The Dual Role of Thymidine Phosphorylase in Cancer Development and Chemotherapy

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Abstract: Thymidine phosphorylase (TP), also known as "platelet-derived endothelial cell growth factor" (PD-ECGF), is an enzyme, which is upregulated in a wide variety of solid tumors including breast and colorectal cancers. TP promotes tumor growth and metastasis by preventing apoptosis and inducing angiogenesis. Elevated levels of TP are associated with tumor aggressiveness and poor prognosis. Therefore, TP inhibitors are synthesized in an attempt to prevent tumor angiogenesis and metastasis. TP is also indispensable for the activation of the extensively used 5-fluorouracil prodrug capecitabine, which is clinically used for the treatment of colon and breast cancer. Clinical trials that combine capecitabine with TP-inducing therapies (such as taxanes or radiotherapy) suggest that increasing TP expression is an adequate strategy to enhance the antitumoral efficacy of capecitabine. Thus, TP plays a dual role in cancer development and therapy: on the one hand, TP inhibitors can abrogate the tumorigenic and metastatic properties of TP; on the other, TP activity is necessary for the activation of several chemotherapeutic drugs. This duality illustrates the complexity of the role of TP in tumor progression and in the clinical response to fluoropyrimidine-based chemotherapy. © 2009 Wiley Periodicals, Inc. Med Res Rev, 29, No. 6, 903–953, 2009

Key words: thymidine phosphorylase (TP); angiogenesis; cancer chemotherapy; fluoropyrimidines; thymidine phosphorylase inhibitors

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1. INTRODUCTION

Thymidine phosphorylase (TP) was first discovered in 1954 as a key enzyme of the pyrimidine salvage pathway,[1] which recovers pyrimidine nucleosides that are formed during RNA or DNA degradation. TP catalyzes the conversion of thymidine and 2′-deoxyuridine to their respective bases and 2-α-D-deoxyribose-1-phosphate (2DDR-1P)[2] (Fig. 1). This reaction is reversible; however, the most important metabolic function of TP is catabolic. TP also has deoxyribosyl transferase activity by which the deoxyribosyl moiety is transferred from a pyrimidine nucleoside to another pyrimidine base, resulting in the formation of a new pyrimidine nucleoside.[3] Besides natural 2′-deoxynucleosides, TP also recognizes several pyrimidines or pyrimidine nucleosides with antiviral and antitumoral activity, such as 5-(*E*)-(2-bromovinyl)-2′-deoxyuridine (BVDU),[4] 5-trifluorothymidine (TFT),[5] 5-fluorouracil (5FU),[6] and 5-fluoro-5′-deoxyuridine (5′DFUR), an intermediate metabolite of capecitabine, which is clinically used against metastatic breast and colon cancer (see Section 7.C).[7]

In 1987, a so-called "new" protein was isolated from human blood platelets.[8] This protein was believed to stimulate endothelial cell growth because it increased the [^{3}H]-thymidine uptake and was therefore named "platelet-derived endothelial cell growth factor (PD-ECGF)." PD-ECGF was also shown to induce endothelial cell migration in vitro and angiogenesis in vivo and in the chicken "chorio-allantoic membrane (CAM)" assay.[9] A few years later, it was reported that recombinant PD-ECGF has TP activity.[10,11] Moreover, analysis of the amino acid sequence of both proteins revealed that PD-ECGF and TP are

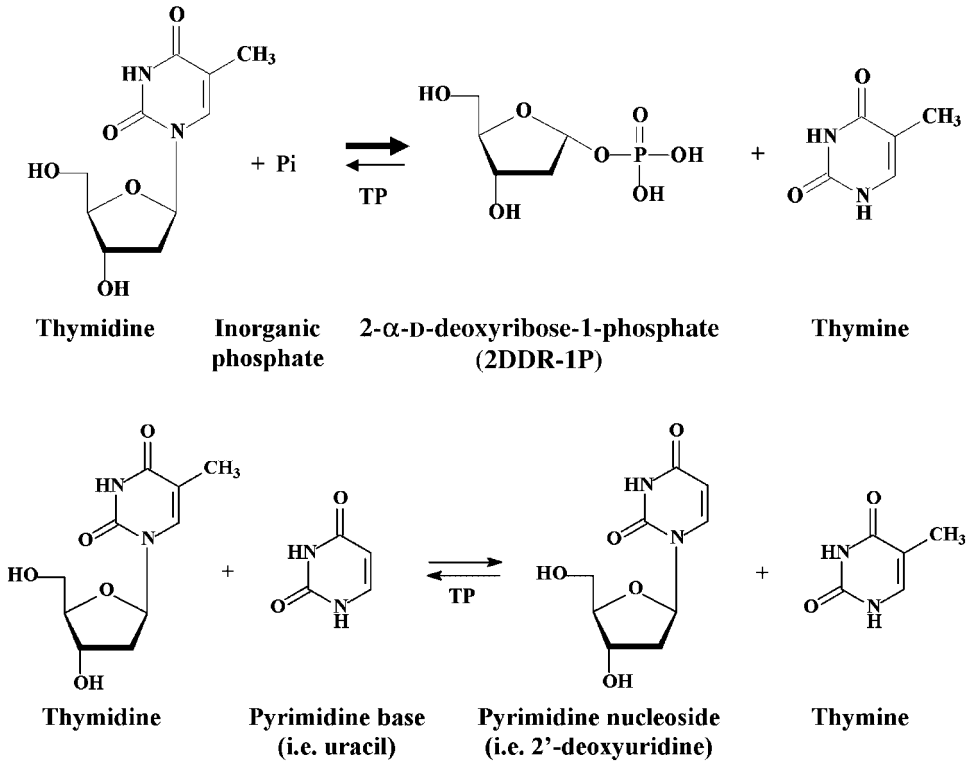

Figure 1. Enzymatic reactions catalyzed by TP. TP catalyzes the reversible conversion of thymidine to thymine and 2DDR-1P. TP also has deoxyribosyl transferase activity by which the deoxyribosyl moiety is transferred from one pyrimidine base to another, resulting in the formation of a new nucleoside.

identical.[12] This leads to the conclusion that the observed increased thymidine uptake was an artifact, caused by the TP activity of PD-ECGF. TP in cell supernatant hydrolyzes serum-derived thymidine, depleting the cells of this metabolite. When the cells are subsequently incubated with [^{3}H]-thymidine, the cells treated with TP take up more of the radiolabelled thymidine than the control cells.[10] Thus, TP is not a growth factor.

A third role for TP has also been described and in this context TP is called gliostatin. In 1992, gliostatin was extracted from human neurofibroma. This protein inhibits the growth of both astrocytes and glial tumor cells.[13] Gliostatin is also shown to promote the survival and neurite outgrowth of rat cortical neurons.[14]

Thus, TP, PD-ECGF, and gliostatin are all synonyms that refer to the same, identical protein. Throughout the literature TP and PD-ECGF are used interchangeably, while the use of the word "gliostatin" is restricted to the context of rheumatoid arthritis (RA) and neurological research.

2. STRUCTURE OF TP

In the mid-1970s TP was purified from both *Escherichia coli* and *Salmonella typhimurium*.[15,16] Several years later, human TP was extracted from the amniochorion.[17] The amino acid sequence of TP is highly conserved during evolution. For example, human TP shares 39% sequence identity with *E. coli* TP.[18]

TP functions as a homodimer consisting of two identical subunits (Fig. 2), with a dimer molecular mass ranging from 90 kDa in *E. coli* to 110 kDa in mammals.[19,20] Detailed structural information on TP was first provided in 1990 by Walter et al. who solved the crystal structure of *E. coli* TP.[21] This analysis revealed that each subunit contains a large mixed α-helical and β-sheet domain (α/β domain) separated from a smaller α-helical domain (α-domain) by a large cleft. The active site consists of the thymine-binding site in the α-domain and the phosphate-binding site across the cleft in the α/β domain. The finding that both sites were about 8 Å apart immediately suggested that a hinge motion of one domain relative to the other was necessary to generate a closed conformation of the enzyme containing a catalytically competent active site,[21,22] as that seen in the crystal structure of the related pyrimidine nucleoside phosphorylase from *Bacillus stearothermophilus*.[23] This closing/opening motion in the presence of substrate, product, and transition state has been simulated using steered molecular dynamics.[24]

It took several more years to have the structure of human TP solved because many crystallization trials failed to produce well-diffracting crystals.[25] Thus, Spraggon et al. reported crystals of human TP for which, despite using a synchroton X-ray source, diffraction was limited to 3.5 Å resolution.[26] Finally, in 2004, Norman et al. successfully solved at 2.1 Å resolution the structure of human TP in complex with the small and potent inhibitor 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl] uracil (TPI) (see Section 6.A).[25] Nonetheless, limited proteolysis with trypsin was found to be necessary and this treatment yielded a structure in which amino acids 409 and 410 were missing and the loop formed by amino acid residues 405–416 was disordered. In these crystals, TP was found as a dimer, with each monomer in the closed, active conformation, and TPI mimicking the substrate transition state. This work provided the first structural insight into the binding mode of an inhibitor to a pyrimidine nucleoside phosphorylase.[25] In 2006, El Omari et al. managed to determine the structure of unproteolyzed human TP at 2.3 Å resolution with the aid of the small-molecule inhibitor KIN59 (see Section 6.B), which helped to obtain good quality diffracting crystals although it could not be located in the electron density map.[27] The asymmetric unit revealed two dimers each displaying the same inter-subunit contacts that were observed in the previous structure and, in

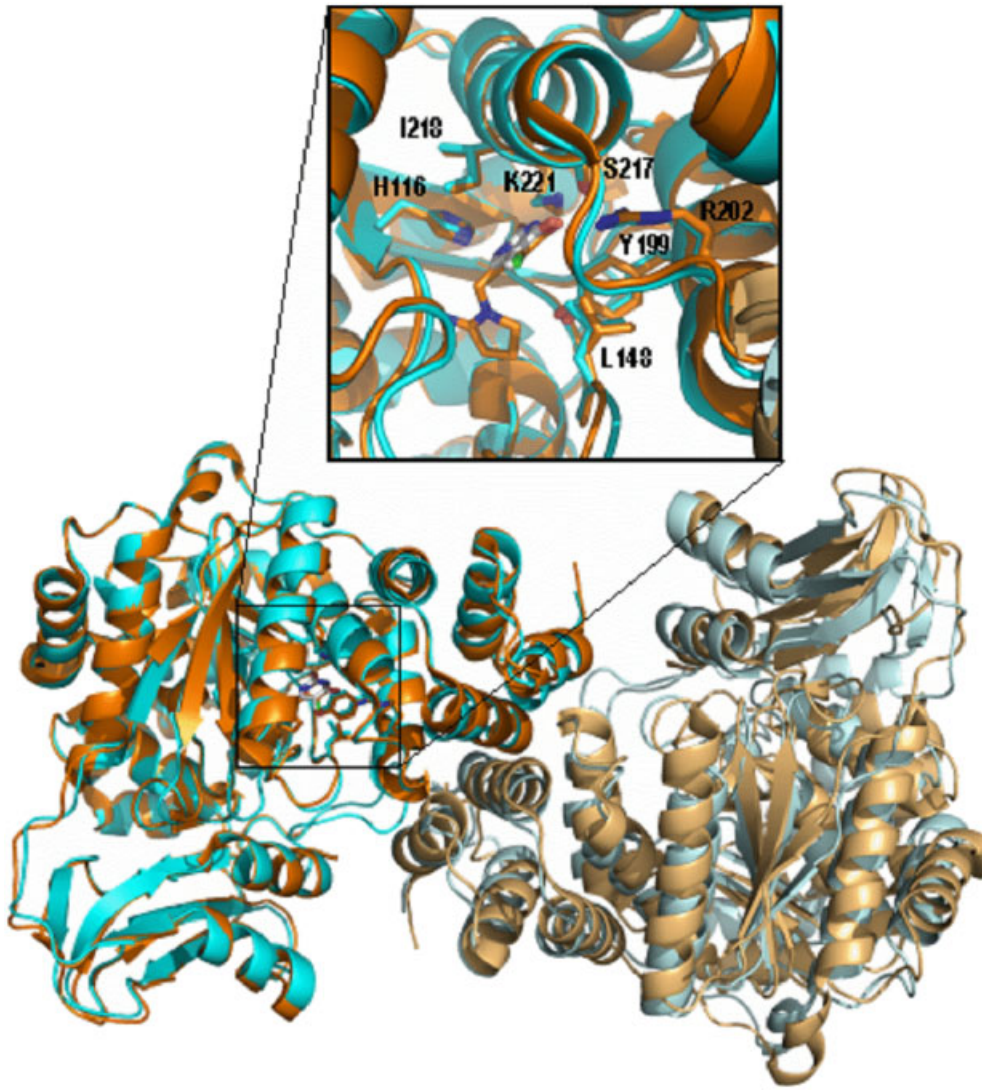

Figure 2. Ribbon representation of human TP showing the dimeric structure and a detail of the active site (boxed) containing either the TPI inhibitor (C atoms in orange) or the thymine product (C atoms in white). The dimer interface consists of a coiled coil formed by helices from each closed-conformation monomer. Superimposition of both available X-ray structures highlights their overall similarity despite belonging to different space groups. The side chains of active-site residues His116, Leu148, Tyr199, Arg202, Ser217, and Lys221 are displayed as sticks.

addition, a fully visible loop made up of residues 405–416.[27] In Figure 2, a ribbon representation of human TP is shown, clarifying the dimeric structure and a detail of the active site containg either the inhibitor TPI or the thymine product. Strikingly, the product thymine was present in three of the four monomers of the asymmetric unit rather than the substrate thymidine, which had been added at the start of the crystallizations.[27] As kinetic studies of *E. coli* TP have shown that phosphate is the first substrate that binds to TP whereas 2DDR-1P dissociates last from the enzyme,[3] this finding suggests that after product release, thymine is able to reassociate with the unliganded enzyme and stabilize the closed conformation, which may explain the mechanism of noncompetitive product inhibition.[27]

3. EXPRESSION OF TP IN HEALTH AND DISEASE

A. The Physiological Role of TP

TP is found in many normal tissues and cells, with high levels in macrophages, stromal cells, glial cells, reticulocytes, some epithelia, tissues of the digestive tract (oesophagus and the rectum), salivary gland, brain, bladder, spleen, lymph, and the lungs.[28–30] Within the cell, TP is present in both the cytoplasm and the nucleus.[28]

Blood platelets are one of the richest sources of TP, which suggests a role for the enzyme in wound healing. TP activity is also detected in plasma and serum, where its presence is probably due to blood platelet damage or cell turnover.[31]

Furthermore, TP plays an important role in the female reproductive cycle. Large quantities of TP are found in the placenta, where two alternative forms of the protein are detected. One is a 27 kDa splice variant of TP,[32] while the other form contains five additional amino acids on the *N*-terminus and is processed at Thr-6 instead of Ala-11.[33] Whether these structural differences also result in functional differences is not clear. High amounts of TP were also discovered in the endometrium, which undergoes extensive angiogenesis during each menstrual cycle. TP shows a characteristic pattern of distribution dependent on the phase of the menstrual cycle: TP expression moves from stroma to epithelium as the cycle progresses[34] and is inversely correlated with oestradiol concentrations.[35] Endometrial TP expression was also raised by human chorionic gonadotropin[36] and by a combination of progesterone and transforming growth factor β1.[34] A marked increase of TP was also detected in decidualized endometrium.[37] During the first trimester of pregnancy, TP is found in the trophoblast together with vascular endothelial growth factor (VEGF), which indicates that both factors play an active role during gestation.[32]

B. TP in Inflammatory Diseases

Various studies indicate that TP is also involved in a wide variety of chronic inflammatory diseases (see Table I). This may be explained by the fact that (i) inflammatory cells such as macrophages contain large amounts of TP (cf. Section 3.D) and (ii) inflammatory cytokines (such as interleukin-1 and tumor-necrosis factor-α) induce TP expression (see Section 5).

TP levels are raised in psoriasis.[38,39] Accordingly, a 20-fold increase in TP activity was found in psoriatic lesions.[39] In inflammatory bowel disease, strong TP expression was observed, predominantly in macrophages and fibroblasts of the inflamed colonic mucosa and the grade of expression augmented with an increasing grade of inflammation.[40,41] In addition, TP was found in the endothelial cells of the inflamed colonic mucosa.[40,41] Furthermore, TP is upregulated in chronic glomerulonephritis (a renal disease characterized by inflammation of the glomeruli) where it probably plays a critical role in the progression of interstitial fibrosis.[42] Moreover, TP is expressed in atherosclerosis. Macrophages, foam cells, and giant cells from both aortic and coronary plaques were found to be immunoreactive for this angiogenic factor, suggesting that TP may play a role in the pathogenesis of atherosclerosis.[43]

TP is also implicated in RA. Higher levels of TP were found in the synovial fluid or sera of patients with RA than in patients with osteoarthritis or normal healthy individuals and serum TP was found to be a useful clinical marker for RA.[44–46] Furthermore, the intra-articular injection of recombinant TP into the knees of rabbits induced RA-like synovitis.[47] Both wild-type TP or mutant (K115E) TP, which lacks enzymatic activity, caused this effect, indicating that not the enzymatic activity of TP but rather the protein itself is implicated in the pathology of RA.[47] Further studies revealed that TP augments its own synthesis through an autocrine mechanism in fibroblast-like synoviocytes (FLS).[48] TP also induced the extracellular secretion of matrix metalloproteinase-1 (MMP-1) and MMP-3, which are the major

Table I. Biological Functions of TP

	References
Physiological role	
Salvage of pyrimidines	1
Female reproductive cycle	32–37
Wound healing	31
Pathological role	
Cancer	63–205
Inflammatory diseases	
Reumatoid arthritis	44–50
Atherosclerosis	43
Psoriasis	38, 39
Inflammatory bowel disease	40, 41
Chronic glomerulonephritis	42
MNGIE	51–58

triggers of cartilage degeneration.[48,49] TP was also found to upregulate both mRNA and protein levels of VEGF, suggesting that both factors have synergistic effects on angiogenesis in RA.[50]

C. The Involvement of TP in MNGIE

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive human disorder associated with multiple deletions of skeletal muscle mitochondrial DNA.[51] This disease is characterized clinically by ptosis, progressive external ophthalmoplegia, gastrointestinal dysmotility, thin body habitus, peripheral neuropathy, myopathy, leukoencephalopathy, and lactic acidosis. Loss-of-function mutations of the TP gene were identified as the possible cause of this disease.[52,53] In MNGIE, the severely reduced TP enzyme activity leads to an increase in the plasma and tissue thymidine and 2′-deoxyuridine levels.[53–55] This may cause unbalanced mitochondrial nucleoside and nucleotide pools, which lead to impaired mitochondrial DNA replication and repair. Therefore, therapies that decrease thymidine levels may be beneficial to MNGIE patients.[53]

In order to unravel the role of TP in MNGIE, mice deficient in the TP gene were generated. As mouse uridine phosphorylase (UP) may also cleave thymidine, $TP^{-/-}UP^{-/-}$ double knockout mice were constructed. In these $TP^{-/-}UP^{-/-}$ mice, all TP activity was abrogated and the plasma thymidine levels were 5-fold higher than in wild-type mice. Surprisingly, no alterations in mtDNA or pathological changes in the muscles of these knockout mice could be observed.[56,57] Only in the brain, mitochondrial DNA depletion, respiratory chain defects and histological alterations could be detected.[57] The brain-specific phenotype in $TP^{-/-}UP^{-/-}$ mice may be due to the relatively modest increases in thymidine or 2′-deoxyuridine levels in mutant mice (5-fold increase in mice versus 100-fold increase in humans). Another possible explanation is the shorter lifespan of mice compared to humans because in humans the average age-at-onset of symptoms of MNGIE is 18.7 years.[58]

Nevertheless, other studies suggest that functional mutations of the TP gene are not sufficient to induce MNGIE. Kumagai et al. postulated that TP gene mutation is not the primary cause of this mitochondrial disease because TP mutations are also found in unrelated healthy individuals.[59] Examination of chromosome 22q13.32 where the TP gene is located, revealed that exon 10 of TP overlaps with the SCO2 gene,[56] which is a cytochrome c oxidase (COX) assembly gene.[60] Mutations in SCO2 have been reported to cause severe COX

deficiency in skeletal muscle leading to mitochondrial disorders characterized by hypertrophic cardiomyopathy and encephalopathy.[61] Thus, more investigations are needed to clarify the exact role of TP deficiency in MNGIE development.

So far no vascular abnormalities have been reported in MNGIE patients nor in $TP^{-/-}UP^{-/-}$ mice.[56,57,62] These data suggest that TP is not of fundamental importance for developmental angiogenesis.

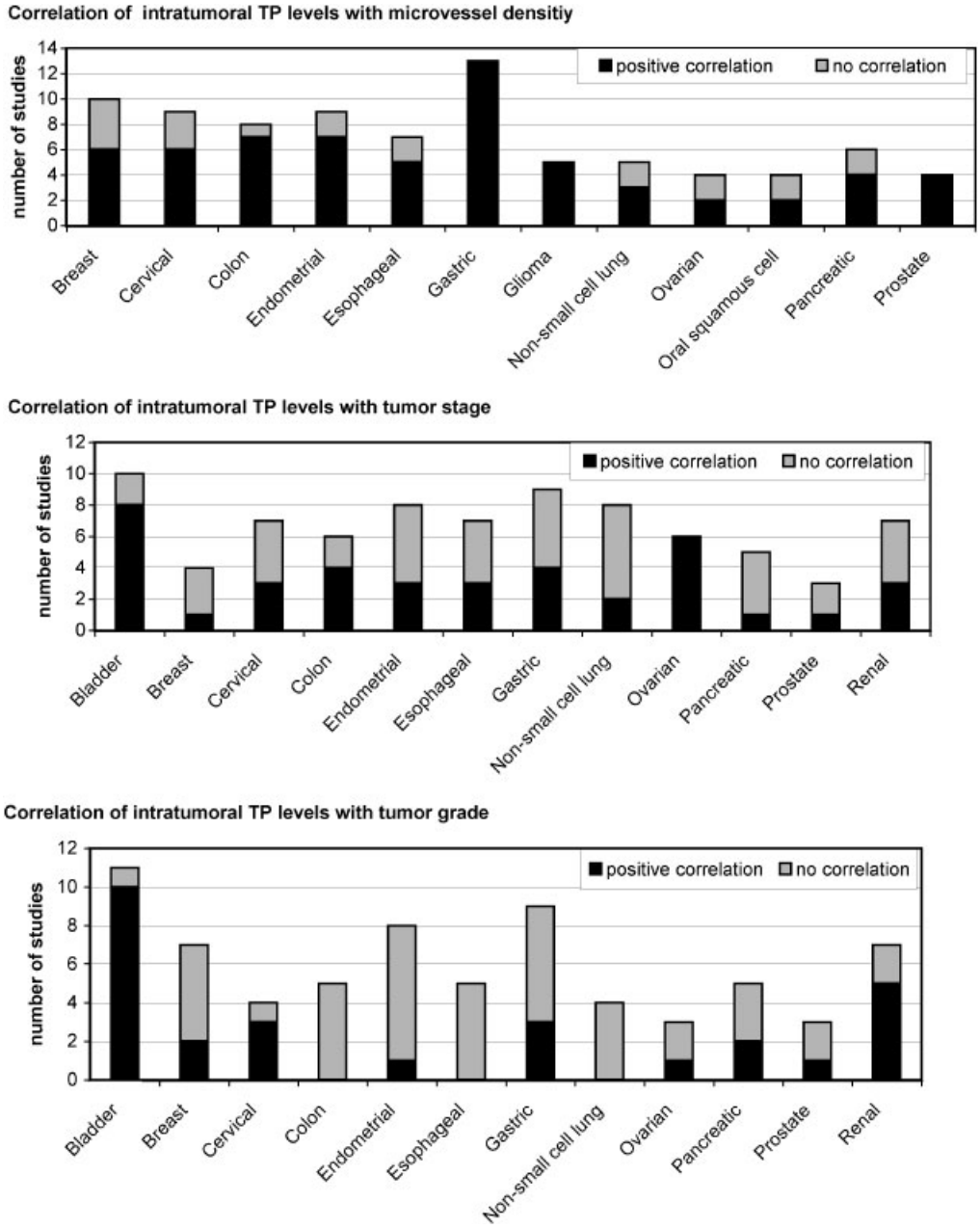

Figure 3. Correlation of TP expression in various cancers with the clinicopathological factors microvessel density, tumor stage, and grade.

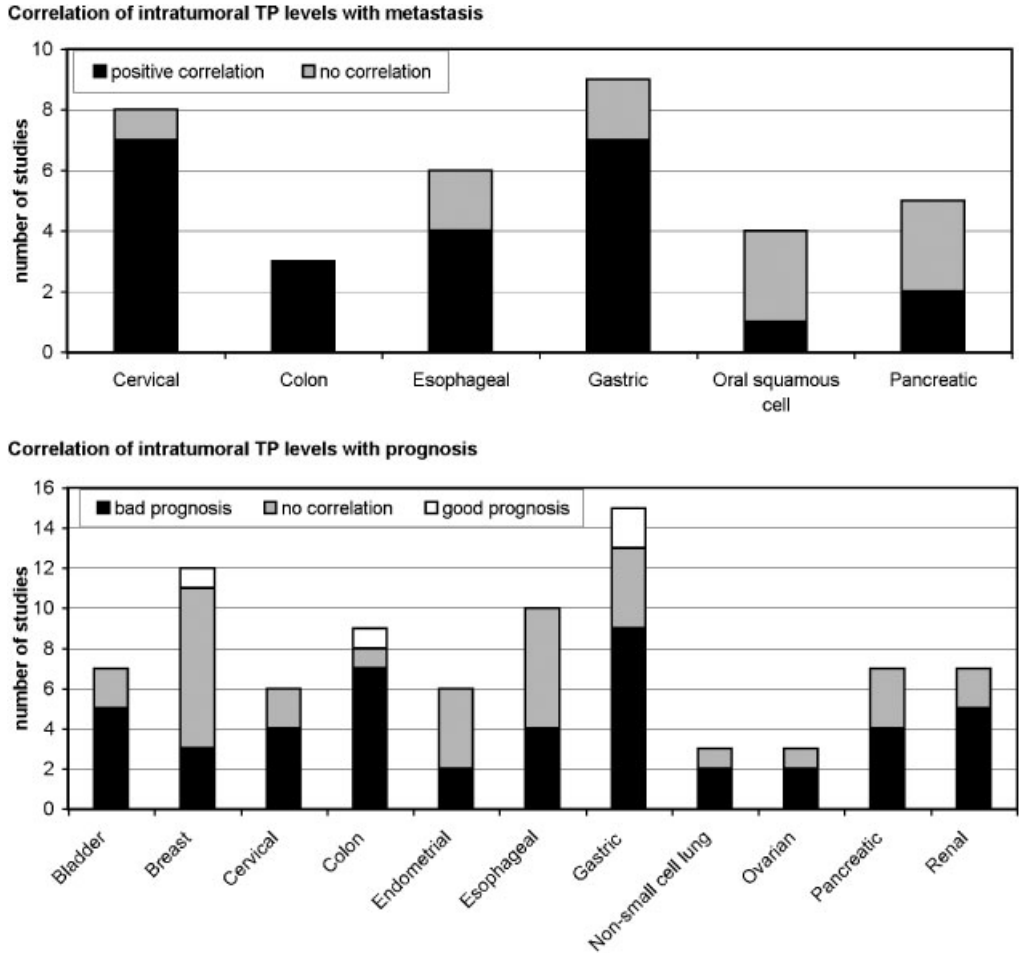

Figure 4. Correlation of TP expression in various cancers with metastasis and prognosis.

D. TP Overexpression in Cancer

Increased TP expression in tumor tissues compared to corresponding nonneoplastic tissue was found in breast,[63] bladder,[64,65] gastric,[29,66,67] colorectal,[29,68] lung,[68,69] esophageal,[68,70] and cervical[68,71] cancers but not in cancers of the liver,[68,72] common bile duct,[68] and the thyroid.[68] TP expression is not only upregulated in solid tumors, elevated levels of TP were also observed in lymph nodes of patients with classical Hodgkin lymphoma where TP levels increased with disease progression.[73] Recently, tumor-reactive T cells from a patient with relapsed multiple myeloma (who was successfully treated with donor lymphocyte infusion after allogeneic stem cell transplantation) were found to be directed against TP.[74] These data identify TP as a potential target for the immunotherapy of hematological tumors.

Numerous studies on cancer patients have examined the relation between TP expression and microvessel density, tumor grade, stage, metastasis, and prognosis. In these studies TP was measured by RT-PCR, immunohistochemistry, or by activity assays. The results of these reports are summarized in Figures 3 and 4.[64–67,70,71,75–205] These figures include only the cancer types for which the association between TP and the respective tumor-related parameter has been investigated in more than three independent studies. Generally, TP expression is correlated with higher microvessel density, higher tumor stage, and more metastasis. An

association of TP with tumor grade is evident in bladder, cervical, and renal cell cancer, but not in the other investigated cancers. Furthermore, in most cases, TP appeared to be associated with poor prognosis, although there are conflicting reports for some cancers. For example, seven of the nine studies on colon cancer reported a significant correlation between TP and bad prognosis, while Saito et al. demonstrated that TP is associated with good prognosis.[142] These discrepancies might be caused by differences in the histological type of cancer, stage (early versus advanced stage of disease), number of patients examined, assay for TP and different methodology for the evaluation of the immunohistochemistry results.[142,206]

Tumors are heterogeneous tissues consisting of unknown variable contributions of tumor, stromal, and infiltrating cells. Besides tumor cells, also endothelial cells, fibroblasts, lymphocytes and especially tumor-associated macrophages (TAM) express TP.[138] TAM are thought to play a key role in stimulating tumor growth and metastasis through the production of various growth factors, proteinases, chemokines, and cytokines.[207] High levels of TP have been demonstrated in TAM of melanoma,[208] gastric,[100,140] glioblastoma,[110] breast,[135,151] colon,[137,209] astrocytic,[178] uterine endometrial,[104] and prostate[210] cancer. In gastric adenocarcinoma,[100] astrocytic tumors,[178] breast,[135] and uterine endometrial cancer,[104] TP expressed in macrophages has been suggested to be correlated with microvessel density and to play an important role in tumor invasiveness.

Elevated TP levels are not only found in the tumor tissue but also in the plasma of cancer patients.[211,212] Already in 1977, Pauly et al. demonstrated that cancer patients had much higher TP activity in the plasma than healthy individuals.[213] He also reported that tumor-bearing animals have elevated TP activity in their ascites and plasma.[214] More recent data indicate that plasma TP concentrations in cancer patients may have a prognostic value. In uterine cervical cancer high serum TP levels correlate with clinical stage, tumor size, lymph node metastasis, and an extremely poor prognosis.[163] High TP concentrations in the blood are also associated with depth of tumor invasion and poor response to treatment in patients with esophageal squamous cell carcinoma.[215] Furthermore, in patients with colorectal cancers, the TP serum level is suggested to be a novel marker to predict occurrence of hematogenous metastasis.[216]

4. TP AND TUMOR DEVELOPMENT

A. Role of TP in Angiogenesis (Fig. 5)

Angiogenesis, the formation of new capillaries from existing blood vessels, is of fundamental importance in several physiological processes, such as embryonic development, wound repair, and reproduction (see Table II). It is a multistep process that involves degradation of the surrounding extracellular matrix (ECM) by proteases, endothelial cell migration, proliferation, and differentiation into mature blood vessels. Cytokines, growth factors, growth factor receptors, enzymes (like TP), components of the ECM, and adhesion molecules each have their own specific role in this well-coordinated process.[217–220] The equilibrium between angiogenic and angiostatic proteins, the so-called angiogenic balance, in the microenvironment controls the rate of new blood vessel formation.[220] Alteration of this angiogenic balance, for example by the uncontrolled release of angiogenic regulators, can lead to several pathological conditions including inflammation, RA, tumor growth, and metastasis.[218,220]

TP induces endothelial cell migration and tube formation in vitro.[9,63,221] This enzyme was also shown to stimulate angiogenesis in the CAM[9,221,222] and in several in vivo models, such as the freeze-injured skin graft,[63] rat corneal,[223] and mouse dorsal air sac[223] assays and in gelatine

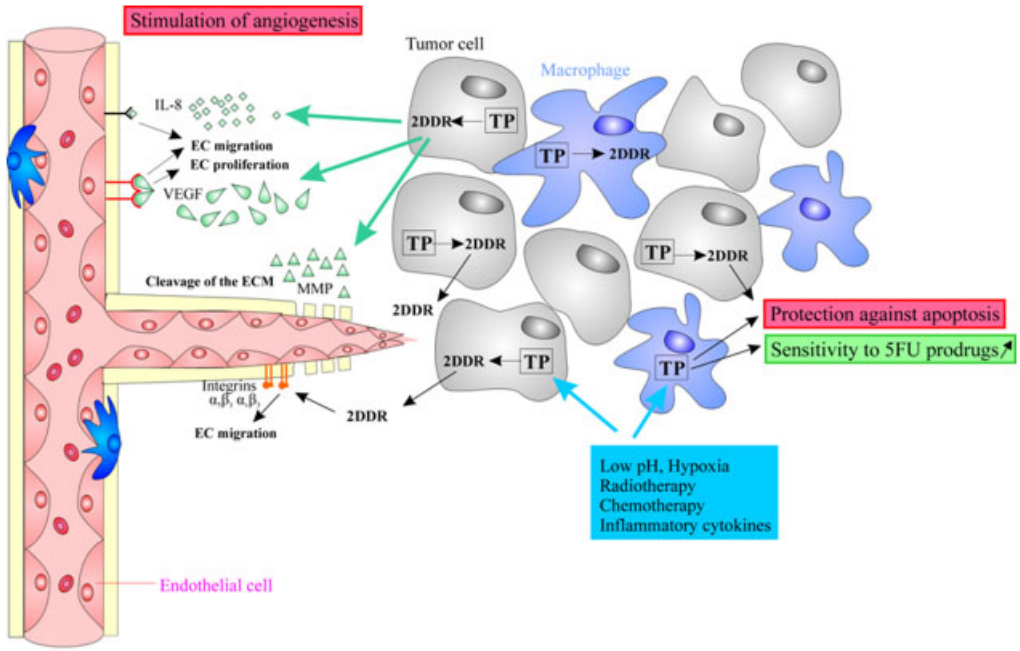

Figure 5. The role of TP in tumor progression. TP expression in tumors can be upregulated by various stress-inducible factors, such as radio- and chemotherapy, inflammatory cytokines, hypoxia, and low pH. Within the tumor tissue, TP is found in both tumor-associated macrophages and tumor cells. TP and its metabolite 2DDR stimulate tumor growth by promoting angiogenesis. TP and 2DDR stimulate the secretion and/or expression of the angiogenic molecules VEGF, IL-8, P-selectin, and various MMPs. 2DDR, which can diffuse outside of the cell, also directly induces endothelial cell migration through activation of the integrins $\alpha_5\beta_1$ and $\alpha_v\beta_3$. TP and 2DDR may also induce tumor progression by protecting the tumor against apoptosis induced by hypoxia, Fas, DNA-damage, and microtubuli-interfering agents. Conversely, tumors do not always profit from elevated TP levels as TP plays a crucial role in the activation of chemotherapeutic drugs such as capecitabine. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table II. Molecular Mediators of TP and/or 2DDR in Angiogenesis

Molecular mediators	Cell type	References
MMP-1	Human bladder RT-112 cells	48, 234
	RA-associated synoviocytes	
MMP-2	Cervical carcinoma cells	256
MMP-3	RA-associated synoviocytes	48
MMP-7	Human PC-3 prostate cancer cells	258
	KK47 bladder cancer cells	
MMP-9	Human PC-3 prostate cancer cells	257, 258
	KK47 bladder cancer cells	
	KB epidermoid cells	
VEGF	Human bladder RT-112 cells	234
Integrins $\alpha_5\beta_1$ and $\alpha_v\beta_3$	HUVEC	225, 240
	EPC	
IL-8	Human bladder RT-112 cells	234
P-selectin	HUVEC	260

sponges subcutaneously implanted in rats or mice.[9,63,221,224] Recently, proteomic research has identified TP as a key regulator of the angiogenic potential of endothelial progenitor cells (EPC).[225] EPC are bone marrow-derived cells, which can differentiate into endothelial cells and

contribute to the repair of blood vessels after a myocardial attack.[226,227] These cells thus offer a promising strategy for the treatment of various cardiovascular diseases.[228] Furthermore, because these cells have the capacity to home to, and invade, tumor tissues, they also show potential as a target for gene therapy against malignant tumors.[229]

Numerous experimental data indicate that the catalytic activity of TP is indispensable for its angiogenic properties: (i) unlike wild-type TP, TP mutants that lack enzymatic activity did not induce the formation of new blood vessels in the gelatine sponge assay;[63,224] (ii) the angiogenic activities of TP could be abolished by using TP-directed neutralizing antibodies,[63] by addition of a specific TP inhibitor such as 5-amino-6-chlorouracil[224] or by downregulating TP by siRNA;[227] and (iii) 2-deoxy-D-ribose (2DDR), which is a degradation product of the TP-metabolite 2DDR-1P, also induces endothelial cell migration and angiogenesis.[230–232] Besides 2DDR, other metabolites of TP have been shown to possess angiogenic properties in vitro. β-amino-iso-butyric acid, which is a degradation product of thymine, stimulated tube formation in the rat aortic assay,[233] while 2DDR-1P induced endothelial cell migration.[230,231,234] Nevertheless, 2DDR is considered to be responsible for the angiogenic activities of TP. de Bruin et al. showed that 2DDR-1P produced by TP-overexpressing Colo320 colon carcinoma cells is rapidly converted to 2DDR.[235] Therefore, it can not be excluded that the biological activity obtained for 2DDR-1P is a result of the conversion of 2DDR-1P to 2DDR. Moreover, Hotchkiss et al. showed that the conversion of 2DDR-1P to 2DDR is indispensable for the induction of endothelial cell migration as addition of an alkaline phosphatase inhibitor, which blocks the dephosphorylation of 2DDR-1P, completely abrogated the chemotactic effects of 2DDR-1P.[230] Moreover, a neutralizing antibody to TP had no effect on endothelial cell migration stimulated by TP-overexpressing cells, even though this antibody completely inhibited migration mediated by recombinant, extracellular TP. These studies demonstrate that TP-mediated endothelial cell migration relies on the intracellular catabolism of thymidine and subsequent extracellular release of 2DDR, which forms a chemotactic gradient.[230] In spite of this, the enzyme purine nucleoside phosphorylase, which also produces 2DDR, has never been reported to possess angiogenic properties.

In several ways, TP is a very exceptional angiogenic molecule. Indeed, usually, angiogenic factors are released into the extracellular space to activate endothelial cells. However, TP lacks an amino-terminal hydrophobic leader sequence required for cell secretion and is therefore mainly found inside the cell.[9] Nevertheless, some tumor cell lines such as the epidermoid carcinoma A431 and stomach cancer MKN74 cell lines do release the protein into the cell culture medium.[30] Also cytokine-treated FLS have been shown to actively secrete TP.[46] The mechanism behind the secretion of TP is possibly a posttranslational process whereby serine residues of TP are covalently linked to phosphate groups of nucleotides, leading to the formation of a nucleotidylated protein that can be secreted.[236] While TP mostly remains inside the cell, its metabolite 2DDR is able to cross the cell membrane and exert its biological effects on other cells. Furthermore, angiogenic factors usually bind to a specific cell surface receptor, which induces a signal transduction cascade followed by a biological response of the cell. However, mammalian cells do not seem to have a receptor for TP nor for 2DDR. Several bacterial receptors for carbohydrates, including for D-ribose, have been identified that play a role in chemotaxis.[237,238] These receptors are histidine kinases, which is a family of receptors that is found in prokaryotes and eukaryotes but not in the animal kingdom.[239] Thus, TP and 2DDR most likely induce angiogenesis through a non-receptor-mediated mechanism.

1. TP stimulates endothelial cell migration

The molecular mechanisms through which TP and 2DDR stimulate endothelial cell migration in vitro are not completely understood. Hotchkiss et al.[240] revealed that TP and 2DDR affect

endothelial cell migration through activation of integrins and their downstream signalling pathways. In human umbilical vein endothelial cells (HUVEC), it was shown that both TP and 2DDR stimulate the formation of focal adhesions and the phosphorylation of tyrosine 397 of focal adhesion kinase (FAK). FAK is a nonreceptor protein–tyrosine kinase that is recruited to focal adhesions by integrin engagement with the ECM. Thus, FAK plays an important role in endothelial cell attachment and migration.[240] Hotchkiss et al. also demonstrated that VEGF, TP, and 2DDR all stimulate HUVEC migration, although through different integrins. TP- and 2DDR-mediated endothelial cell migration and FAK phosphorylation were blocked by antibodies against either integrin $\alpha_5\beta_1$ or $\alpha_v\beta_3$, whereas VEGF-induced migration was only inhibited by the $\alpha_v\beta_3$ antibody.[225,240] The cell surface expression of integrin $\alpha_5\beta_1$ and the cellular expression of integrin β_3 were increased by TP and 2DDR.

Also other investigators tried to unravel the signalling pathways through which 2DDR activates endothelial cells. Seeliger et al. demonstrated that rapamycin completely abrogates 2DDR-mediated HUVEC migration and tube formation in the rat aortic ring assay, probably by blocking 2DDR-induced p70/s6 kinase activation.[241] The intracellular p70/s6 kinase is known to induce endothelial cell migration after activation of the mammalian target of rapamycin.[242] It has been shown that p70/s6 kinase activation is induced after interaction of integrins with ECM components and that this activation requires FAK.[243,244]

2. TP induces the expression and/or secretion of other angiogenic factors

Various studies have demonstrated that TP and 2DDR promote the expression and secretion of several angiogenic factors (see Table II). Human bladder carcinoma cells transfected with TP (RT112-TP) secrete higher amounts of VEGF, interleukin-8, and MMP-1 than mock-transfected RT112 cells in the presence of thymidine.[234] RT112-TP cells incubated with thymidine also showed an elevated expression of heme oxygenase-1 (HO-1), HO-1 is an enzyme that catalyzes the degradation of heme to carbon monoxide, iron, and biliverdin.[245] The expression of HO-1 can be induced by hypoxia, cytokines, and several angiogenic factors such as VEGF and stromal cell derived factor-1 (SDF-1).[246–249] Recent data indicate that HO-1 also possesses proangiogenic properties: it promotes endothelial cell proliferation, protects endothelial cells from apoptosis, and induces the secretion of several angiogenic mediators such as VEGF.[248–250] Among the different end products of the enzyme reaction of HO-1, carbon monoxide is proposed to be responsible for the angiogenic actions of HO-1,[248,249] although recently also biliverdin has been reported to stimulate the synthesis of angiogenic mediators.[251] Not only in RT112-TP cells an elevated expression of HO-1 occurred, also in TP-overexpressing vascular smooth muscle cells (VSMC) an induction of HO-1 was observed.[252] An excess of thymine, which acts as a scavenger for the formed 2DDR-1P, prevented the induction of HO-1. Brown et al.[234] suggested that 2DDR is a strongly reducing sugar that may generate oxygen radical species during the early stages of protein glycation. It was hypothesized that 2DDR binds to an amino group (preferentially at a lysine, arginine or the *N*-terminal amino acid) of a protein during a nonenzymatic reaction, the so-called Maillard reaction. This may lead to the formation of a Schiff base, which can then rearrange to an α-hydroxyketone. This unstable reaction intermediate autoxidizes during which reaction free oxygen radicals are produced. Thus, through the formation of 2DDR, TP may induce oxidative stress in TP-overexpressing tumor cells causing these cells to secrete angiogenic factors, such as VEGF.[234] A recent study demonstrates that TP may induce VEGF secretion through another mechanism. Transcription of VEGF is known to be driven by hypoxia-inducible factor-1α (HIF-1α). Under hypoxic conditions, the transcription factor HIF-1α is upregulated and increases the expression of several target genes by forming a dimer with HIF-1β, which recognizes the hypoxia responsive elements in the promoter

region.[253] In RT112 cells, TP activity augments the levels of HIF-1α during in vitro hypoxia and TP and HIF-1α acted together to induce VEGF secretion.[254]

Not only MMP-1, but also other MMPs have been shown to be upregulated by TP. MMPs degrade the ECM surrounding tumor and endothelial cells and therefore promote tumor cell invasion, migration, and metastasis.[255] In several cervical carcinoma cell lines, TP expression correlated significantly with the mRNA level of MMP-2 and with cell invasion in vitro.[256] Human epidermoid carcinoma cells (KB) transfected with TP expressed more MMP-9,[257] while the TP-overexpressing PC-3 prostate and KK47 bladder cancer cells had higher levels of MMP-7 and MMP-9 under hypoxia than the mock-transfected control cells.[258] TP induced the expression and extracellular secretion of MMP-1 and MMP-3 in cultured human RA-associated synoviocytes.[48] Also clinical data provide evidence for a correlation between TP and MMP expression. In breast cancer, TP was associated with higher levels of activated MMP-9.[259] Moreover, in human bladder cancers, TP correlated significantly with the expression of MMP-1, MMP-9, and urokinase plasminogen activator,[258] which also plays a role in ECM degradation.

cDNA microarray analysis showed that the cell adhesion molecule P-selectin is upregulated in HUVEC treated with TP.[260] A correlation between tumor cell P-selectin expression and TP was also assessed in human breast cancers by immunohistochemistry.[260] P-selectin is a vascular adhesion molecule mostly found on endothelial cells that mediates the interactions of endothelial cells and leukocytes during inflammation.[261] It is also believed to play a vital role in tumor growth and metastasis, including the promotion of angiogenesis[262] and the movement of tumor cells through the endothelial cell layer.[261]

A study on breast cancers revealed that angiopoietin-1 (Ang-1) is inversely related to TP.[263] Ang-1, which is a ligand of the tyrosine kinase receptor Tie-2,[264] is a survival factor for endothelium and stabilizes vascular networks.[265–267] It maintains the integrity of the capillaries by recruiting and stabilizing nonendothelial support cells such as pericytes.[265] Thus, the loss of Ang-1 results in pericyte–endothelial cell contact destabilization, which possibly enables TP and 2DDR to act on the ''free'' endothelial cells and induce angiogenesis.[263]

3. TP is a promising target for the treatment of vascular obstructive diseases

As TP is an angiogenic molecule, its upregulation could be applied to treat diseases caused by reduced angiogenesis or a disturbed blood flow. In 2005, Li et al.[268] showed that gene therapy using TP is an effective treatment against chronic myocardial ischemia in dogs. The plasmid-mediated gene transfer of TP stimulated angiogenesis and arteriogenesis in chronically ischemic myocardium, decreased the infarct size, restored the myocardial blood flow, and improved myocardial function.[268] This experimental gene therapy also proved to have a long-term beneficial effect in the treatment of chronic ischemic myocardium.[269]

As VSMC play an important role in vessel maturation during angiogenesis, and deregulated growth or motility of VSMC contributes to the pathogenesis of vascular obstructive diseases (such as ischemia), the effect of TP on VSMC migration and proliferation was investigated. TP-overexpressing VSMC migrated and proliferated more slowly than mock-transfected VSMC. The decreased VSMC proliferation was correlated with TP-induced HO-1 expression. In TP-overexpressing VSMC the cyclin-dependent kinase inhibitor (p27KIP1) was upregulated and the cell cycle was arrested at the G1 phase. Thus, surprisingly, TP inhibits proliferation and migration of VSMC, while it stimulates the migration of endothelial cells. This apparently conflicting role of TP may be due to the opposite effect of HO-1 in endothelial cells and VSMC. As described above, TP induces HO-1 both in endothelial cells and VSMC. HO-1 has been shown to induce endothelial cell proliferation and migration,[249,250] while it inhibits VSMC growth.[270] In balloon-injured rat carotid arteries adventitial TP gene delivery significantly reduced neointimal VSMC migration and neointima

formation.[252] Furthermore, adventitial delivery of the TP gene also prevented intimal hyperplasia of vein grafs in rabbits.[271] TP thus reduces the neointimal mass and inhibits further neointimal outgrowth and is therefore a promising target in occlusive vascular diseases.

B. TP Induces Metastasis

TP was found to increase the metastatic potential of several experimental and human tumors. Moreover, in various cancers high TP expression correlates with metastasis (Fig. 4). Takao et al. demonstrated that TP-expressing KB carcinoma cells show more basement membrane invasion than their mock-transfected counterparts.[272] Intrasplenic injection of KB/TP cells in nude mice resulted 4 weeks after injection in significantly more metastatic nodules in the livers than injection with KB/CV cells.[272,273] The stimulation of metastasis by TP-over-expressing cells could be dramatically inhibited by the TP inhibitor TPI or by 2-deoxy-L-ribose (2DLR), a stereoisomer of 2DDR.[272,273] Finally, in mice xenografted with the human melanoma cancer cell line A-07, lung colonization and spontaneous metastasis were inhibited by treatment with neutralizing antibodies against TP.[274]

C. TP Protects Cancer Cells Against Apoptosis

Moghaddam et al. reported in 1995 that TP-expressing breast carcinomas have a higher growth rate without an increase in microvessel density than breast cancers that do not express TP.[63] Furthermore, a clinical study of human colon carcinomas showed that TP is a prognostic factor independent of angiogenesis.[121] These data suggest that TP may stimulate tumor growth through a mechanism different than angiogenesis. Uchimiya et al.[223] investigated in a mouse model the anti-apoptotic effect of TP by injecting KB/TP cells or mock-transfected KB cells (KB/CV) into nude mice.[223] The apoptotic index in KB/TP tumors was significantly lower than in KB/CV tumors, indicating that TP protects cells against apoptosis.[223,273,275] Also numerous clinical studies give evidence for the anti-apoptotic effect of TP. TP expression is correlated with a reduction in apoptotic cells in colon,[144] gastric,[276] esophageal,[82,184] ovarian,[277] and oral squamous cell[175] carcinomas but not in cervical cancers[169,184] or in astrocytic tumors.[165,178]

A correlation between TP expression and apoptosis was first demonstrated in vitro by using human epidermoid carcinoma KB cells. KB cells transfected with TP (KB/TP) were resistant to hypoxia-induced apoptosis. This advantage was abrogated when the cells were treated with TPI, which inhibits the enzymatic activity of TP, leading to the conclusion that the enzymatic activity of TP is indispensable for protection against hypoxia-induced apoptosis.[278] Also the metabolites of the TP reaction, 2DDR, and thymine, partially prevented hypoxia-induced apoptosis in KB cells. A potential molecular basis for the inhibition of hypoxia-induced apoptosis was first suggested by Ikeda et al.[279] In human leukemia (HL-60) cells, 2DDR inhibited numerous hypoxia-induced pro-apoptotic events, such as activation of caspase 3 and 9, mitochondrial cytochrome c release, loss of mitochondrial transmembrane potential, phosphorylation of p38 mitogen-activated protein kinase, and downregulation of the anti-apoptotic proteins Bcl-2 and Bcl-xl.[279,280] Furthermore, 2DDR also prevented the upregulation of the transcription factor HIF-1α.[279] Also in the human leukemia cell line (Jurkat cells) overexpression of TP inhibited the upregulation of HIF-1α and the HIF-1α-inducible, pro-apoptotic factor BNIP3.[281] This is in contradiction with the results of Brown et al. who showed that TP-activity augments the levels of HIF-1α in RT-112 cells during in vitro hypoxia.[254] It is however possible that different tumor cell lines demonstrate considerable variation in induction of HIF-1α when subjected to TP. Another explanation might be that in HL-60 cells, 2DDR was added extracellularly, while in RT-112 cells the effect was observed in cells transfected with TP.

Besides hypoxia-induced apoptosis, TP suppresses apoptosis induced by Fas, microtubule-interfering, and DNA damage-inducing agents such as cisplatin (see Table III).[282–286] TP exerts these protective effects independent of its enzymatic activity.[282,283,286] Furthermore, in the presence of UV-light, which causes DNA damage, KB/TP cells had higher amounts of both Akt and phosphorylated Akt than KB/CV cells. Akt activation was significantly decreased by phosphatidylinositol 3 kinase (PI3K) inhibitors, suggesting that the Akt/PI3K pathway is implicated in TP-induced resistance against DNA damage.[284] Finally, it was demonstrated that TP protects cells against Fas-induced apoptosis by inhibiting caspase-8 cleavage, Bcl-2 phosphorylation, and cytochrome c release.[285]

5. REGULATION OF TP EXPRESSION

The TP gene is localized on chromosome 22q13[287] and is composed of ten exons dispersed over a 4.3 kb region. The TP promoter lacks a "TATA" and a "CCAAT" box, sequences recognized by RNA polymerase II, prevalent in most eukaryotic genes.[288] Instead, it contains a cluster of six to nine SP1-binding motifs, just upstream of the transcription start site.[288,289] The transcription factor SP1 is activated by protein kinase A, which is in turn activated by cyclic adenosine monophosphate (cAMP).[290] SP1 sites are also involved in the transcription of VEGF.[291] Indeed, various studies confer the tendency for VEGF and TP to be co-expressed. A significant correlation was found between expression of VEGF and TP in breast,[118,124,292] colorectal,[293] non-small cell lung,[69,133] head and neck squamous cell,[294] endometrial,[79] astrocytic,[165] lung,[295] and cervical[296] carcinomas. The co-expression of TP and VEGF may also be explained by the fact that TP increases the expression of VEGF by inducing oxidative stress or by upregulating the transcription factor HIF-1α, as described in Section 4.A.2. However, no relation between VEGF and TP expression was found in gastric,[80] gallbladder,[297] bladder,[298] and esophageal squamous cell[191] carcinomas.

Besides SP1 sites, the promoter region of the *TP* gene contains other transcription factor-binding sites, such as an interferon-stimulated response element (ISRE)[299] and a γ-activated sequence-like element (GAS).[300] In HT29 colon carcinoma cells, interferon-γ (IFN-γ) induces TP expression through these ISRE sequences,[299] while in U937 monocytes IFN-γ promotes TP expression by increasing the binding of the signal transducer and activator of transcription 1 (STAT1) to the GAS sequence, suggesting that IFN-γ induces TP expression by activation of the Janus kinase (JAK)/STAT pathway.[300] This observation is in line with the findings of Yao et al. who reported that the JAK inhibitor AG-490 blocks both IFN-induced STAT1 phosphorylation and TP expression in glioblastoma cells.[301] Furthermore, in clinically resected colon carcinomas eight of nine tumors tested had both high STAT1 protein levels and TP activity.[302] The IFN-induced TP gene expression is also regulated by post-transcriptional mechanisms. Schwartz et al. demonstrated that IFN induces an increase in TP mRNA and that the TP mRNA levels remained elevated for up to 72 hr, suggesting that IFN promotes TP mRNA stability. Analysis of the TP mRNA sequence revealed the presence of a pyrimidine-rich sequence at the 3′- end that is similar to a motif that has been reported to increase the mRNA stability in other genes such as VEGF.[299]

Interferons are not the only inflammatory cytokines that upregulate TP. Also tumor necrosis factor-α (TNF-α) and interleukins induce the expression of TP.[46,289,303,304] In THP-1 monocytes TP expression was increased by TNF-α and this induction could be mimicked by an antibody against TNF-α receptor 2.[303,304] It was also shown that the TNF-α-induced increase in TP mRNA was inhibited by an inhibitor of transcription factor nuclear factor-κB (NF-κB).[304] Correspondingly, de Bruin et al. showed that prolonged exposure of human macrophage THP1 and U937 cells to sulfasalazine, an anti-inflammatory drug and inhibitor

Table III. Molecular Mechanisms of the Anti-Apoptotic Effect of TP

Apoptosis induced by	Involvement of catalytic activity of TP	Cell type	Anti-apoptotic actions of TP	Ref.
Hypoxia	Yes	KB epidermoid cells	–	278
		HL-60 leukemia cells	*Inhibition of* Caspases 3 and 9 activation Cytochrome c release p38 MAPK phosphorylation Bcl-2 and Bcl-xl downregulation HIF-1α upregulation	279, 280
		Jurkat leukemia cells	*Prevention of upregulation of* HIF-1α BNIP3	281
DNA damage caused by cisplatin	No	Jurkat leukemia cells	*Inhibition of* Caspases 3 and 9 activation Cytochrome c release	282
DNA damage caused by UV	No	KB epidermoid cells	Akt activation	284
Fas	No	KB epidermoid cells	*Inhibition of* Caspase-8 cleavage Bcl-2 phosphorylation Cytochrome c release	285
Microtubule interfering agents	No	Jurkat leukemia cells	*Suppression of* Bcl-2 phosphorylation FasL expression	283

of NF-κB, resulted in downregulation of TP and IL-8 along with elimination of their induction by TNF-α and IFN-γ.[305] Thus, the transcription factor NF-κB is involved in the induction of TP expression.

In cultured FLS, TNF-α, IL-1α, IL-1β, IL-6, and IL-8 stimulated the expression of TP.[46,306] The IL-1β-induced expression of TP was inhibited by treatment with the anti-rheumatic drugs dexamethasone and aurothioglucose, while methotrexate or sulfasalazine had no influence on the TP levels.[306] This suggests that dexamethasone and aurothioglucose may inhibit RA through inhibition of TP expression. Also in OUMS-27 chondrosarcoma cells the secretion of TP was augmented by IL-1β in a dose-dependent manner. This effect could be blocked by selective inhibition of the p38 mitogen-activated protein kinase (p38 MAPK) pathway.[49]

Cancer treatments, such as X-ray irradiation and chemotherapeutic agents (including paclitaxel, docetaxel, doxorubicin, oxaliplatin, cyclophosphamide, and mitomycin C) have been reported to dramatically increase the tumor TP levels (see Table III). This is probably due to the fact that these therapies induce cytokines (such as TNF-α, IFN-γ, IL-1) that stimulate TP expression.[302,307–309]

Recently, evidence was provided that TP expression is also regulated at the transcription level by epigenetic modifications, such as methylation and histone deacetylation.[310] High

expression of TP was associated with complete demethylation of the CpG dinucleotides located in the TP promoter, which was demonstrated in breast carcinoma SKBR-3 cells. Low TP expression was correlated with hypermethylation of the CpG islands as in DLD-1 colon carcinoma cells. In DLD-1 cells, the expression of TP could be activated by demethylation with 5-aza-2′deoxycytidine and to a lesser extent by histone deacetylation with trichostatin-A.[310]

Finally, also microenvironmental stress conditions, such as hypoxia and low pH stimulate the expression of TP.[311] This clarifies why TP can be found in those parts of the tumor that are adjacent to necrotic areas or after occlusion of the tumor blood supply.[311] In conclusion, stress-related factors, such as hypoxia and cytokines, induce the expression of TP, which indicates that this enzyme is a product of inflammation or microenvironmental stress.

6. TP INHIBITORS

Already in 1971, Judah Folkman postulated that tumor growth is angiogenesis-dependent and that tumor development and metastasis could be abolished by blocking the tumor blood supply.[312] Currently, anti-angiogenic drugs, such as the VEGF-antibody bevacizumab (Avastin, Genentech/Roche, Basel Switzerland) and the kinase inhibitors sorafenib (Nexavar, Bayer, Leverkusen, Germany), and sunitinib (Sutent, Pfizer, New York, NY) are being used in cancer treatment while dozens of other anti-angiogenic molecules are evaluated clinically[219,313] (for an update see: http://www.cancer.gov/cancertopics/factsheet/Therapy/angiogenesis-inhibitors). However, the benefits from these anti-angiogenic therapies are at the best temporary and mostly followed by resistance development of the tumor. Although tumor resistance may be caused by various mechanisms such as poor pharmacokinetics, limited drug uptake, increased drug efflux, and mutation of the target proteins, tumor resistance may also be caused by circumvention of the angiogenic blockade by activation and/or upregulation of alternative pro-angiogenic pathways in the tumor.[313,314] For example, a study on glioblastoma patients treated with the VEGF receptor inhibitor cedinarib (Recentin, Astra Zeneca, London, UK) showed that the tumors evaded the anti-angiogenic therapy by upregulating the angiogenic fibroblast growth factor-2 (FGF-2) and stromal cell derived factor-1α (SDF-1α).[315] Therefore, there is an urgent need to develop anti-angiogenic drugs directed at different angiogenic targets. As TP plays a fundamental role in cancer angiogenesis, many laboratories have tried to synthesize potent TP inhibitory drugs. Some of these molecules have been tested preclinically and clinically, but currently no product has been approved yet for clinical use. Therefore, only a few relevant inhibitors will be discussed. For a more extensive review on TP inhibitors, we refer to reference.[316]

A. Pyrimidine Analogues

For more than 30 years the only compounds known to inhibit TP were uracil derivatives, such as 6-aminothymine (6AT) and 6-amino-5-bromouracil (6A5BU) (Fig. 6). These molecules have 50% inhibitory concentration (IC_{50}) values against the enzyme in the submicromolar range.[317] When it became clear that TP is not only an enzyme involved in the nucleoside salvage pathway but is also implicated in angiogenesis, numerous laboratories aimed at synthesizing more potent TP inhibitors.

In 2000, Fukushima et al. identified "5-chloro-6-[1-(2-iminopyrrolidinyl)methyl] uracil hydrochloride (TPI)" (Fig. 6), the most potent inhibitor of human TP so far, with an IC_{50} value of 35 nM.[318] This molecule was shown to abrogate several biological actions of TP. For example, TPI inhibited TP-induced angiogenesis in the mouse dorsal air sac assay. It also significantly reduced the tumor growth rate and microvessel density and increased

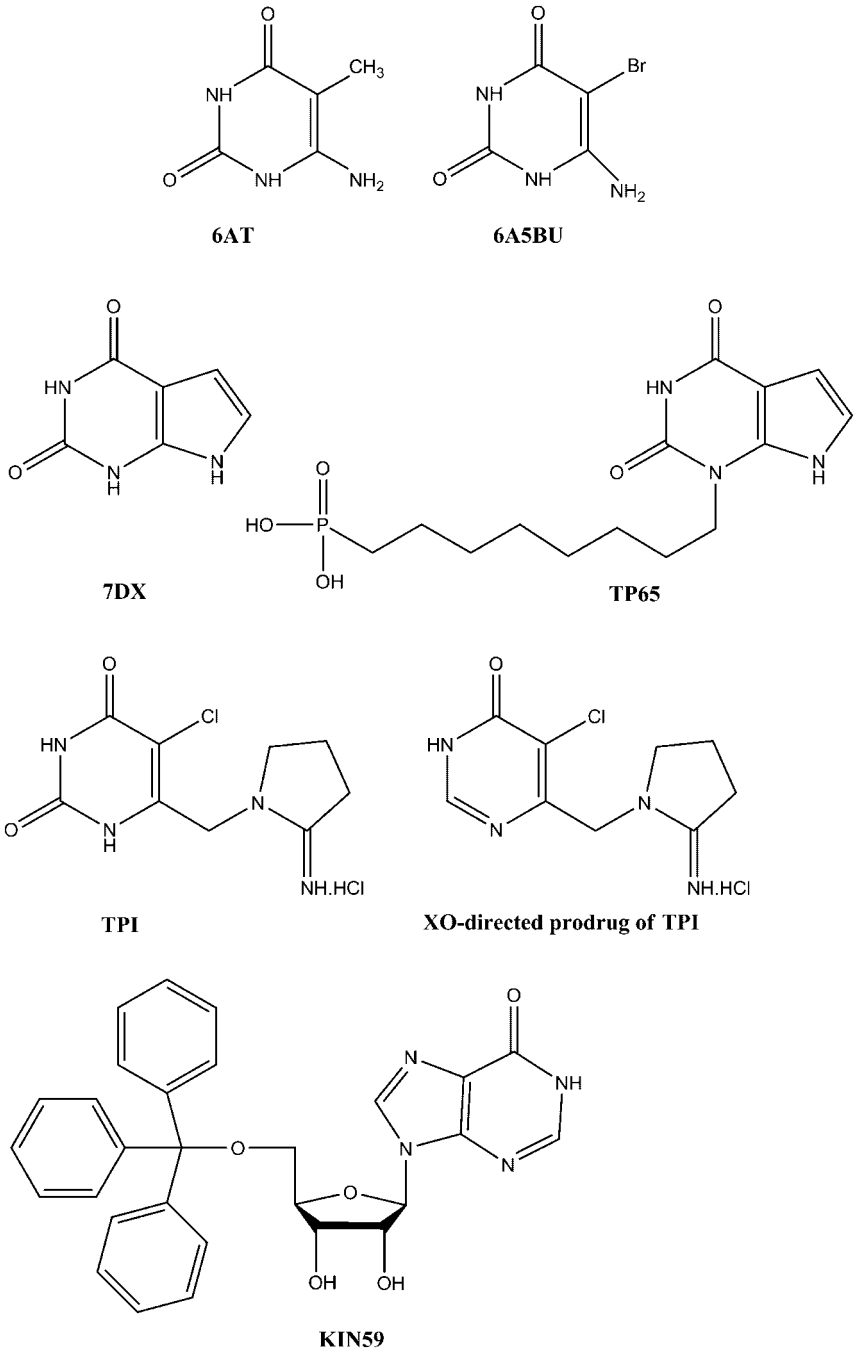

Figure 6. Chemical structure of some illustrative inhibitors of TP.

the apoptotic index of KB/TP xenografted tumors.[275] Furthermore, oral administration of TPI suppressed macroscopic liver metastases of highly metastatic KB/TP cells and also the level of human β-globin as a molecular marker of micrometastases in the livers of the mice.[272] The fact that TPI is orally bio-available and has a strong nanomolar

inhibitory activity against TP suggests that this molecule might be a promising antitumor agent.

B. Purine Analogues

In 1998, Balzarini et al.[319] described 7-deazaxanthine (7-DX) (Fig. 6) as the first purine derivative with inhibitory activity against a pyrimidine nucleoside phosphorylase (i.e. TP). The three-dimensional structure of *E. coli* TP was used for the rational modelling and design of 7-DX, which can be regarded as a pyrimidine at which a second ring was added to create extra stabilization. 7-DX not only efficiently inhibited the enzymatic activity of TP; it was also able to prevent neovascularization in the CAM assay.[319]

The available crystal structure of *E. coli* TP has also led to the rational design of compounds that interact both with the thymine and the phosphate-binding site, the so-called multisubstrate analogue inhibitors of TP. These types of molecules consist of a base, interacting with the nucleoside-binding site and a phosphonate moiety that may bind to the phosphate-binding site. The distance between the thymine- and the phosphate-binding site of *E. coli* TP is estimated to be around 10 Å, therefore the thymine and the phosphonate moiety of these novel inhibitors were linked to each other with a spacer of 6–9 methylene entities. These compounds interact with both substrate-binding sites, and thus "freeze" the enzyme in an open, inactive conformation.[320,321] TP65, which contains an alkyl phosphonate moiety covalently linked to 7-DX (Fig. 6), is such a multisubstrate inhibitor of TP, with an IC_{50} value in the micromolar range. This molecule could also abrogate biological activities of TP, such as angiogenesis in the CAM assay and the formation of microvascular sprouts from endothelial cell aggregates in a fibrin gel.[222]

Another purine derivative that inhibits TP is 5′-*O*-tritylinosine (KIN59) (Fig. 6). KIN59 consists of a purine base (hypoxanthine), a ribose sugar and a trityl group at the 5′-position of the ribose. The trityl group of KIN59 has proven to be crucial for its inhibitory activity against TP and its anti-angiogenic effect in the CAM assay.[322,323] KIN59 is in several ways a very unusual TP inhibitor. In the CAM assay, KIN59 not only prevented the formation of new blood vessels but also promoted the degradation of small pre-existing immature blood vessels. This effect was not due to unspecific cell toxicity. Furthermore, in contrast to all previously described TP inhibitors, this molecule does not compete with the natural substrates for binding to either the nucleoside- or the phosphate-binding site of TP, but interacts with a new, yet unknown, allosteric site of the enzyme in a non-competitive fashion.[322] In order to identify the amino acids that interact with KIN59, computer-assisted modelling of the KIN59-TP complex was performed (unpublished data). This in silico analysis revealed a cavity in which KIN59 could fit in the vicinity of the Gly405-Val419 loop. In this pocket the amino acid Asp203 was found to play an important role for loop stabilization required for efficient enzyme catalysis. Site-directed mutagenesis of Asp203 to alanine yielded a TP with ~60-fold reduction in phosphorolytic capacity (V_{max}/K_m) relative to the wild-type enzyme. Furthermore, KIN59 was not able to inhibit the enzymatic activity of the mutant TP, while the competitive inhibitors 6AT and 6A5BU maintained their inhibitory capacity.

C. Prodrugs of TP Inhibitors

Reigan et al. have explored a xanthine oxidase (XO) prodrug strategy. XO activity and expression are increased in hypoxic conditions. Moreover, increased XO activities are found in colorectal and prostate tumors as compared to their corresponding normal tissues. Therefore, 2′-nitro prodrugs of potent 2′-aminoimidazolyl TP inhibitors were developed. These prodrugs may become selectively activated by XO in the tumors and thus may exert

their TP inhibitory activity specifically within the hypoxic regions of the tumors.[324] Also XO-sensitive prodrugs of 6A5BU, 7-DX, and TPI (Fig. 6) have been synthesized.[325,326] The in vivo efficacy of these prodrug molecules remains to be investigated.

D. 2-Deoxy-L-Ribose

2-Deoxy-L-ribose (2DLR) is a stereoisomer of 2DDR. It is not a direct TP inhibitor because it does not inhibit the enzymatic activity of TP. However, this molecule is able to affect the biological effects of TP. Indeed, 2DLR suppresses the anti-apoptotic effect of 2DDR and prevents 2DDR-induced chemotaxis and tubulogenesis of bovine aortic endothelial cells in vitro. In vivo, 2DLR was able to abrogate TP-induced angiogenesis in the rat corneal assay and in the mouse dorsal air sac assay.[223] Moreover, oral administration of 2DLR could significantly reduce the growth of KB/TP cells transplanted into nude mice and suppressed invasion and metastasis induced by KB/TP cells.[273] Also the TP-induced activation of MMP-9 and secretion of IL-8 and VEGF could be blocked by 2DLR.[257,273] The molecular basis of the biological effects of 2DLR remains, however, to be resolved.

7. TP IN FLUOROPYRIMIDINE CHEMOTHERAPY

A. 5-Fluorouracil (5-FU)

In 1957, Heidelberger et al. discovered the antitumor activity of 5-fluorouracil (5FU) (Fig. 7).[327] Fifty years after its first synthesis, 5FU remains extensively used in the treatment of colorectal cancer. The possible metabolic pathways of 5FU are depicted in Figure 8. 5FU can elicit its antitumor activity by inhibiting thymidylate synthase (TS), which is responsible for the de novo thymidylate production and is thus a rate-limiting enzyme in DNA synthesis.[328] First, 5FU needs to be converted by TP to 5-fluoro-2′-deoxyuridine (FdUrd). This action of TP can only take place if there is enough co-substrate (i.e. 2DDR-1P) for TP. Indeed, increase in the 2DDR-1P availability by addition of 2′-deoxy-pyrimidine nucleosides or 2′-deoxypurine nucleosides greatly enhances TP-mediated 5FU sensitivity of tumors.[329–331] FdUrd is further converted by thymidine kinase (TK) to 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP). FdUMP can also be derived from FdUDP that is formed by reductive synthesis of FUDP, a reaction catalyzed by ribonucleotide reductase. FdUMP binds to both 5,10-methylenetetrahydrofolate (CH_2THF) and TS leading to the formation of a ternary complex, which inhibits TS activity.[332,333] As a result, dTTP pools get depleted, affecting DNA synthesis. FdUMP can also be further phosphorylated to FdUTP, which can be mis-incorporated into the DNA. Alternatively, 5FU may be converted directly or indirectly to 5-fluorouridine 5′-monophosphate (FUMP), which can be incorporated into RNA (upon conversion to its 5′-triphosphate derivative), resulting in inhibition of protein synthesis.[328] The direct conversion of 5FU to FUMP by orotate phosphoribosyl transferase (OPRT) is considered to be the most important pathway for the activation of 5FU. 5FU cytotoxicity is not only determined by the above-described anabolic pathways but also by 5FU catabolism, i.e. 5FU is degraded by dihydropyrimidine dehydrogenase (DPD), which is abundantly found in the liver. This reaction is so fast that the plasma half-life of 5FU is only 6–20 min, i.e. more than 80% of the administered 5FU is catabolized by DPD.[334]

Several strategies have been explored to increase the anticancer activity of 5FU. One of these strategies is to enhance the binding of FdUMP to TS, which increases the cytotoxicity of 5FU. Administration of leucovorin (LV, Fig. 9), which is converted to CH_2THF, increases the intracellular pools of CH_2THF and stabilizes the FdUMP/TS complex. Studies have demonstrated that the addition of LV to bolus 5FU improves response rates compared to

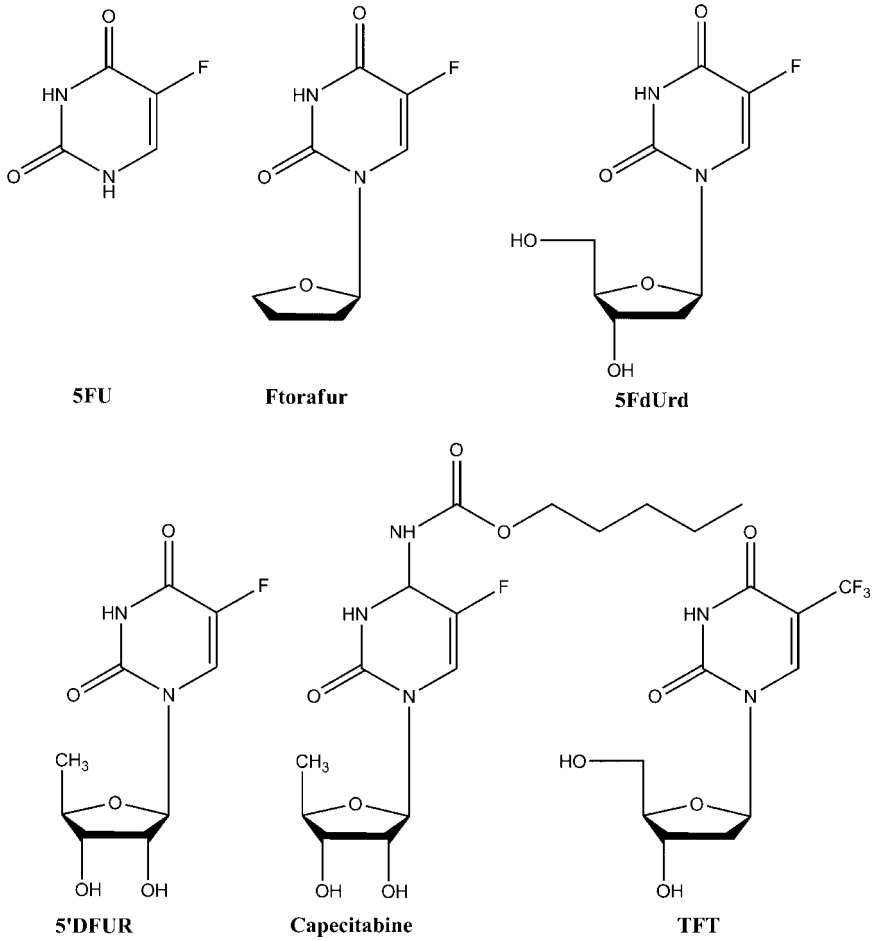

Figure 7. Chemical structure of TFT, 5FU, and 5FU prodrugs.

single agent 5FU treatment (23 versus 11%) in patients with advanced colorectal cancer.[335] 5FU/LV can be combined with oxaliplatin in a formulation known as FOLFOX, which is a frequently used therapy against metastatic colorectal cancer.[336] Another approach to boost the bioavailability of 5FU is the inhibition of DPD, which causes the rapid breakdown of the fluorinated nucleobase derivative. The use of DPD inhibitors enables the oral use of 5FU because they almost completely prevent 5FU degradation in the gastrointestinal tract. 5-chloro-2,4-dihydropyrimidine (CDHP) is an example of such a frequently used DPD inhibitor,[337] but also other DPD inhibitors such as (*E*)-5-(2-bromovinyl)uracil (BVU)[338,339] and 5-ethynyluracil (eniluracil)[340] have been reported. Efficient inactivation of DPD by oral administration of eniluracil has been observed in primary and metastatic colorectal cancer.[341] However, eniluracil is currently withdrawn from further development because several studies showed that the combination of oral eniluracil and 5FU had lower activity compared to intravenous 5FU/LV treatment.[342]

B. 5FU-Prodrugs

As 5FU has a poor oral bioavailability and is rapidly degraded by DPD, 5FU has to be administered via bolus injection or continuous intravenous administration. As these

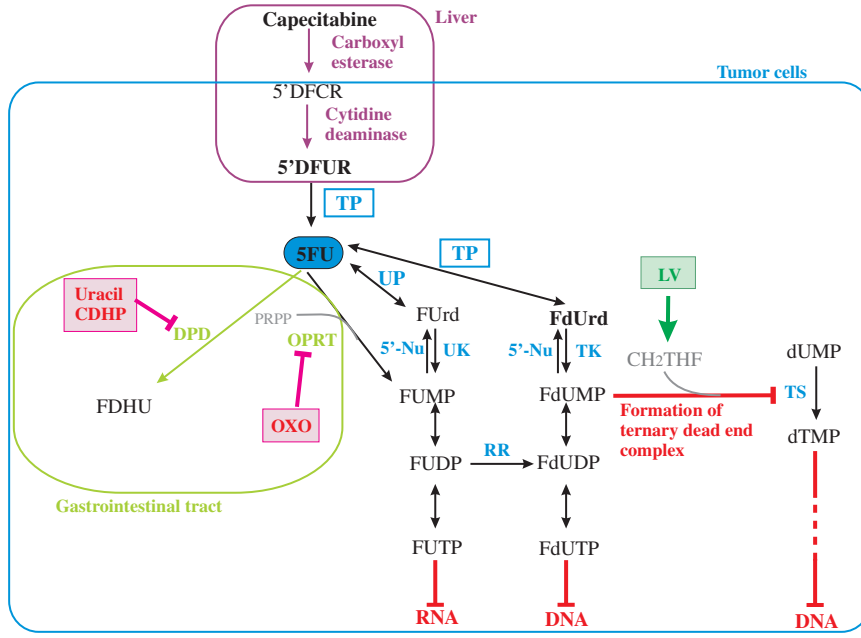

Figure 8. Schematic representation of the metabolic pathways of 5FU and the 5FU prodrugs 5′DFUR and capecitabine. Capecitabine is converted to 5′DFCR and subsequently to 5′DFUR by carboxyl esterase and cytidine deaminase, respectively. Both enzymes are abundantly present in the liver. 5′DFUR is preferentially activated in the tumor cells by TP to 5FU. 5FU exerts it cytotoxic effect through three different pathways and mechanisms. First, 5FU can be converted by TP to FdUrd and subsequently by TK to FdUMP, which forms a covalent ternary complex with TS. This results in the inhibition of TS function and eventually DNA synthesis. FdUMP can also be synthesized after conversion of FUDP to FdUDP by ribonucleotide reductase (RR). FdUrd can also be converted to FdUTP, which can be misincorporated into the DNA. Finally, 5FU inhibits RNA synthesis by its conversion to FUTP. The cytostatic action of 5FU is limited by its degradation by DPD, an enzyme abundantly present in the gastrointestinal tract. This scheme also shows the actions of molecules that modulate 5FU cytotoxicity. Uracil and CDHP prevent degradation of 5FU by inhibiting the catabolic enzyme DPD. OXO specifically inhibits OPRT in the gastrointestinal tract, thereby preventing activation (ribophosphorylation) of 5FU in the normal mucosa and thus limiting the gastrointestal toxicities of 5FU. LV increases the intracellular levels of CH_2THF, thereby enhancing TS inhibition by FdUMP. 5′DFCR, 5-fluoro-5′deoxycytidine; 5′DFUR, 5-fluoro-5′-deoxyuridine; 5FU, 5-fluorouracil; 5′-Nu, 5′-nucleotidase; CDHP, 5-chloro-2,4-dihydropyrimidine; CH_2THF, 5,10-methylenetetrahydrofolate; DPD, dihydropyrimidine dehydrogenase; dUMP, 2′-deoxyuridine 5′-monophosphate; dTMP, 2′-deoxythymidine 5′-monophosphate; FDHU, 5-fluorodihydrouracil; FdUMP, 5-fluoro-2′-deoxyuridine 5′-monophosphate; FdUDP, 5-fluoro-2′-deoxyuridine 5′-diphosphate; FdUrd, 5-fluoro-2′-deoxyuridine; FdUTP, 5-fluoro-2′-deoxyuridine 5′-triphosphate; FUDP, 5-fluorouridine 5′-diphosphate; FUMP, 5-fluorouridine 5′-monophosphate; FUTP, 5-fluorouridine 5′-triphosphate; LV, leucovorin; OPRT, orotate phosphoribosyl transferase; OXO, potassium oxonate; PRPP, phosphoribosyl pyrophosphate; THF, tetrahydrofolate; TK, thymidine kinase; TP, thymidine phosphorylase; TS, thymidylate synthase; UP, uridine phosphorylase; UK, uridine kinase; RR, ribonucleotide reductase. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

infusional 5FU regimens are a costly, labor intensive approach and uncomfortable for the patients, large efforts have been made to design effective 5FU analogues suitable for oral administration. Ftorafur was the first designed oral 5FU prodrug (Fig. 7). This molecule is used in several combinations to improve its bioavailabilty.[206] UFT, a 4:1 combination of uracil and Ftorafur, allows higher levels of circulating 5FU by saturating DPD with its natural substrate uracil. UFT is worldwide approved for the treatment of patients with colorectal cancers.[342] Initially, TP was thought to play a role in the activation of Ftorafur.[343] However, Ftorafur toxicity was not decreased by the TP inhibitor TPI or increased in cells with high TP levels.[344] It is currently thought that this compound is converted by cytochrome P450 enzymes, which are expressed in the liver and in some colon carcinoma cell lines.[345,346] The successor of UFT is S-1, which is a combination of Ftorafur, CDHP and potassium oxonate (OXO) in a molar ratio of 1:0.4:1. CDHP is a potent and reversible inhibitor of DPD. OXO reduces 5FU gastrointestinal toxicity by inhibiting OPRT, which activates 5FU

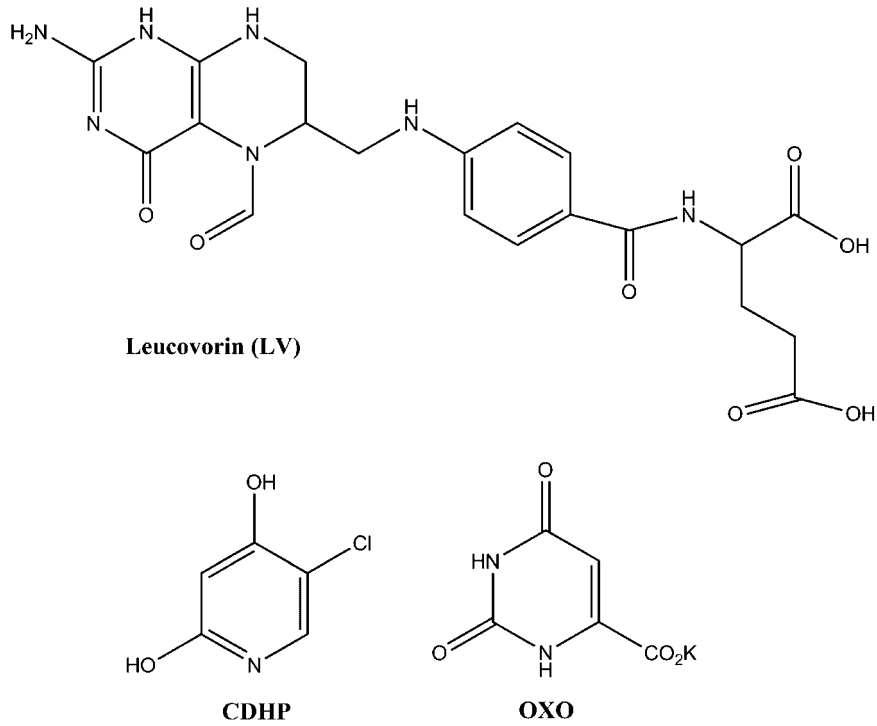

Figure 9. Chemical structure of products that modulate 5FU cytotoxicity: leucovorin, CDHP, OXO.

in the digestive tract.[337] OXO specifically accumulates in the gastrointestinal tract, thus preventing the activation of 5FU in the normal mucosa but not in the tumor.[347]

5-fluoro-2′-deoxyuridine (FdUrd, floxuridine) is the deoxyribose metabolite of 5FU. As seen in Figure 8 it is a precursor of FdUMP, which inhibits TS.[348] FdUrd can also be converted to 5FU in the liver by TP.[349] Like 5FU, this molecule has a short plasma half-life (15 min) and causes gastrointestinal toxicity.[348] Due to the higher toxicity, higher costs, and the equal efficacy of bolus injection of FdUrd compared to bolus 5FU, the use of FdUrd has been very limited.[348] It is only occasionally used as a chemotherapeutic agent for hepatic arterial infusion in the treatment of unresectable liver metastases caused by colorectal cancer.[349]

Another prodrug of 5FU is doxifluridine (5′-deoxy-5-fluorouridine, 5′DFUR), which requires TP for its one-step conversion to 5FU. Numerous in vitro studies showed that transfection of TP cDNA into tumor cells dramatically increases the sensitivity of the cells to 5′DFUR.[6,350–353] Some studies also reported that TP enhances the activity of 5FU, but to a lesser extent than that of 5′DFUR. This is due to the fact that 5′DFUR only requires TP for the conversion to 5FU, while 5FU activation is mediated by at least three different pathways. As TP expression is also high in the gastrointestinal tract, 5′DFUR therapy resulted in dose-limiting toxicity, such as diarrhea.[354,355]

Capecitabine (Xeloda, *N*4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine), an oral prodrug of 5FU, was designed to circumvent the gastrointestinal toxicity of 5′DFUR and to generate 5FU preferentially at the tumor site.[7] The conversion of capecitabine to 5FU requires three distinct steps (see Fig. 8). Once oral capecitabine has passed the intestines in its intact form, it is hydrolyzed to 5′-deoxy-5-fluorocytidine (5′DFCR) by carboxylesterase in the liver. The second step is the conversion to 5′DFUR by cytidine deaminase, which is

localized in the liver and in various tumor types. Finally, 5′DFUR can be converted to 5FU in the tumors by TP[7] (and UP[356,357]). As TP is highly expressed in the tumor tissue, it permits the targeted intratumoral release of 5FU and consequently minimizes systemic toxicity.[358–360] For example, in patients with colorectal cancer, it was proven that following oral administration of capecitabine, the 5FU concentration in the tumor tissue was 3.2 times higher than in adjacent nontumorigenic tissue and 21 times more elevated than in plasma.[358] Furthermore, phase III trials enrolling patients with metastatic colorectal cancer showed that single agent capecitabine treatment is at least as effective as 5FU/LV therapy and is associated with significantly fewer clinically relevant toxicities.[359–362] As capecitabine is at least as active as the 5FU/LV standard and better tolerated by the patients, it has become one of the most prescribed oral chemotherapeutic agents. Currently, capecitabine is approved by the US Food and Drug Administration (FDA) as an adjuvant in stage III Dukes' C colorectal cancer and as first-line monotherapy in metastatic colon cancer. The drug is also accepted for use against metastatic breast cancer in combination with docetaxel after failure of anthracycline-based treatment or as monotherapy when patients have failed paclitaxel-based therapy. At the moment, the combination of capecitabine with different other anticancer agents such as bevacizumab, enzastaurin, and sorafenib is being evaluated in clinical trials.[303,363–365]

As TP is the rate-limiting enzyme for the activation of capecitabine, it might be a useful predictor of tumor response to capecitabine-based chemotherapy. In colorectal[366] and advanced non-small cell lung[367] cancer, TP expression was associated with tumor response to capecitabine, while in patients with breast[368] and gastric[369] cancer the TP/DPD ratio showed a significant correlation with response to capecitabine therapy.

C. Combination of TP-Inducible and TP-Targeted Therapy

As it has been shown in numerous transfection experiments that the antitumoral activity of 5′DFUR and capecitabine depends on their activation by TP, this enzyme is used as a target to enhance the anticancer activity of these fluoropyrimidines. As described in Section 5, TP levels can be elevated by several anticancer treatments, such as X-ray irradiation and chemotherapeutic agents (i.e. taxanes, mitomycin C, cyclophosphamide). It has been hypothesized that combination of TP-inducing therapies (such as taxanes) with TP-targeted therapy (such as capecitabine) would improve the clinical efficacy of these fluoropyrimidines. In the WiDr colon and MX-1 mammary human cancer xenograft models, the combination of X-ray irradiation with either capecitabine or 5′-DFUR showed a better antitumor effect than either radiation or chemotherapy alone.[309] Furthermore, several clinical trials have demonstrated a synergy between capecitabine and TP-inducible chemotherapy. For example, in a large randomized phase-III trial on metastatic breast cancer it was demonstrated that the addition of capecitabine to docetaxel therapy results in an increased response rate, time to progression, and survival compared to standard treatment alone.[370] Also the combination with other TP-inducible therapies, such as irinotecan, oxaliplatin, cisplatin, cyclophosphamide, paclitaxel, mitomycin C, and irradiation resulted in improved survival and time-to-progression compared to the monotherapy.[7] The combination of capecitabine plus oxaliplatin (XELOX regimen) now represents a new standard of care for metastatic colon carcinoma.[371]

D. TFT

The fluoropyrimidine nucleoside 5-trifluorothymidine (TFT) was originally synthesized by Heidelberger et al. in 1964.[372] TFT is phosphorylated by TK to its active monophosphate TFT-MP, which inhibits TS.[373] In contrast to FdUMP, TFT-MP does not form a ternary complex with TS, but binds covalently to the active site of TS.[374] TFT-MP can also be

further phosphorylated to TFT-TP, which can be incorporated into the DNA leading to cell death due to DNA strand break formation.[375] Since 1980, TFT has been used for the topical treatment of epithelial keratitis caused by herpes simplex virus.[376] This molecule has also been evaluated as an antitumor agent but the clinical studies were discontinued because of the high toxicity of TFT and its rapid degradation by TP that inactivates TFT by converting it to its inactive base.[5,377] Given as a single agent, the plasma half-life of TFT is less then 20 min.[378] Therefore, TFT was recently chosen to be combined with TPI, a very potent inhibitor of TP (see Section 6.A). This oral combination therapy, called TAS-102, combines TFT and TPI in a 1:0.5 molar ratio.[379] The application of TFT together with TPI bypasses TFT degradation by TP resulting in increased TFT plasma levels compared to TFT alone. TAS-102 thus improves the bioavailability and thereby the efficacy of TFT.[318] Another advantage of TAS-102 is that TPI might also abrogate the angiogenic properties of TP. Furthermore, TAS-102 can also be used against cancers that are resistant to 5FU, as shown by in vitro studies and tumor implants in nude mice.[375,380] So far, several phase I clinical trials using TAS-102 have been completed. The toxicities observed were granulocytopenia, nausea, vomiting, diarrhea, fatigue, and rash. A phase I clinical trial demonstrated that TAS-102 is active against heavily pretreated metastatic breast cancers.[381] However, in a recent phase I trial, where TAS-102 was administered daily on a 5-day-a-week schedule to patients with solid (mostly colorectal) tumors, patients treated with TAS-102 showed no objective response although stable disease was seen in 18 of 61 patients.[382] Currently, phase II trials of TAS-102 alone or in combination with other therapies against breast, gastrointestinal, and other solid tumors are ongoing.[381,382] The combination of TFT together with oxaliplatin was tested in vitro in various colon carcinoma cell lines and strong synergism was observed. These results provide a motivation for the clinical study of TAS-102 together with oxaliplatin in the treatment of colorectal cancer.[383]

E. Mycoplasmal TP and Fluoropyrimidines

TP is not only present in humans; TP activity was also detected in different *Mycoplasma* species.[318,384,385] Mycoplasmas are the smallest self-replicating bacteria, which can cause respiratory and urogenital diseases.[386] Most mycoplasmal infections remain, however, unidentified, because many people seem to be chronically infected without apparent clinical symptoms.[387] Mycoplasmas might also play a role in cancer.[388,389] Mycoplasmal infections are associated with leukaemia and ovarian and cervical cancer.[390–392] In particular, the species *M. hyorhinis* is frequently found in tissues of gastric, colon, esophageal, lung, and breast cancer, but not in analogous nontumorigenic tissue.[393] Chronic and persistent infections with mycoplasmas affect many biological characteristics of mammalian cells and can even lead to malignant transformations.[394–396] The *M. hyorhinis*-encoded protein p37 was shown to alter gene expression, growth, and migratory potential of prostate cancer cell lines in vitro.[397–399] p37 was also found to promote cancer cell invasiveness and metastasis by activation of MMP-2 and by phosphorylation of the epidermal growth factor receptor.[399] Moreover, in an experimental metastasis mouse model, p37-encoding adenovirus-infected mouse melanoma B16F10 cells formed more metastatic lesions than the parental cell line.[399]

Recently, it was shown that TP encoded by *M. hyorhinis* not only catalyzes the conversion of thymidine to thymine, but also efficiently recognizes FdUrd, TFT, and 5′DFUR.[389,400] As a result, the cytostatic activity of FdUrd and TFT was significantly decreased in MCF-7 breast carcinoma cells infected with *M. hyorhinis* compared to control MCF-7 cells. The sensitivity to 5FU was not altered by mycoplasma infection while 5′DFUR was at least 30-fold more cytostatic in mycoplasma-infected MCF-7 cells, suggesting that

mycoplasma-encoded TP activated this molecule. Addition of the TP inhibitor TPI or the mycoplasma-specific antibiotic plasmocin could restore the altered cytostatic activity.[389,400] Also HCT116 colon cancer cells infected with mycoplasma were 5- and 100-fold more resistant to 5-FU and FdUrd, respectively, than the parental noninfected cells.[401] These data demonstrate that the presence of mycoplasma and thus mycoplasmal TP may severely affect the cytostatic efficacy of FdUrd, TFT (and 5FU), suggesting that the combination of these anticancer agents with a specific antibiotic against mycoplasmas might improve the efficacy of these drugs.[389,400]

8. CONCLUSIONS AND PERSPECTIVES

There is compelling evidence that the intracellular enzyme TP plays an essential role in tumor progression. TP stimulates tumor growth by protecting tumor cells from apoptosis and by inducing angiogenesis. Although many investigators have tried to unravel the molecular mechanisms through which TP exerts these biological effects, more research needs to be done to gain more detailed insight into the signal transduction cascades induced by TP and its product metabolite 2DDR. This knowledge could offer crucial information for the rational development of strategies to inhibit the protumoral actions of TP. Indeed, as TP expression is elevated in numerous tumor types, inhibition of TP activity might offer a potential strategy in the battle against cancer. In this respect, more potent TP inhibitors need to be synthesized and (pre)clinically evaluated as TPI is currently the only available TP inhibitor that is being investigated in clinical trials. As such, the results of the phase II clinical trials with TAS-102 will be of particular interest, as they will provide important information on the impact of TP inhibitors on tumor growth in cancer patients.

Recent data have shown that tumors treated with anti-angiogenic monotherapy seem to escape drug treatment by upregulating and/or activating alternative angiogenic pathways. Therefore, future strategies should favor the concomittant attack of multiple targets by combining different anti-angiogenic molecules or anti-angiogenic molecules with radio- and/or chemotherapy. Thus, TP presents an alternative target or an additional target to existing anticancer therapies. The potential use of TP inhibitors is not limited to cancer as TP is also involved in many inflammatory diseases, such as RA. Also these patients might thus benefit from treatment with TP-inhibitory drugs and the application of TP inhibitors in the treatment of inflammatory diseases should be further explored.

However, the use of TP inhibitors in the treatment of cancer should be carefully and cautiously considered, because TP activity is required for the activation of the commonly used 5FU-prodrug capecitabine. Thus, TP inhibitors should not be combined with such TP-activation-dependent therapeutic agents. One possible strategy to overcome this problem might be to inhibit the downstream mediators of TP instead of directly inhibiting TP activity per se. 2DLR has been shown to inhibit the biological actions of TP without affecting its enzymatic activity and therefore it might be a good candidate for combination with fluoropyrimidine chemotherapy. However, as TP fulfills a key role in capecitabine activation, most investigations have explored the combination of TP-upregulating therapies with capecitabine Clinical trials combining capecitabine with TP-inducible therapies showed that combination of both therapies results in a higher antitumor efficacy than monotherapy of either agents.

In conclusion, TP may play a dual role in cancer development and therapy: TP stimulates tumor growth but at the same time it is also required to activate the chemotherapeutic agent capecitabine. Depending on the type of the tumor and the nature of the therapeutic agents, cancer patients may benefit from TP-inducible therapies or TP inhibitory drugs.

9. ABBREVIATIONS

BVDU	5-(*E*)-(2-bromovinyl)-2′-deoxyuridine
CAM	chorio-allantoic membrane
CDHP	5-chloro-2,4-dihydropyrimidine
CH_2THF	5,10-methylenetetrahydrofolate
2DDR	2-deoxy-D-ribose
2DDR-1P	2-deoxy-D-ribose-1-phosphate
5′DFUR	5-fluoro-5′-deoxyuridine
2DLR	2-deoxy-L-ribose
DPD	dihydropyrimidine dehydrogenase
ECM	extracellular matrix
EPC	endothelial progenitor cell
FAK	focal adhesion kinase
FdUMP	5-fluoro-2′-deoxyuridine 5′-monophosphate
FdUrd	5-fluoro-2′-deoxyuridine
FLS	fibroblast-like synoviocytes
5FU	5-fluorouracil
HIF-1α	hypoxia-inducible factor-1α
HO-1	heme-oxygenase-1
HUVEC	human umbilical vein endothelial cell
IC_{50}	50% inhibitory concentration
IFN-γ	interferon-γ
IL	interleukin
LV	leucovorin
MMP	matrix metalloproteinase
MNGIE	mitochondrial neurogastrointestinal encephalomyopathy
OPRT	orotate phosphoribosyl transferase
OXO	potassium oxonate
PD-ECGF	platelet-derived-endothelial cell growth factor
PRPP	phosphoribosyl pyrophosphate
RA	rheumatoid arthritis
RR	ribonucleotide reductase
TAM	tumor-associated macrophage
TFT	5-trifluorothymidine
TK	thymidine kinase
TP	thymidine phosphorylase
TPI	5-chloro-6-[1-(2-iminopyrrolidinyl)methyl] uracil hydrochloride
TS	thymidylate synthase
UP	uridine phosphorylase
VEGF	vascular endothelial growth factor
VSMC	vascular smooth muscle cell
XO	xanthine oxidase

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