Angiogenesis and anti-angiogenesis: Perspectives for the treatment of solid tumors

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Summary

Angiogenesis is the formation of new blood vessels from pre-existing ones. Many solid tumors depend on an extensive newly formed vascular network to become nourished and to expand. Tumor cells induce the formation of an extensive but aberrant vascular network by the secretion of angiogenic factors. A proper context is needed for the endothelial cells to respond. To create this proper context, the tumor often uses the body's own repair system to accelerate angiogenesis and the subsequent tumor expansion. The angiogenic response is governed by the interaction of angiogenic growth factors and cytokines with specific receptors on the endothelium, as well as by the interaction of these cells with their surrounding matrix, which is regulated by matrix-degrading proteases and adhesion molecules such as integrins. A number of agents have been discovered and developed that aim to inhibit angiogenesis and to convert the tumor to a dormant state. They have proven to be effective in animal studies. At present their efficacy in man is under evaluation.

Key words: angiogenesis, angiogenesis inhibition, angiogenic growth factors, endothelium, matrix-degrading proteases, pancreas

Introduction

Angiogenesis is the outgrowth of new blood vessels from existing ones. It occurs during development, but normally stops at maturity. In the healthy adult it is only found in the endometrium and ovaries during the menstrual cycle, and in conditions associated with tissue repair and inflammation. Angiogenesis is increased in a number of diseases including cancer, rheumatoid arthritis and diabetic retinopathy. This increase is accompanied by changes in the behavior of endothelial cells, which are reflected in a large increase in their proliferation rate, increased migration and invasion into the extracellular matrix, and the formation of new tubular structures. The increased vascular bed nourishes the malignant tissue and accelerates the growth of many tumors. In the last two decades not only have the mechanisms and factors that underlie the angiogenic process become known, but insight has also grown into the possibilities that inhibition of the angiogenic process may contribute to the treatment of solid tumors [1-3].

Angiogenesis: A perspective for tumor treatment

In 1971 Folkman [4] proposed that angiogenesis was required for tumor growth. He and other investigators also anticipated that the interaction between the tumor cells and the existing endothelium causes an alteration in the behavior of endothelial cells, by which they become induced to participate in angiogenesis. On the basis of experiments on the induction of tumors in the pancreatic islets of transgenic mice Hanahan and Folkman [5,6] observed that the transition from hyperplastic islets to neoplastic islets was preceded by angiogenic activity. On the basis of this and clinical observations on tumor vasculature the hypothesis was put forward that an "angiogenic switch" was required before the tumor could expand its volume to greater than several cubic mm [7]. In addition to this, the formation of a new vascular bed around the tumor also facilitates the escape of tumor cells, a small number of which may develop into metastases. These observations underscore the initial perspective postulated by Folkman that if tumor angiogenesis could be inhibited, tumor growth may be arrested, and that if the new angiogenic vascular bed could be induced to collapse, the tumor might suffocate and shrink. The important role of angiogenesis in tumor development and progression was confirmed by many clinical studies that showed a positive association between tumor angiogenesis and tumor aggressiveness in carcinoma of the breast, lung, and prostate, malignant melanoma and other solid tumors, though in a minority of studies this could not be demonstrated [8-10]. Furthermore, in animal studies induction of angiogenesis by angiogenic growth factors caused a tumor to expand more rapidly, while inhibition of angiogenesis by different types of treatment caused reduction of tumor growth [11,12]. These observations have further strengthened the idea that anti-angiogenic compounds may supply an additional treatment to retard or reduce the growth of primary solid tumors and their metastases.

Tumor angiogenesis: Developmental and repair-associated angiogenesis

A number of investigators have isolated growth factor proteins from tumors that have angiogenic properties. These factors include basic fibroblast growth factor (FGF-2), vascular permeability factor / vascular endothelial growth factor (VPF/VEGF), hepatocyte growth factor (HGF) and transforming growth factor-β (TGF-β) [1,2]. Other factors have been added to this list (Table 1). These angiogenic factors induce cell proliferation and/or cell migration and invasion. The importance of several of these factors was
Mechanisms contributing to angiogenesis

The process of angiogenesis is generally thought to involve (a) activation, and subsequent liberation of endothelial cells by degradation of their basement membrane, (b) migration of endothelial cells into the extracellular matrix, accompanied by (c) proliferation of endothelial cells, and finally (d) lumen formation and organization of the migrated cells into a new capillary structure with a new basal membrane. While proliferation and survival of endothelial cells is needed to keep up with the increased mass of the new vascular structures, the other processes mainly depend on the interaction of the cells with their surrounding matrix and the modification of this interaction by proteases and adhesion molecules.

Much has been learned from in vitro models in which microvascular endothelial cells are induced to form capillary networks in a three-dimensional matrix of fibrin or collagen [26]. Form these studies it has become clear that in particular the proteases of the urokinase/plasmin and the matrix metalloproteinases systems can play a crucial role in angiogenesis [27,28]. These proteases and the formation of capillary structures can be induced in bovine endothelial cells by angiogenic factors themselves [27]. However, formation of capillary structures by human microvascular endothelial cells requires the simultaneous exposure to an angiogenic factor (bFGF, VEGF or HGF) and the cytokine TNFα [29]. When this process was studied in a fibrin matrix, it was completely inhibited by antibodies against u-PA or u-PA receptor, or by the inhibition of plasmin, indicating that cell-bound u-PA and plasmin activation play a crucial role.

In line with this are the in vivo observations that tumor angiogenesis can be inhibited by the amino terminal fragment of u-PA, which displaces u-PA from its cellular receptor, but has no plasmin-activating ability [19,20]. In a matrix consisting of collagens the matrix-degrading metalloproteinases (MMPs) play a similar role [28,30]. It should be noted, however, that proteolytic activity causing detachment of cell-matrix interactions can only contribute to cell migration and invasion if it is accompanied by the formation of new binding sites, e.g. by newly induced αvβ3- and αvβ5-integrins. Inhibition of proteases and interference with integrin binding have been used as a lead to develop compounds that inhibit angiogenesis [21,22]. If no new interaction sites are made, the endothelial cell will detach and go into apoptosis. Angiogenic factors not only stimulate endothelial proliferation, but also act on the expression of proteases and cell adhesion molecules, directly or in conjunction with other cytokines.

Inhibition of angiogenesis

The quiescent nature of the adult vasculature and the fact that certain tissues, such as cartilage are not vascularized suggest that natural inhibitors of angiogenesis may exist. Furthermore, it is well known that after the formation of a granulation tissue, which is highly vascularized and occurs in inflammation and wound healing, the vessels regress again. The so-called 'pruning' of vessels is also observed in development and is a basic mechanism of the body to balance the tissue need of blood vessels. If one could
understand this response of the body, one may also gain the ability to induce tumor blood vessels to regress leaving the tumor mass no other choice than to die or shrink. As summarized in Table 2 a number of natural inhibitors of angiogenesis have become recognized during the last years. Interestingly, many of them are proteolytic fragments of larger molecules. Apparently a defense system has developed in evolution that recognizes uncontrolled matrix proteolysis from the matrix proteolysis that is needed for angiogenesis.

It was again the clinical observation that primary tumors suppress metastases that brought Folkman and O'Reilly to the hypothesis and first evidence that this suppression may partly be due to inhibition of angiogenesis in the metastases by the primary tumor. From the urine and tumor cells of tumor-bearing mice they isolated angiostatin and subsequently endostatin, two potentially potent angiogenesis inhibitors, which are at present being further investigated [31,32]. In particular the initial results with repeated endostatin treatment of mouse tumors were impressive, because this repeated treatment did not only shrink the tumor every time that it was restarted, but the tumor also failed to recover after 3 to 5 periods of treatment and went into a completely dormant state [33]. These observations stress the potency of anti-angiogenic treatment, but many further studies will be needed before these experiments can be translated into a similar clinical perspective.

A more direct clinical perspective is at present being evaluated for a number of pharmacological inhibitors of angiogenesis [34]. These inhibitors mainly aim to reduce the efficacy of angiogenic growth factors by blocking their receptors or signal transduction, or at interfering with matrix-degrading metalloproteinases and the efficacy of specific integrins. Inhibitors of matrix-degrading proteases, such as marimastat, AG3340, BAY-9566, CGS-27023A and carboxyamidotriazole act both on tumor cell invasion and angiogenesis [35]. Other inhibitors, such as TNP-470/AGM-1470, thalidomide [37] and 2-methoxyestradiol [38] act on proliferating endothelial cells, as do antagonists of angiogenic growth factors binding and signal transduction. Their present status ranges from preclinical to Phase II/III. An alternative approach, also aiming to interfere with the newly formed tumor vessel bed is the induction of a vascular collapse in the tumor. The tumor vascular bed usually appears as a chaotic network of blood vessels, with many abnormal structures, such as sinusoids, tortuositities and dead-ended vessel parts [39]. Perfusion of the vascular bed is very heterogeneous, which hampers drug delivery. Furthermore, the vessels are often leaky [40]. However, because of the high interstitial pressure in the tumor, hydraulic conductivity is absent and drugs only penetrate into the tumor by diffusion [41]. This markedly hampers the delivery of large cytotoxic molecules to the tumor. The unusual aspect of the tumor vasculature has suggested to many investigators that the endothelium of these blood vessels must assume tumor-characteristic features, to which drugs can be delivered. Increased expression of the protein endoglycan and local expression of tissue factor on the endothelium have been reported [42,43]. Their presence has been used in experimental animals to precipitate coagulation in the tumor vasculature in order to deprive the distal tumor bed of blood supply [44]. New compounds are presently in development.

<table>
<thead>
<tr>
<th>Table 2: Inhibitors of angiogenesis</th>
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<td><strong>Naturally occurring anti-angiogenic factors:</strong></td>
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<tr>
<td>Platelet factor-4</td>
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<tr>
<td>Thrombospordin-1</td>
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<tr>
<td>Interferons</td>
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<td>IL-1, IL-12</td>
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<td>Vascular endothelial growth inhibitor (VEGF)</td>
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<td>2-methoxyestradiol</td>
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<td>Tissue inhibitors of MMPs (TIMPs)</td>
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<td>Proliferin related protein</td>
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<td><strong>Pharmacological inhibitors of angiogenesis:</strong></td>
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<tr>
<td>Thalidomide</td>
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<td>TNP-470/AGM-1470</td>
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<td>Carboxyamidotriazole</td>
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<td><strong>Inhibitors of tumor invasion and probably angiogenesis:</strong></td>
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<tr>
<td>AG3340, Marimastat, BAY 9566, CGS-27023A</td>
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<td>and other synthetic MMP inhibitors</td>
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<td>Amino terminal fragment of u-PA (ATF)</td>
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From the perspective of present clinical practise the local perfusion with TNFα and melphalan in limbs is probably the procedure which comes closest to the idea of collapsing the tumor vascular bed as a tumor therapy.

**Consequences for pancreatic tumors**

A limited number of studies has focused on the expression of angiogenic factors in normal and malignant human pancreas. They have shown the presence of VEGF-A, the VEGF-A receptors, VEGF-R1 (flt-1) and VEGF-R2 (kdr), FGF-2 (bFGF) and FGF-R1, thymidine phosphorylase (PDECGF) and angiogenin [45-49]. The expression of avB3-integrin was also demonstrated [50]. Thymidine phosphorylase and angiogenin were shown to be associated with the aggressiveness of pancreatic tumors [47,48]. However, the factors recognized at present are probably not a complete list of angiogenic factors in these tumors, as there are many others which have not yet been investigated. No intervention studies have be done in patients to establish that the factors presently encountered had a causative contribution rather than being the consequence of the malignant disease. Only one study on vessel count in human pancreatic tumors is available. This study was unable to find a significant difference between vessel count and severity of the disease, but it only evaluated tumor specimens of 22 patients [51]. On the basis of animal experiments it is likely that pancreatic tumors depend similarly on an extended vascular bed as do many other solid tumors, and that inhibition of angiogenesis reduces tumor expansion [52]. However, future studies will have to demonstrate whether this is also the case in man.

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References


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