanTP and BsPYNP, but not in EcTp.[27]

This information about the tertiary structure of humanTP provides a basis for further structure-based drug design studies.

4. TP and D-dRib inhibit hypoxia-induced apoptosis

We recently proposed a second, non-angiogenic role for TP in the progression of solid tumors. We found that the proportion of apoptotic cells in TP over-expressing tumors (KB/TP) was

significantly lower than in control KB/CV (TP-negative clone transfected with the vector alone) tumors under hypoxic conditions, as assessed by the TUNEL assay. TPI treatment abolished the difference in response between the two tumors.[28] Furthermore, both TP and D-dRib inhibited down-regulation of the pro-apoptotic bcl-2 and bcl-X_L, mitochondrial cytochrome *c* release and caspase 3 activation that were induced in HL60 cells under hypoxic conditions.[29] These data indicate that TP confers resistance to apoptosis induced by hypoxia, and that the enzymatic degradation of thymidine by TP is required for the inhibition of hypoxia-induced apoptosis. The finding that TP can confer resistance to apoptosis, in addition to its angiogenic activity, may explain why breast carcinoma cells that overexpress TP show enhanced growth, but no increased vessel density *in vivo*.[21] The dual activities of TP may also explain why patients with TP-positive tumors have a poorer prognosis than those with TP-negative tumors, and why microvessel density is not a significant prognostic factor in colorectal and renal cell carcinomas.[30, 31] However, further study is required to elucidate the exact mechanism of apoptosis inhibition by TP, as inhibition of Fas and cisplatin-induced apoptosis does not appear to require TP enzymatic activity.[32, 33]

5. The molecular basis for the function of D-dRib

Our findings described above suggest that D-dRib is a downstream mediator of TP functions. However, the mechanism of D-dRib action is unclear. It has been suggested that D-dRib may be an important energy source under hypoxic conditions.[34] If this is the case, then the mechanism by which the D-dRib analog 2-deoxy-L-ribose (L-dRib) suppresses tumor growth under hypoxic conditions could be via inhibition of D-dRib entry into glycolysis.[34] A second possibility, suggested by the same group, is that D-dRib may play a role in regulation of the expression levels of the angiogenic factors VEGF and interleukin-8 (IL-8) mRNA. TP activity can augment VEGF and IL-8 production and release from KB cells. Thus, thymidine induces oxidative stress in TP-overexpressing carcinoma cells and promotes secretion of VEGF and IL-8, as well as induction of matrix metalloproteinase-1[35]. The secreted protein levels of VEGF and IL-8 from KB/TP cells were higher than those from KB/CV cells under hypoxic conditions. Our results have confirmed and extended these findings, and we have shown that the addition of D-dRib to KB/CV cells under hypoxic conditions enhances the expression levels of VEGF and IL-8 mRNA. In contrast, treatment of KB/TP cells with L-dRib under hypoxic conditions led to a reduction in the secreted protein levels of both VEGF and IL-8.[36]

The biological functions of D-dRib may, however, be more complex than described above. Recent evidence suggests that D-dRib affects endothelial cell migration via activation of integrin downstream signaling pathways. Both TP and D-dRib stimulated the formation of focal adhesions and the activating tyrosine 397 phosphorylation of focal adhesion kinase (FAK) in human umbilical vein endothelial cells.[37] TP and D-dRib increased the association of both FAK and the focal adhesion-associated protein vinculin with integrin $\alpha5\beta1$ and, in intact cells, increased the co-localization of FAK with $\alpha5\beta1$. The induction of endothelial cell migration and FAK phosphorylation by TP and D-dRib was blocked by antibodies to the integrins $\alpha5\beta1$ and $\alpha v\beta3$, thereby directly linking the migration and signaling components of TP and D-dRib action. Cell surface expression of $\alpha5\beta1$ was also increased by TP and D-dRib. These experiments demonstrate a direct effect of TP and D-dRib on signaling pathways associated with endothelial cell migration. Recent investigations are beginning to shed some light on the signaling pathways through which D-dRib might exert its effects on integrin function. Rapamycin completely abrogated D-dRib-induced endothelial cell migration and aortic ring formation, correlating with a blockade of D-dRib-induced p70/s6 kinase activation in endothelial cells.[38] It has recently been demonstrated that FAK functions upstream of phosphatidylinositol 3-kinase (PI3K)/Akt in transducing a $\beta1$ integrin viability signal in collagen matrices.[39] D-dRib may therefore transmit signals for viability and angiogenesis, at least in part, through the FAK/PI3K/Akt signaling pathway.

6. TP (−/−) UP(−/−) double knockout mouse

In order to elucidate the physiological roles of TP, we generated mice deficient in the TP gene. Although TP activity was abrogated in the liver of these mice, it was fully maintained in the small intestine. Murine UP, unlike human UP, cleaves thymidine, as well as uridine. We therefore generated TP-UP double knockout ($TP^{-/-}UP^{-/-}$) mice. All TP activity was abrogated in $TP^{-/-}UP^{-/-}$mice. Plasma thymidine levels were more than 5-fold higher in $TP^{-/-}UP^{-/-}$ mice compared with wild-type mice, whereas plasma thymidine levels of $TP^{-/-}$ mice were only 2-fold higher than those of wild-type mice.[40] This is in accordance with previous data showing that treatment with TP inhibitors that completely inhibit the enzyme activity of TP enhances plasma thymidine levels. It has been reported that loss-of-function mutations in the TP gene lead to an increase in plasma thymidine levels and to the appearance of mitochondrial DNA abnormalities and mitochondrial neurogastrointestinal encephalomyopathy (MNGIE).[41] Although, we could not observe alterations of mitochondrial DNA or pathological changes in the muscles of the $TP^{-/-}UP^{-/-}$mice, brain MRI revealed high-intensity lesions on the T2 map in five out of six brains from $TP^{-/-}UP^{-/-}$mice. Ultrastructural observation revealed that the myelin sheaths of the enlarged fibers in the $TP^{-/-}UP^{-/-}$ mice have segmental dilatation in the tangential view and uneven protrusions in the transverse view. The ultrastructure of the axons was conserved and such dilatation and protrusion were due to abnormal myelin structures.[40] The major gross defect in $TP^{-/-}UP^{-/-}$mice was therefore neurological. This double knockout mouse may be useful to elucidate the role of TP expressed in cancer cells in tumor progression.

7. Overexpression of TP in various malignant tumors

The role of TP in modulating angiogenesis, as well as apoptosis under hypoxic conditions, suggested that TP plays an important role in malignant tumors. This idea has been borne out by a number of investigations. A comparison of the activity and expression of TP in carcinomas of the esophagus, stomach, colorectum, pancreas and lung versus that in the adjacent non-neoplastic tissues indicated that TP activity was significantly higher in the carcinomas. The expression level of TP, detected by immunoblotting, correlated well with the activity of TP.[42] The proportion of TP-positive tumors in differentiated gastric adenocarcinomas was also higher than that in undifferentiated gastric adenocarcinomas. Patients with TP-positive carcinomas had a poorer prognosis than those with TP-negative differentiated adenocarcinomas, suggesting that TP must play some role in the development or malignancy of tumors.

In studies aimed at analyzing a potential correlation between TP and malignancy it was shown that the mean microvessel count in TP-positive colorectal carcinoma specimens was higher than that in TP-negative carcinoma specimens. The number of TP-positive tumors was in accordance with the microvessel count.[30] Positive TP counts also showed highly significant statistical associations with tumor size, extent of invasion, lymph node metastasis, lymphatic invasion and venous invasion in colon cancer. Cox regression analysis revealed that TP expression was prognostic for poor disease outcome after adjustment for Dukes' stage and microvessel count.[30] TP was also shown to be expressed in infiltrating cells in most of the colon cancer specimens, but rarely in tumor epi-

thelium, suggesting that infiltrating cells expressing TP may contribute to angiogenesis.[43] In renal cell carcinomas (RCCs) higher levels of TP expression were associated with more extensive angiogenesis, poor clinical and laboratory findings, and unfavorable clinical outcome.[29]

Tumor-infiltrative macrophages or lymphocytes in the lymph nodes, alveolar macrophages and Kupffer cells also express high levels of TP.[10, 42] TP was expressed mainly in the invasive edges of tumors and was expressed more frequently in macrophages than in tumor cells (Fig. 2). TP expressed in macrophages has been suggested to be correlated with microvessel count and to play an important role in tumor invasiveness and progression in differentiated gastric adenocarcinoma (Fig. 3).[44] Some cytokines, tumor necrosis factor, IL-1α, and IFN-γ, have been reported to upregulate TP expression,[45] and IFN-γ was suggested to be the most effective cytokine in enhancing the expression of TP in cultured human monocyte U937 cells.[46] The presence of the sequence containing the γ-activated sequence-like element was essential for IFN-γ-dependent activation of the TP gene.[46] IFN-γ could therefore be a mediator of TP expression in infiltrated monocyte/macrophages. Tumor-associated macrophages that express TP may thus be a good target for chemotherapy of TP-positive solid tumors.

8. The role of TP in invasion and metastasis

Inhibitors of TP and D-dRib have been used to analyze the role of TP in invasion and metastasis in *in vivo* model systems. TPI inhibited the high chemotactic motility and basement membrane invasion of TP-overexpressing KB/TP cells. In nude mice, oral administration of TPI suppressed not only macroscopic liver metastases of highly metastatic KB/TP cells, but also the level of human β-globin, a molecular marker of micrometastases, in the liver of mice. These findings demonstrate that TP plays a key role in the invasiveness and metastasis of TP-expressing solid tumors and suggest that TPI might be a novel antimetastatic agent for blood-borne metastasis.[47]

D-dRib promotes angiogenesis and chemotaxis of endothelial cells and also confers resistance to hypoxia-induced apoptosis in some cancer cell lines. L-dRib, a stereoisomer of D-dRib, both inhibits D-dRib-dependent anti-apoptotic effects and suppresses the growth of KB/TP cells transplanted into nude mice. L-dRib also has the ability to suppress invasion and metastasis of KB/TP cells.[48]

A correlation between the expression and activity of TP and invasion and metastasis is also suggested from the analysis of clinical samples. TP-positive differentiated adenocarcinomas invaded more deeply than the TP-negative ones, but this was not the case with undifferentiated adenocarcinomas. TP was expressed mainly in the invasive edges of tumors where it would be most likely to exert an effect on angiogenesis.[44] Significant correlations were observed between TP expression and extrapancreatic neural plexus invasion and the presence of postoperative hepatic metastases in patients with ductal adenocarcinoma of the pancreas.[49] TP mRNA was elevated in invasive bladder cancer, being 33-fold higher than in superficial tumors and 260-fold higher than in normal bladder.[50] Transfection of TP into the RT112 bladder cancer cell line transformed it from a superficial to an invasive phenotype.[51] These results strongly suggest that TP is involved in invasion and metastasis of some solid tumors.

9. Correlation between TP activity and sensitivity to chemotherapy

The enzymatic activity of TP has been suggested to modify pyrimidine antimetabolites used for tumor therapy. Patterson *et al*. reported that recombinant TP directly catalyses the phospho-

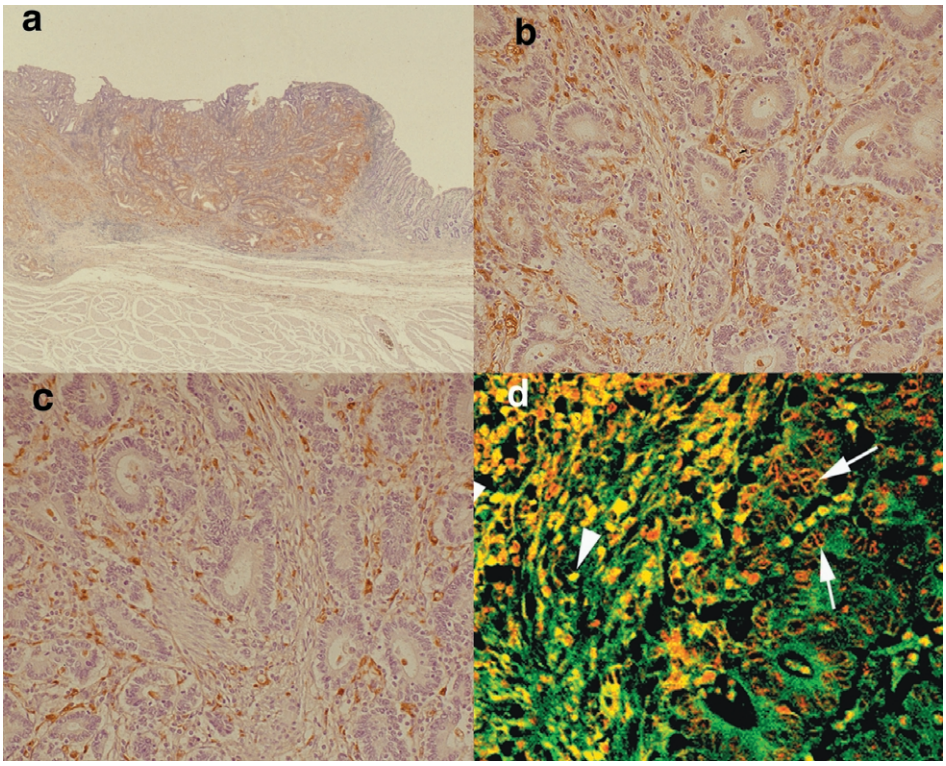

Fig. 2. Immunostaining of TP and CD68. a) TP was expressed strongly at the invasive edge of the tumor in a ×40 field. b) TP staining (×100). c) CD68 staining (×100). d) Double staining for TP and CD68 (×100). The cells that expressed TP were stained red (arrows), and the stromal cells that expressed CD68 (specific for macrophages) were stained green. Many stromal cells were stained yellow (arrowheads), suggesting that they express TP.

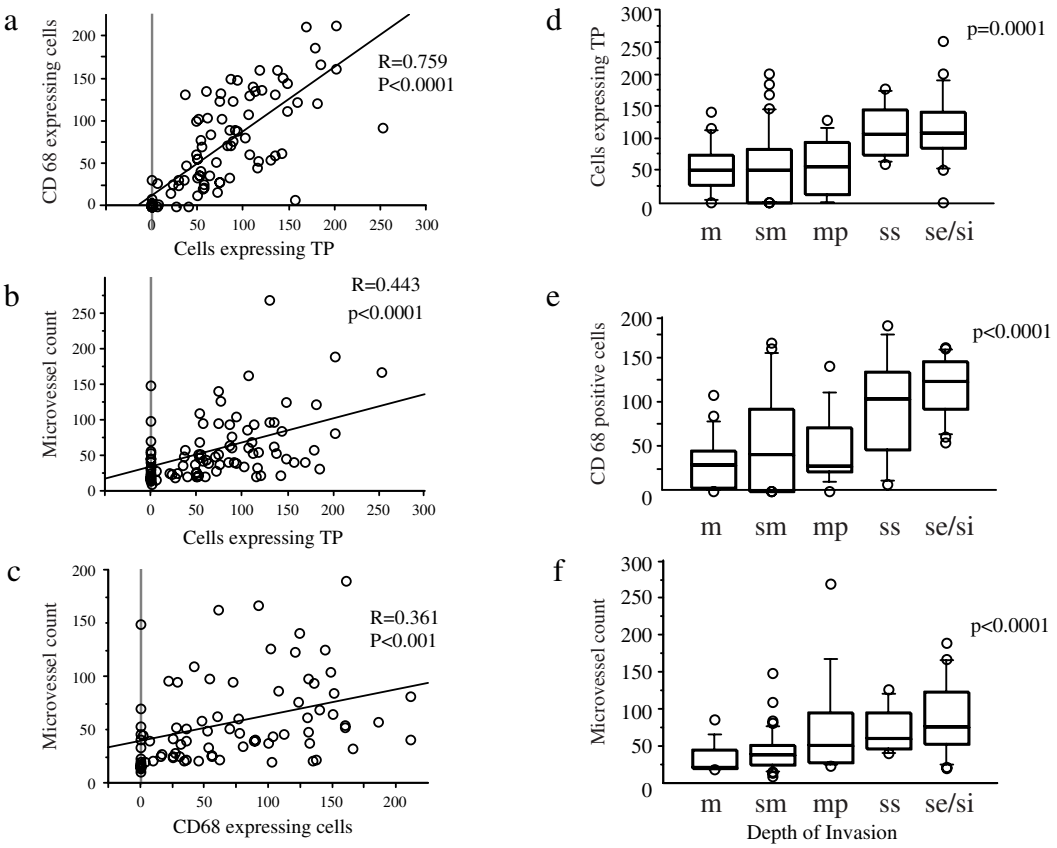

Fig. 3. Relation between the expression of TP and CD68 and the microvessel count, and relation between depth of invasion and TP or CD68 expression and microvessel count in differentiated adenocarcinoma. a) Correlation between cells expressing TP and CD68. b) Correlation between cells expressing TP and the microvessel count. c) Correlation between cells expressing CD68 and the microvessel count by Spearman rank correlation test (R) and linear regression analysis. Correlation between depth of invasion and d) cells expressing TP; e) cells expressing CD68; and f) the microvessel count (Mann-Whitney *U* test). The boxes correspond to the interquartile ranges, with the lower boundary representing the 25th percentile and the upper boundary representing the 75th percentile. The lines in the boxes represent the mean values. The whiskers represent the 10th and 90th percentiles. The open circles represent outliers. m: mucosa; sm: submucosa; mp: muscularis propria; ss: subserosa; se: serosa exposed; si: serosa infiltrating.

rolytic cleavage of the prodrug 5′-deoxy-5-fluorouridine (5′-DFUR) to 5-FU suggesting that it may enhance the efficacy of the administered drug.[52] We have previously shown that the sensitivity of KB/TP cells to doxifluridine and tegafur was higher than that of KB/CV cells.[53] Transfection of TP cDNA into other cultured cell lines, such as MCF-7, C26 and SW480, also enhanced their sensitivity to the prodrug 5′-DFUR.[52, 54, 55] The *in vivo* growth of the s.c. transplanted SW480/TP tumor in nude mice was significantly suppressed by i.p. injection of 5′-DFUR compared with that in control mice that received phosphate-buffered saline (PBS) treatment.[55] A role for TP in regulating 5′-DFUR conversion to 5-FU in tumors has also been implied, based on a study of the interplay between TP and dihydropyrimidine dehydrogenase (DPD), an enzyme that degrades 5-FU to inactive molecules. Measurement of the TP/DPD ratio in tumor tissue obtained from surgically resected samples from 93 patients with primary gastric cancer showed a significant correlation with 5′-DFUR sensitivity.[56] Furthermore, treatment of cancer patients with taxol, taxotere, cyclophosphamide, mitomycin C or X-rays increased the level of TP in tumors and synergistically enhanced the antitumor activities of prodrugs of 5-FU.[57–60]

The ability of TP to enhance the activities of antitumor drugs makes it a key enzyme for combination chemotherapy of tumors. A phase II study of capecitabine and docetaxel combination chemotherapy has already started in patients with advanced gastric cancer.[61] A phase II study of combination chemotherapy of older women with metastatic breast cancer using capecitabine in combination with taxane has indicated that this combination would be an effective front-line therapeutic agent.[62]

Conclusions

TP is expressed in various malignant tumors and plays an important role in angiogenesis, tumor growth, invasion and metastasis of TP-expressing tumors. The enzymatic activity of TP is required for TP-induced angiogenesis and is therefore a good target for the development of anti-tumor drugs. A novel, specific TP inhibitor, TPI, inhibited TP-enhanced angiogenesis, tumor growth and metastasis.

There are a number of drawbacks to using inhibitors of TP as antitumor or anti-angiogenic agents. Firstly, inhibition of TP may lead to high plasma thymidine levels that can have adverse effects on replication or repair of mitochondrial DNA. Secondly, treatment of tumors with inhibitors of TP in combination with 5-FU or its prodrugs is impossible, because TP is one of the enzymes correlated with activation of 5-FU.

One potential method to overcome these problems is to inhibit the downstream mediators of TP function rather than to directly inhibit TP activity. D-dRib is a downstream mediator of TP function, and L-dRib, a stereoisomer of D-dRib, inhibited angiogenesis, tumor growth and metastasis enhanced by TP (Fig. 4). L-dRib and its analogs are therefore potentially useful agents for the suppression of TP-dependent angiogenesis, tumor growth and metastasis.

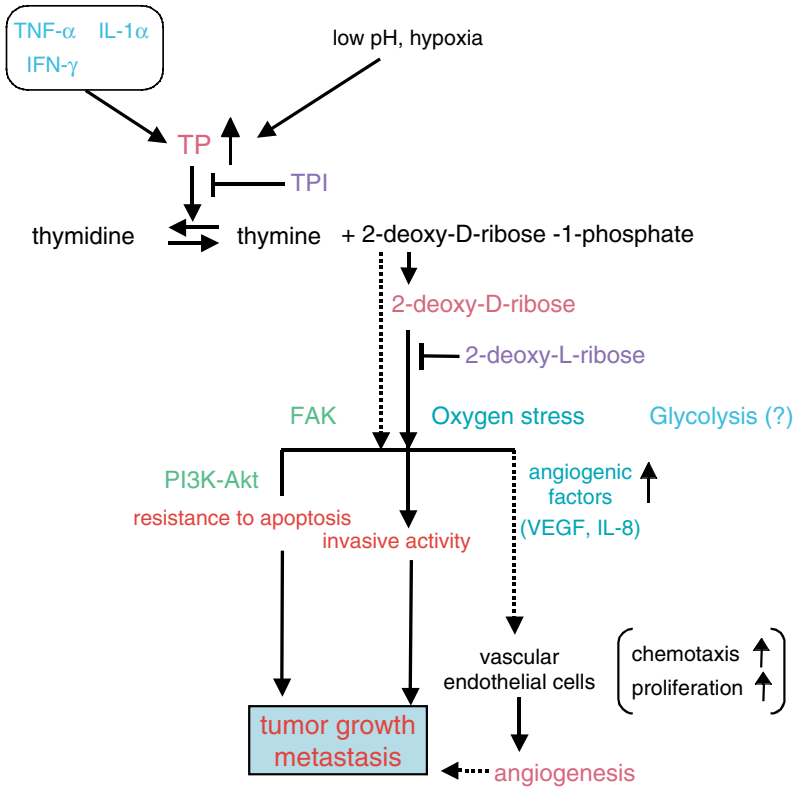

Fig. 4. Schematic representation of the roles of TP and D-dRib in tumorigenesis and the inhibition by L-dRib. The expression of TP is induced by cytokines, hypoxia, or low pH in various tumor cells. TP catalyzes the reversible conversion of thymidine to thymine and 2-deoxy-D-ribose-1-phosphate. D-dRib is produced by dephosphorylation of 2-deoxy-D-ribose-l-phosphate. D-dRib is a downstream mediator of TP and confers resistance to hypoxia-induced apoptosis, enhances chemotaxis of vascular endothelial cells and causes angiogenesis and metastasis. L-dRib can suppress these various biological effects of D-dRib, leading to an inhibition of tumorigenesis.

Elucidation of the exact molecular basis for the function of D-dRib is important to obtain a better insight into the function of TP and to develop other, clinically useful inhibitors of TP function.

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