REVIEW ARTICLE

Thymidine phosphorylase, 2-deoxy-D-ribose and angiogenesis

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Angiogenesis is the term used to describe the formation of new blood vessels from the existing vasculature. In order to attract new vessels, a tissue must release an endothelial-cell chemoattractant. 2-Deoxy-D-ribose is produced *in vivo* by the catalytic action of thymidine phosphorylase (TP) on thymidine and has recently been identified as an endothelial-cell chemoattractant and angiogenesis-inducing factor. TP, previously known only for its role in nucleotide salvage, is now known to be angiogenic. TP expression is elevated in many solid tumours and in chronically inflamed tissues, both known areas of active angiogenesis. There

is evidence that TP is also involved in physiological angiogenesis such as endometrial angiogenesis during the menstrual cycle. The majority of known endothelial-cell chemoattractants are polypeptides that bind to endothelial-cell-surface receptors. In contrast, 2-deoxy-D-ribose appears to lack a cell-surface receptor. Glucose is another sugar that acts as an endothelial-cell chemoattractant. The migratory activity of glucose is blocked by ouabain. It is possible that 2-deoxy-D-ribose and glucose stimulate endothelial-cell migration via a similar mechanistic pathway.

ANGIOGENESIS

Endothelial-cell migration, but not proliferation, is essential for angiogenesis. Angiogenesis is rare in the healthy adult, occurring only during wound healing, the female reproductive cycle and in regions that have become inadequately perfused due to an increased metabolic load. Angiogenesis is an essential component of solid-tumour growth and the progression of chronic inflammatory diseases such as rheumatoid arthritis and psoriasis. Numerous reviews of angiogenesis have been published over the past few years, as have two recent books [1,2].

It has been known for some time that endothelial-cell migration without proliferation is sufficient for an angiogenic response. Thus Knighton (cited in [3]) irradiated whole rabbits such that cell proliferation was completely blocked. Subsequent implantation of an angiogenic stimulus into the cornea elicited a strong response [3]. It has also been shown that capillary extension can proceed for at least 2 days by endothelial-cell migration alone [4]. The proliferative index of tumour endothelium, although substantially higher than in normal tissues, is still quite low in spontaneous human tumours [5].

THYMIDINE PHOSPHORYLASE (TP) AND NUCLEIC ACID HOMOEOSTASIS

TP was first purified and characterized as an enzyme involved in nucleic acid homoeostasis. A role for TP in nucleic acid metabolism was well established long before its angiogenic activity was discovered. TP was first described almost 45 years ago [6], and purified in the mid-1970s from both *Escherichia coli* and *Salmonella* [7,8]. *E. coli* TP is a homodimer of 45 kDa subunits [9]. Eukaryotic TP, a 47 kDa subunit homodimer, was first purified to homogeneity from the human amniocorion [10,11].

The TP catalysed reaction that plays a role in maintaining the balance of the nucleotide pool is:

Although the reaction is reversible, nucleic acid homoeostasis is dependent upon the catabolic reaction which drives the salvage pathway shown in Scheme 1. The salvage pathway ensures that the pyrimidine-nucleotide pool is sufficiently large for efficient DNA repair and replication.

The effect of substrate and product on TP activity suggest that it evolved as a catabolic enzyme. Thus Iltzsch et al. [12], showed that TP has several thymine-binding sites. When these sites are occupied, the enzyme is inhibited. Inhibition by end products is a common control mechanism; however, it is most unusual for an enzyme to be inhibited by its substrate. It was also reported that thymidine and phosphate co-operatively enhance TP activity. This is consistent with these being substrates. The TP gene is induced by thymidine in prokaryotes [13], but there is no report of this in eukaryotes. Again, one can postulate that this control mechanism would only evolve if thymidine were usually the substrate and not the product. The catabolic reaction is also responsible for the angiogenic activity (see below).

TP RELEASES AN ENDOTHELIAL-CELL CHEMOATTRACTANT: A NEW ROLE FOR AN OLD ENZYME

Platelet-derived endothelial-cell growth factor

In 1987 a 'novel' angiogenic protein was isolated from platelet lysate [14]. It was named 'platelet-derived endothelial-cell growth factor' (PD-ECGF; not to be confused with platelet-derived growth factor, 'PDGF'). As the name implies, it was thought to be a classic polypeptide growth factor that would bind to a cognate cell-surface receptor and elicit a cellular response. PD-ECGF was first purified to homogeneity in 1989 [15] and its crystal structure elucidated, though not to a particularly high resolution [16]. The PD-ECGF gene was isolated [17]. This had not previously been achieved for TP.

Abbreviations used: TP, thymidine phosphorylase; PD-ECGF, platelet-derived endothelial-cell growth factor; VEGF, vascular endothelial growth factor; PKC, protein kinase C; fUra, 5'-fluorouracil.

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Scheme 1 Thymidine transport and metabolism in the mammalian cell.

TP catalyses the breakdown of thymidine to the base thymine and the sugar 2-deoxy-D-ribose 1-phosphate. The latter can only leave the cell once it has been dephosphorylated. TP clears cytosolic thymidine from the cytoplasm and drives the nucleoside salvage pathway. TP promotes genetic stability by keeping the nucleotide pool topped up. Thymidine entry into the cell is rapid and non-energy-dependent. If thymidine levels outside the cell are not limiting, TP should not encounter a shortage of substrate within the cell. An up-regulation of TP activity will therefore also mean an increase in production of the angiogenic 2-deoxy-D-ribose. Compounds outside the cell are shown in red, inside the cell in black. The cell membrane is shown in pink. fdU is 5'-fluoro-2'-deoxyuridine.

The assertion that PD-ECGF is an endothelial-cell mitogen was based on endothelial-cell radiolabelled-thymidine uptake [18]. However, true growth curves were not reported. In 1992, a protein sequence search revealed that PD-ECGF shared significant sequence identity with *E. coli* TP [19]. This raised the possibility that the thymidine-uptake data were an artefact, and this was subsequently shown to be the case. Thus PD-ECGF in cell supernatants hydrolyses serum-derived thymidine, depleting the cells of this metabolite. A subsequent pulse of radiolabelled thymidine is rapidly taken up by the cells compared with controls. It was shown that PD-ECGF/TP is angiogenic by stimulation of endothelial-cell migration. It is not an endothelial-cell growth factor [20]. The name 'PD-ECGF' is therefore a misnomer and for the remainder of this review the protein will be referred to as 'TP'.

TP induces angiogenesis by catalytic release of 2-deoxy-D-ribose from thymidine

The angiogenic activity of TP is dependent upon its enzymic activity, as mutagenesis of the enzyme's active-site residues abolished the angiogenic activity [20,21]. The TP enzymic inhibitor 6-amino-5-chlorouracil has also been reported to block the angiogenic activity of TP [22].

There are two possible reasons for a cell being attracted to an area of high enzyme activity. Either the substrates for the catalysed reaction are chemorepellant, or the products are chemoattractant. The first possibility is theoretically appealing

for TP, as millimolar concentrations of thymidine are cytostatic as a result of inhibition of ribonucleotide reductase [23]. Endothelial cells may therefore have evolved a chemorepellent response to thymidine. On examination thymidine is not, however, an endothelial-cell chemorepellant [24]. Instead, TP is angiogenic by catalytic release of the endothelial-cell chemoattractant 2-deoxy-D-ribose [22,25].

TP lacks a signal sequence required for cell secretion. It is therefore most likely to attract endothelial cells by operating inside a second cell type. TP releases the monosaccharide 2-deoxy-D-ribose 1-phosphate. Dephosphorylation permits departure from the cell as the cell-permeant 2-deoxy-D-ribose. It has been shown that addition of thymidine to platelets (which have high levels of TP) gave rise to thymine and 2-deoxy-D-ribose (but not 2-deoxy-D-ribose 1-phosphate) in the extracellular medium [26]. Clearly, the phosphate can be removed within the cytoplasm.

In the late 1970s, plasma TP levels were shown to be elevated in cancer patients [27]. This does not, however, prove that TP has an extracellular action. Substantial cell necrosis and lysis are thought to occur within solid tumours, and intracellular enzymes will therefore spill into the plasma, raising plasma TP levels.

TP is angiogenic because of the same intracellular enzyme activity that drives the nucleotide salvage pathway. Recently, a third role for TP has been reported, and the mechanism of action is surprisingly different. TP directly binds a specific receptor on the surface of the target cell. In this guise, TP is known as gliostatin [28]. Gliostatin is both a neurotrophic factor and an inhibitor of glial-cell proliferation. It is thought to play a crucial role in the formation of the blood/brain barrier and in the later stages of repair following brain injury [29]. TP secretion requires a post-translational modification in which the serine residues of TP are covalently linked to the phosphate groups of ATP [30]. This activity appears to be unrelated to the angiogenic activity of TP.

TP IN PATHOLOGY

Elevated TP activity generates increased 2-deoxy-D-ribose

Elevated TP levels do not necessarily lead to increased 2-deoxy-D-ribose; for example, the supply of substrate thymidine might be limiting. Several pieces of evidence suggest that this is, however, not the case. Wohlheuter et al. were the first to uncouple the transport of thymidine from its intracellular metabolism and showed that thymidine transport across the cell membrane is fast and energy-independent [31]. Once in the cell, thymidine may be cleaved by TP (Scheme 1). The primary source of plasma thymidine is thought to be cell turnover [32]. Cell turnover will be fast in a prevascular tumour, where high rates of cell division balance equally high rates of cell death. For these reasons, one can assume that intracellular TP activity is roughly proportional to the amount of 2-deoxy-D-ribose produced. *In vivo* models have shown that elevated TP leads to an increase in tissue vascular density. Thus, Ha-ras-transformed 3T3 cells transfected with TP cDNA form tumours with an increased vascular density [33] compared with controls. Similarly, MCF-7 cells transfected with TP cDNA and xenografted showed that TP enhances not only the vascular density but also the tumour growth rate [20,34] (Figure 1).

TP expression has been shown to be a reliable prognostic indicator in bladder carcinoma. Thus, the TP levels in invasive tumours were 33-fold higher than in superficial tumours and 260-fold higher than in normal bladder [35]. High TP expression in the primary tumour is a risk factor for both hepatic metastasis and lymph-node metastasis in gastric carcinoma [36,37]. TP

Figure 1 TP enhances tumour growth *in vivo* but has no effect on growth *in vitro*

Effect of overexpression of TP on MCF-7 breast-carcinoma growth. (a) TP activity of wild-type (WT) cells (transfected with vector alone) and TP-transfected (TP-7) cells. (b) Growth of WT (○) and TP-7 (●) cells in 1% foetal-calf serum *in vitro*. (c) Growth of WT (○) and TP-7 (●) cells when xenografted into BALB/c *nu/nu* mice.

expression is significantly raised in non-small-cell lung carcinomas, where it correlates with both tumour vascularization and patient prognosis [38]. This is not, however, the case in small-cell lung carcinoma [39]. Areas of high blood flow in ovarian tumours show increased TP expression, and this has been shown to correlate with malignancy [40,41]. TP is also a prognostic indicator in Non-Hodgkin's lymphoma [42]. 2-Deoxy-D-ribose-mediated angiogenesis may be part of the pathology of AIDS, as TP levels are elevated in Kaposi's sarcoma [43]. Finally, in primary human breast cancer, TP expression correlates with the vascular density of the tumours, in contrast with the expression of six other angiogenic factors that showed no such relationship [44].

TP activity is raised in chronic inflammation

TP is also overexpressed in a wide range of chronic inflammatory diseases. TP levels are higher in rheumatoid (chronic inflammatory) arthritis than in osteoarthritis [45,46]. TP expression is also raised in psoriatic lesions. This was initially thought to be because of TP's role in nucleotide salvage: rapidly proliferating keratinocytes requiring higher TP levels to maintain the nucleotide balance [47]. Recently, however, TP overexpression in the psoriatic lesion has been shown to increase the vascular density of thickened psoriatic skin [48].

There is much evidence that inflammatory cells such as macrophages can overexpress TP. TP levels are highest at the edge of gastric ulcers [49]. It is the edge of a site of chronic inflammation which contains the highest concentration of inflammatory cells. A tumour is also a site of chronic inflammation. In some cancers, TP overexpression is seen in the invading macrophages, but not the tumour cells themselves [50]. TP levels in macrophages that have invaded colon tumours correlate well with microvessel density and are a good prognostic indicator [51,52]. As TP overexpression is seen in many chronic diseases, it is important that we understand both the regulation of TP expression and the action of 2-deoxy-D-ribose on the endothelial cell.

THE REGULATION OF TP EXPRESSION

The intracellular molecular mechanisms regulating TP gene expression are not well understood. In recent months, however, it has been discovered that similar mechanisms control transcription of the TP gene and the gene encoding vascular endothelial growth factor (VEGF). The extracellular factors which act upon the cell to up-regulate TP expression are better understood. These are summarized in Scheme 2.

Transcriptional control of TP expression

Apart from steroid-hormone-responsive elements (of importance in endometrial angiogenesis), the upstream promoter of the TP gene contains only SP1-binding sites [17]. The transcription factor SP1 is activated by protein kinase A, which is in turn activated by cAMP [53]. TP transcription is not, however, controlled by this signal-transduction pathway alone. Thus phorbol esters, which activate protein kinase C (PKC), also elevate TP expression [54].

SP1 sites are also involved in VEGF transcription [55]. Indeed, in breast cancer there is a tendency for VEGF and TP to be co-expressed [56]. In psoriasis, where TP is known to be over-expressed, VEGF levels are also high [57]. A tendency for VEGF and TP to be co-expressed may hold consequences for the patient. One would expect co-expression of an endothelial-cell proliferative factor and an endothelial-cell chemotactic factor to co-operatively increase blood-vessel formation. This appears to be the case in primary human breast tumours. High expression of both TP and VEGF is a particular strong prognostic marker in primary human breast tumours [58]. The frequency of hepatic recurrences in gastric carcinoma is significantly raised when the primary tumour overexpresses both VEGF and TP [59]. In some cancers, however, VEGF and TP are not co-expressed. In bladder cancer, for example, TP levels are higher in invasive than in superficial tumours, whilst VEGF is more strongly expressed in superficial tumours [35]. Control of transcription not involving SP1 sites must operate in these cases.

Tumour cells initiate angiogenesis as a result of change in oncogene and tumour-suppressor gene expression, whose gene products turn the angiogenic switch on and off respectively. For

Scheme 2 Elevation of TP levels in pathological and physiological angiogenesis

The transcriptional and post-transcriptional control of TP expression is not well understood. Environmental influences that raise TP activity above the level required for efficient nucleotide salvage are, however, becoming better characterized. TP is not just a nucleotide-salvage enzyme that is only up-regulated in disease states: TP is up-regulated in tumours and chronic inflammation through mechanisms which also control physiological angiogenesis. The cytokines interferon- γ and TNF- α , released by inflammatory cells during wound healing, up-regulate TP in many carcinoma lines. They may also raise TP expression in the inflammatory cells themselves. Vascularization of hypoxic regions is also partially controlled by TP, which is up-regulated in response to low oxygen and low pH. It is unclear whether TP is up-regulated by genetic lesions in tumour cells.

example, the tumour suppressor p53 elevates production of anti-angiogenic thrombospondin and inhibits expression of VEGF [60,61]. There is as yet no evidence that oncogene or tumour-suppressor gene products control TP expression. In both bladder and breast carcinoma, TP levels are independent of the p53 status of the carcinoma cells [62,63].

TP is up-regulated by both the low oxygen partial pressure and low pH seen in poorly perfused areas [64]. The pattern of TP expression in a diseased area often indicates that TP up-regulation is due to the hypoxia caused by the disease state rather than the disease state itself. For instance, in high-grade non-Hodgkin's lymphoma, it is the normal dendritic cells in the immediate vicinity of the tumour which show elevated TP expression [65].

Post-transcriptional control of TP expression

TP levels may not only be controlled at the transcriptional level. Modulation of the TP mRNA stability could also play a role. For instance, the cytokine interferon- α causes a 5-fold rise in TP mRNA levels, but an 8-fold rise in TP enzyme activity [66]. This implies that there is at least some post-transcriptional control of TP expression

TP EXPRESSION IN PHYSIOLOGICAL ANGIOGENESIS

TP and the female reproductive cycle

The endometrium undergoes extensive angiogenesis during each menstrual cycle (see Rees and Bicknell [67]). TP is the only angiogenic factor whose levels have been shown to vary during

the course of the menstrual cycle [68]. The TP promoter contains half-palindromic oestrogen response motifs [69], and endometrial TP expression is raised by the unique combination of progesterone and transforming growth factor- β 1 [68]. Two alternative forms of TP are found in the placenta. As stated above, the eukaryotic TP subunit normally has a molecular mass of 47 kDa. In the placenta the 47 kDa protein has an additional five amino acids on its N terminus: processing occurs at Thr⁶, not Ala¹¹ [18,70]. In addition, the placenta also contains a 27 kDa splice variant [71]. It is not known whether homodimers of this TP isoform are enzymically active.

Mutation and the regulation of TP expression

In addition to hypoxia and cytokines, genetic changes may elevate TP. Elevated TP will favour tumour progression for two reasons: (i) increased angiogenesis and (ii) increased thymidine salvage.

The evidence for TP gene mutations in tumour cells is limited. The fact that normal cells bordering the tumour often show raised TP levels indicates that raised TP expression in the cancer cell is usually triggered by environmental and not genetic change. Loss of chromosome 22, on which the TP gene is sited, has been observed in Kaposi sarcoma, a disease characterized by up-regulation of TP [72], arguing against a genetic role for TP up-regulation. Only one potential example of genetic up-regulation of TP can be found in the literature. Normal TP mRNA is 1.8 kb in length. The human epidermal carcinoma cell line A431, however, has 3.0 and 3.2 kb transcripts. The increased length is due to a very long 5' leader sequences. This leader contains seven or eight copies of the SP-1-binding site present in the normal promoter of the 1.8 kb transcript [73]. This genetic change could conceivably raise TP levels.

THE ACTION OF 2-DEOXY-D-RIBOSE ON THE ENDOTHELIAL CELL

Prokaryotes, eukaryotes and cell-surface pentose-sugar receptors

Figure 2 shows that the endothelial migratory activity of deoxyribose is highly stereospecific. Thus, while the D isomer shows strong activity, the L isomer is inactive. In contrast with eukaryotic cells, prokaryote migration towards soluble sugars is comparatively well understood. *Escherichia coli* spends its life either tumbling in circles or swimming in a relatively straight line. The choice between these two states is controlled by histidine kinase receptors on the cell surface. These measure the rate at which the concentration of chemoattractant alters in the extracellular medium. If levels of the chemoattractant are rising, the bacterial cell will continue swimming in a straight line. If the concentration of chemoattractant starts to fall, however, the cell begins tumbling. It then chooses a new swimming direction at random (reviewed in [74]). *E. coli* has many cell-surface receptors for sugars, including a specific one for D-ribose [75]. At least five bacterial receptors for D-ribose have been identified, including some that are transmembrane.

Eukaryotic cells in the multicellular organism (with the exception of macrophages) do not generally migrate. Temporal measurement of changes in chemoattractant concentration is therefore not a feasible method of controlling chemotaxis. Static eukaryotic cells must instead determine the direction of a gradient of chemoattractant by comparing chemoattractant concentrations at opposite ends of the cell. This spatial mechanism is possible in the eukaryotic cell because of its large size. Once the eukaryotic cell has established the direction from which a soluble chemoattractant derives, it will begin migration by extension of

pseudopodal processes from the end of the cell at which the chemoattractant concentration is greatest.

The majority of receptors are on the cell surface. To date all endothelial-cell chemoattractants with a defined mechanism of action are polypeptides which bind to cognate cell-surface receptors [76]. Despite these fundamental differences between pro- and eu-karyotic chemotaxis, virtually all known examples of eukaryotic chemotaxis are mediated via receptors. It was therefore expected that endothelial-cell chemotaxis towards an area of high TP activity would be mediated by a cell-surface 2-deoxy-D-ribose receptor, possibly remotely similar to that in *E. coli*.

Using 2-deoxy-D- ^3H ribose we have been able to obtain no evidence for specific binding to endothelial cells [34]. It remains possible that the cell may employ a non-receptor-mediated mechanism to sense a 2-deoxy-D-ribose gradient.

Entry of sugar into the endothelial cell may permit it to sense a sugar gradient

In 1993, Vogel et al. [77] examined the migration of corneal (not vascular) endothelial cells in response to gradients of several simple sugars. The results showed that endothelial cells only migrated towards sugars that could be utilized as a readily accessible source of energy. No binding studies were performed, but it was shown that migration towards glucose is dependent upon the Na^+/K^+ -ATPase pump. This implies that chemoattractive sugars must enter the cell, for blocking the Na^+/K^+ will reduce glucose entry into the cell via symport transport with Na^+ . It was also shown that incubation of cells in physiologically high concentrations [25 mM] of glucose down-regulated Na^+/K^+ -ATPase activity, and it is possible that there is a negative-feedback loop that regulates glucose entry into the cell.

The cells used in the study had been cultured in physiological levels of nutrients and would have had a normal level of intracellular ATP. They would have sufficient energy, even in the absence of glucose entry into the cell, to migrate in response to a sugar ligating an extracellular receptor. Thus it appears that the entry of glucose into the cell may do more than simply provide energy for migration: it might conceivably allow the endothelial cell to sense the sugar concentration gradient.

Sugar entry may drive local pseudopod formation

Pseudopodia extend from the end of the cell at which the chemoattractant concentration is highest. The highest rate of glycolysis is also seen at this end of the cell. Glycolysis occurs in the cytoplasm where the glycolytic enzymes are attached to the cytoskeleton [78]. Any rise in intracellular ATP will therefore be localized and at the cell periphery. A local rise in intracellular ATP should mean more actin-ATP in that area. Actin-ATP is essential for the first stages of pseudopod formation. Locally elevated ATP levels will provide more substrate for the initial, ATP-dependent, steps of glycolysis; further elevation of the local ATP concentration might also activate the Na^+/K^+ pump, causing a greater regional influx of glucose.

Figure 2 2-Deoxy-D-ribose, but not the enantiomer 2-deoxy-L-ribose, stimulates microvascular (HMEC-1) endothelial-cell migration [96]

Endothelial-cell migratory and angiogenic activity of 2-deoxyribose. (a) HMEC-1 cell migration in a Boyden chamber in response to 2-deoxy-D-ribose, 2-deoxy-L-ribose and a basic-fibroblast-growth-factor (bFGF) control. (b and c) Chorioallantoic membrane of the chick after a control (b) and 2-deoxy-D-ribose (c) implant.

Another study which supports these ideas compared chemotaxis and phagocytosis in granulocytes [79]. Both are energy-dependent processes relying upon cytoskeletal changes, but glucose uptake is only elevated during chemotaxis. In phagocytosis the extra energy is obtained by glycogenolysis. This implies that glucose entry is not required to supply the energy for cell migration. Instead it may be carrying out an additional function, such as providing information on sugar gradients and the energy environment. It has been shown that elevated glucose activates PKC in the endothelial cell [80]. A PKC inhibitor (GF-109203X) has been shown to block migration in response to glucose [81].

2-Deoxy-D-ribose as a cellular energy source

Vogel et al. [77] did not test whether corneal endothelial cells migrate towards 2-deoxy-D-ribose. There is, however, good reason to suspect that 2-deoxy-D-ribose attracts cells by acting as an energy source. Deoxyriboses are only ever degraded; ribose itself is used in the nucleotide-synthetic reaction. Thus 2-deoxy-D-ribose enters exclusively the following catabolic pathway, it is phosphorylated to 2-deoxy-D-ribose 5-phosphate that is then cleaved by deoxyribose-phosphate aldolase (4.1.2.4) to acetaldehyde and glyceraldehyde-3-phosphate, which enter glycolysis. Each molecule of 2-deoxy-D-ribose yields half of the ATP obtained from a molecule of glucose. The enantiomer 2-deoxy-L-ribose, which does not enter glycolysis, fails to stimulate endothelial-cell migration [34].

2-Deoxy-D-ribose has been shown to be an important energy source for pig erythrocytes *in vitro*. Pig erythrocytes lack glucose transporters, but contain glycolytic enzymes [82,83]. 2-Deoxy-D-ribose and D-ribose are the only sugars which maintain ATP levels in pig erythrocytes [84]. We note, however, that this does not necessarily mean that 2-deoxy-D-ribose is the main energy source for pig erythrocytes *in vivo*. The cells take up 2-deoxy-D-ribose more rapidly than D-ribose. This suggests that pentose sugars may enter the cell without a transporter, as 2-deoxy-D-ribose is the less polar molecule. If this is true, ouabain should not block endothelial-cell migration towards 2-deoxy-D-ribose. A pentose-sugar transporter specific for D-ribose and 2-deoxy-D-ribose has been identified in promastigotes of the parasitic protozoan *Leishmania donovani*, but not in other organisms [85]. High D-ribose concentrations inhibit glycolysis [84]. This is intriguing, as endothelial-cell migration decreases when the 2-deoxy-D-ribose concentration increases above 100 μ M (R. Choudhuri and R. Bicknell, unpublished work).

2-Deoxy-D-ribose is not present in normal serum [86]. Thus, the release of 2-deoxy-D-ribose from the tumour will create a gradient along which the endothelial cell may migrate.

Sugars also attract endothelial cells through traditional cell-surface receptors

Migration by macrophages towards the sugars fucose, mannose and galactose is far greater if they are coupled to BSA. This chemotactic response is presumably cell-surface-receptor-mediated [87]. 2-Deoxy-D-ribose can be rephosphorylated to 2-deoxy-D-ribose 1-phosphate outside the cell. 2-Deoxy-D-ribose 1-phosphate is unable to cross the plasma membrane, but has been shown to stimulate endothelial-cell migration at a concentration of just 50 nM. It is therefore possible that 2-deoxy-D-ribose 1-phosphate acts on endothelial cells through a cell-surface receptor [34].

TP in oncology: a double-edged sword

The finding that TP's promoter contains oestrogen response elements is of interest in the light of the observation that the anti-oestrogen tamoxifen inhibits breast-tumour growth by inhibiting angiogenesis [88]. As we have already seen, TP and 2-deoxy-D-ribose may be one of the more significant angiogenic systems in breast cancer [44]. It is possible that tamoxifen may exert its anticancer effect by down-regulating TP in breast carcinoma.

Surprisingly, node-positive breast-cancer patients with elevated tumour TP levels show greater relapse-free survival [89]. This is because chemotherapeutic drugs, which are nucleic acid homologues [5'-fluorouracil (5 Ura) for example], require activation by TP to give the derivatives that inhibit thymidylate synthase, leading to cell death. TP-overexpressing tumour cells show a greater susceptibility to these chemotherapeutic agents than do normal cells, which contain lower levels of the enzyme [90,91]. Inhibition of TP in a colon-tumour cell line decreases 5 Ura toxicity by 79 % [92]. In addition, the gastrointestinal toxicity of pyrimidine antimetabolite chemotherapy can be reduced by oral administration of acyclothyridine, a competitive inhibitor of TP [93]. Patterson et al. [94] transfected TP cDNA into MCF-7 cells. This increased the sensitivity of these cells to 5 Ura, proving that it is TP which directly increases analogue drug activation. The increased activation of chemotherapeutic agents may even lead to the killing of tumour cells expressing normal levels of TP, simply because they sit next to a cell expressing high levels of TP. This 'bystander effect' may increase the effectiveness of pyrimidine antimetabolite drugs against tumours where only a subset of cells overexpress TP [95].

Our understanding of factors promoting TP overexpression may be used to devise double therapies that employ both a pyrimidine antimetabolite and an agent which stimulates TP overexpression in the tumour. Interferon- α , one of the cytokines which elevate TP levels, has been shown to potentiate the antitumour activity of 5 Ura against colon cancer *in vitro*, *in vivo* and in clinical trials [66]. It may be prudent to routinely determine tumour TP levels in patients. Those patients with tumours that express high levels of TP would then be best suited to treatment with pyrimidine analogues.

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