Therapeutic potentials of angiostatin in the treatment of cancer

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ABSTRACT

The discovery of specific endothelial inhibitors such as angiostatin and endostatin not only increases our understanding of the functions of these molecules in the regulation of physiological and pathological angiogenesis, but also provides an important therapeutic strategy for cancer treatment. Recent studies have demonstrated that the angiostatin protein significantly suppresses the growth of a variety of tumors in mice. However, the dosages of angiostatin protein used in these animal studies seem to be too high for clinical trials. In addition, repeated injections and long-term treatment with angiostatin are required to reach its maximal antitumor effect. In this article, I will discuss several alternative approaches that may become feasible to move angiostatin therapy from animal experiments into the clinic. In particular, I will emphasize the therapeutic potentials of angiostatin gene therapy and more potent angiogenesis inhibitors that are related to angiostatin.

The vasculature remains quiescent in the adult mammal, except for transient processes of neo-vascularization in the female reproductive system. In response to an appropriate growth stimulus, endothelial cells can degrade the basement membrane locally. Simultaneously, the quiescent endothelial cells change their morphology, proliferate, migrate, invade into the surrounding stroma tissue, form microtubes, sprout new capillaries, and reconstitute the basement membrane. This complex process of angiogenesis implies the presence of multiple controls of the system, which can be switched on and off within a short period.

Angiogenesis is involved in the development and progression of pathogenic processes in a variety of disorders, including diabetic retinopathy, psoriasis, rheumatoid arthritis, cardiovascular diseases and cancer. For the last three decades, a large body of work by a number of laboratories has provided both direct and indirect evidence that tumor growth and metastasis are accompanied by the growth of new blood vessels. At the pre-vascular stage, a solid tumor rarely grows larger than 2-3 mm³ and may contain a few million cells. Cells in pre-vascular tumors may proliferate as rapidly as those in vascularized expanding tumors. However, the growth rate of cells in avascular tumors reaches an equilibrium with their death rate. Once a tumor is vascularized, it grows exponentially and often reaches an uncontrollable volume. The infiltration of new blood vessels in tumors not only supplies nutrients and oxygen for tumor cells, but also removes the waste products produced by tumor cells. In addition, endothelial cells can communicate directly with tumor cells by producing tumor growth promoting factors. The inter-relationship between endothelial cells and tumor cells is shown in Figure 1.

It appears that a switch to the angiogenic phenotype requires a local change in the balance between angiogenic factors and angiogenic inhibitors. Among angiogenic factors, families of fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF) are most commonly expressed in tumors. While FGFs display their biological effects on a variety of cell types, VEGF/VPF appear to be the most selective growth factors acting on endothelial cells. These two families of angiogenic factors can promote angiogenesis in a synergistic manner. Levels of expression of VEGF mRNA and protein are markedly up-regulated in the majority of human tumors including tumors of bladder, breast, lung, gastrointestinal tract, ovary and prostate, and glioblastoma, hemangioma, and retinoblastoma. High concentrations of bFGF were found in both serum and urine of patients with various cancers. Although up-regulation of angiogenic factors is necessary for a tumor to switch on its angiogenic phenotype, production of angiogenesis inhibitors has to be simultaneously down-regulated. Increasing evidence demonstrates that down-regulation of angiogenesis inhibitors is equally important as up-regulation of angiogenic factors in the switch to the angiogenic phenotype of a tumor. For example, an angiogenesis inhibitor, thrombospondin-1, has been found to be down-regulated in a number of tumors.
While biological functions and molecular mechanisms of angiogenic factors including FGF and VEGF families have been extensively characterized, little is known about the molecular aspects of angiogenesis inhibitors. During the last few years, several endogenous angiogenesis inhibitors have been discovered as shown in Table 1. Among these inhibitors, angiostatin and endostatin have been reported to be the most potent and specific inhibitors of angiogenesis.9,10 Consequently, these two inhibitors and most other angiogenesis inhibitors also suppress tumor growth in animals. However, high dosages of angiostatin and of most other inhibitors are required for cancer treatment and these doses seem to be too high for clinical administration in cancer patients. In addition, repeated injections of angiostatin and long-term treatment have to be administered in order to reach high efficacy. Thus, alternative approaches need to be developed to overcome these problems.

Angiostatin

Discovery

Inhibition of metastatic tumor growth by primary tumor mass has been observed in some clinical malignancies. The removal of certain primary tumors in patients, such as breast and colon carcinomas, can be followed by a rapid growth of distant metastases. In a similar animal metastatic model, angiostatin was isolated from both serum and urine of mice bearing a transplantable murine Lewis lung carcinoma (3LL) in syngeneic C57Bl6/J mice, thus providing the most compelling explanation for this phenomenon.9 The endothelial inhibitory activity was purified by an in vitro assay of inhibition of basic fibroblast growth factor (bFGF)-stimulated bovine capillary endothelial cell proliferation. Angiostatin is a circulating angiogenesis inhibitor produced in association with primary Lewis lung tumor growth. This 38 kD inhibitor accumulates in the circulation in the presence of a growing primary tumor and disappears from the circulation after removal of the primary tumor. Thus, resection of the primary Lewis lung carcinoma results in depletion of circulating angiostatin and promotes neovascularization and growth of lung micrometastases.

Structure and generation

Microsequence analysis of angiostatin purified from both urine and serum samples of tumor (3LL)-bearing animals revealed greater than 98% identity with an internal fragment of mouse plasminogen with an N-terminus at amino acid valine98.9 Based on its molecular weight, mouse angiostatin is predicted to contain a C-terminus at approximately amino acid 440 of plasminogen. Thus, angiostatin should contain the first four of five triple loop disulfide-linked structures, known as the kringle structures of plasminogen. Proteolytic fragments, generated by in vitro limited elastase proteolysis of human plasminogen, which are compatible to the murine angiostatin, contain inhibitory activity on endothelial cell proliferation.9 Purified human angiostatin in the initial studies contained three molecular weight species of apparent molecular masses of 40, 42, and 45 kD, and each of

Table 1. Endogenous angiogenesis inhibitors.

<table>
<thead>
<tr>
<th>Inhibitors</th>
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<tr>
<td>Angiostatin</td>
<td>O’Reilly et al., 1994</td>
</tr>
<tr>
<td>Chemokine gro-β</td>
<td>Cao et al., 1995</td>
</tr>
<tr>
<td>Endostatin</td>
<td>O’Reilly et al., 1997</td>
</tr>
<tr>
<td>Interferon-α</td>
<td>Ribatti et al., 1996</td>
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<tr>
<td>Interleukin-12 (IL-12)*</td>
<td>Veest et al., 1995</td>
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<tr>
<td>Chemokine IP-10</td>
<td>Angelillo et al., Streider et al., 1995</td>
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<tr>
<td>Platelet factor-4 (PF-4)</td>
<td>Maione et al., 1990</td>
</tr>
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<td>Prolactin-RP</td>
<td>Jackson et al., 1994</td>
</tr>
<tr>
<td>Soluble FGF-R</td>
<td>Hennenken et al., 1994</td>
</tr>
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<td>Thrombomospondin-1 (TSP-1)</td>
<td>Good et al., 1990</td>
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<td>TIMPs (TIMP-1,2,3)</td>
<td>Moses et al., 1990</td>
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<td>16-kD prolactin</td>
<td>Clap et al., 1993</td>
</tr>
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<td>Kringel 5 of plasminogen</td>
<td>Cao et al., 1997</td>
</tr>
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<td>Kringel 1-5 of plasminogen</td>
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<tr>
<td>Troponin-1</td>
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*IL-12 does not inhibit endothelial cell growth in vitro.
the three bands could inhibit endothelial cell proliferation similarly. Microsequence analysis of each of the three bands revealed an identical N-terminus at amino acid 97 or 99, suggesting that the C-terminal length of these fragments is variable. Although the intact plasminogen molecule contains the same kringle structures, it is inactive in inhibition of endothelial cell growth, in vivo neoangiogenesis and metastatic tumor growth. These data imply that angiostatin may contain a different dimensional structure than the kringle structure of plasminogen. It is also speculated that the endothelial inhibitory activity is hidden in the intact plasminogen molecule.

Although angiostatin is detected in vivo in association with 3LL Lewis lung tumor growth, it is unlikely that tumor cells produce angiostatin directly because they lack a detectable amount of mRNA for angiostatin and plasminogen (Cao et al., unpublished observation). Angiostatin can be generated in vitro by limited elastase proteolysis of plasminogen. A recent study has uncovered the mechanism responsible for the in vitro production of angiostatin in syngeneic C57Bl6/J mice bearing Lewis lung carcinomas (3LL). The mediator of angiostatin production in 3LL-Lewis lung carcinoma has been found to be a tumor-infiltrating macrophage. Immunohistochemical staining of subcutaneous tumor sections discovered the infiltration of macrophages and high levels of expression of metalloelastase (MME) mRNA was detected in tumor tissues. Successive passages of cultures established from tumors demonstrated that the generation of angiostatin was directly correlated with the macrophage-derived metalloelastinolytic activity. The incubation of plasminogen with 3LL cells cultured in vitro did not produce angiostatin, whereas cocultures of macrophages with 3LL cells converted plasminogen to angiostatin. Further studies indicate that the metalloelastinolytic activity in macrophages can be up-regulated by the cytokine, GM-CSF, secreted by 3LL tumor cells. Thus, 3LL tumor cells communicate with macrophages through the secretion of GM-CSF which increases the production of elastase activity and thereby the generation of angiostatin.

It appears that MME released from tumor-infiltrating macrophages is not the only source responsible for angiostatin production in tumors. Three human prostate carcinoma cell lines (PC-3, DU-145 and LN-CaP) have been found to produce proteolytic activity that generates angiostatin from plasminogen or plasmin. The enzymatic activity released by these cell lines is independent from that of tumor infiltrating macrophages. Studies with protease inhibitors demonstrate that a serine protease is essential for angiostatin generation. Thus, at least two known sources of proteases exist in association with tumors that can convert plasminogen to angiostatin. MME released from tumor-infiltrating macrophages and serine proteases produced directly from tumor cells.

### Antiangiogenic and antitumor effects

Purified angiostatin specifically and reversibly inhibits proliferation of endothelial lineages, including bovine capillary endothelial (BCE), bovine aorta endothelial (BAE), human umbilical vein endothelial (HUVE) and malignant mouse hemangioendothelioma (EOMA) cells in a dose-dependent manner. In contrast, concentrations of angiostatin for maximal inhibition of endothelial cell proliferation are not inhibitory on a variety of normal and neoplastic non-endothelial cell lines, including 3T3 fibroblasts, bovine aorta smooth muscle cells, bovine retinal pigment epithelial cells, human fetal fibroblasts, and 3LL murine Lewis lung carcinoma cells. Thus, angiostatin specifically inhibits endothelial cell proliferation.

Studies of smaller fragments of human angiostatin on inhibition of endothelial proliferation demonstrate that a functional difference is present among individual kringle structures. For example, appropriately folded recombinant kringle 1 exhibits potent inhibitory activity on BCE cells. Recombinant kringle 2 and kringle 3 also display significant endothelial inhibition. In contrast, kringle 4 exerts ineffective inhibition on endothelial cell proliferation. These data indicate that the endothelial inhibitory activity of angiostatin is shared by kringle 1, kringle 2 and kringle 3 but probably not by kringle 4. Among the tandem kringle arrays, the recombinant kringle 2-3 fragment exerts inhibitory activity similar to kringle 2 alone. However, relative to kringle 2-3, marked enhancement of inhibition is observed when individual kringle 2 and kringle 3 are added together to endothelial cells. This implies that it is necessary to open the inter-kringle disulfide bridge between kringle 2 and kringle 3 in order to obtain the maximal inhibitory effect of kringle 2-3. In view of variable lysine binding affinity of homologous domains, it appears that lysine binding capability does not correlate with relative inhibitory effects of the kringle containing fragments. Folding studies indicate that the anti-proliferative activity of angiostatin is largely abolished after reduction/alkylation. Thus, appropriate folding of kringle structures as tandem domains held together by intra-chain and inter-chain disulfide bonds is essential for angiostatin to maintain its full anti-endothelial activity.

In the chick embryo chorioallantoic membrane (CAM), purified human angiostatin induces avascular zones over a concentration range of 0.1-100 µg/embryo. The dose-dependent inhibition reaches saturation at approximately 100 µg/embryo. Systemic administration of human angiostatin in mice implanted with basic fibroblast growth factor (bFGF or FGF-2) in corneal micropockets (80-100 ng bFGF/cornea) significantly inhibited corneal neovascularization induced by bFGF. At the concentration of 50 mg/kg/day for 4 hours, angiostatin inhibits new vessel growth by 85% compared to growth in controls.
Previous studies showed that systemic administration of human angiostatin potently inhibits the growth of transplanted human and murine primary tumors in mice. The growth of three aggressive primary murine tumors (Lewis lung carcinoma, T241 fibrosarcoma and reticulum cell sarcoma) is inhibited by an average of 84% at doses of 100 mg/kg/day. These tumors exhibit poor response to other therapies. Inhibition of primary tumor growth becomes apparent at 10 mg/kg/day and increasing doses of angiostatin correlate with increased anti-tumor efficacy. Systemic treatment of human tumors growing in immunodeficient mice produces an almost complete suppression of primary tumor growth. Angiostatin inhibits the growth of human breast carcinoma by 95%, colon carcinoma by 97% and prostate carcinoma by almost 100%. In the colon and breast carcinoma bearing mice, tumors re-grow within two weeks after withdrawal of angiostatin treatment. Angiostatin treatment does not result in weight loss or other toxicity in mice even in those that receive 100 mg/kg/day or in immunocompromised mice receiving treatment for as long as 60 days. The lack of toxicity suggests that the effective anti-tumor therapy by angiostatin functions directly against the endothelial compartment of a tumor, which demonstrates the importance of the endothelial cell compartment in controlling tumor growth.

Histologic studies reveal that indices of tumor cell proliferation and apoptosis reach a net balance in angiostatin-treated human dormant tumors. Angiostatin treatment does not result in weight loss or other toxicity in mice even in those that receive 100 mg/kg/day or in immunocompromised mice receiving treatment for as long as 60 days. The lack of toxicity suggests that the effective anti-tumor therapy by angiostatin functions directly against the endothelial compartment of a tumor, which demonstrates the importance of the endothelial cell compartment in controlling tumor growth.

Similarly, primary tumor-produced and systemically administered angiostatin can induce dormancy of lung metastases of Lewis lung carcinoma bearing mice. Metastases are dependent on angiogenesis in at least two steps of the metastatic events. First, metastatic tumor cells must exit from a primary tumor which has been vascularized. Second, upon arrival at their target organ, metastatic tumor cells must undergo neovascularization in order to grow to a clinically detectable size. In the presence of angiogenesis inhibition, metastatic tumor cells form microscopic perivascular cuffs around the pre-existing microvessel from which they probably left the circulation. The colonies of dormant lung micrometastases rarely expand beyond 0.3 mm in diameter, they proliferate as rapidly as fast growing metastases, they undergo a high apoptotic rate and they lack neovascularization. The mechanism by which angiostatin therapy leads to an increased death rate of tumor cells is not known. It is speculated that complete inhibition of tumor angiogenesis may result in a loss of survival factors essential for tumor cells. These tumor cell survival factors can be either from the circulation as endocrine factors, and/or from the endothelial cell as paracrine factors. Alternatively, a decreased tumor cell number in a dormant tumor may also limit the production of survival factors for tumor cells such as autocrine factors.

Angiostatin gene therapy

Although angiostatin is a potent inhibitor of angiogenesis and tumor growth, the need of high dosages, repeated injections and long-term administration of this protein into the body have made it less attractive for clinical trials. In order to develop alternative strategies for therapy, we have investigated the possibility of angiostatin gene therapy. We transfected mouse angiostatin cDNA into murine T241 fibrosarcoma cells and stable cell lines expressing the secreted form of angiostatin were established. Despite high levels of expression in various clones, angiostatin had no direct influence on tumor-cell growth in vitro. implantation of stable clones expressing angiostatin in C57B1/6J mice produced inhibition of primary tumor growth by an average of 77%. Reduction of tumor growth correlates with reduced vascularization, suggesting that the antitumor effect of angiostatin is mediated by inhibition of angiogenesis. After removal of primary tumors, the pulmonary micrometastases in about 70% of mice remain in a microscopic dormant and avascular state for months. The tumor cells in the dormant micrometastases exhibit a high rate of apoptosis balanced by a high rate of proliferation.

Knowing that expression of angiostatin cDNA in tumor cells potently inhibited the growth of primary tumors and metastases, we cloned the same angiostatin cDNA into a retroviral vector that was used to transduce RT2 rat glioma cells. The conditioned medium of glioma cells transduced with angiostatin produced a significant inhibition of endothelial cell growth and the inhibition was depleted by a specific antibody to the recombinant angiostatin. Transduction of RT2 cells by the retrovirus containing angiostatin did not affect tumor cell growth in vitro. The angiostatin-transduced cells were microsurgically implanted into the subrenal capsule of nude mice. Two weeks after implantation, small, pale tumor nodules were observed in the angiostatin-transduced tumor implants, whereas large, red and hypervascularized tumors were present in the vector-transfected control tumor cells. Immunohistochemical analysis revealed a significant reduction of blood vessels in the angiostatin-transduced tumors as compared with that seen in the control tumors. To ensure that the anti-angiogenic and antitumor effect was not limited to the subrenal model, we evaluated tumor growth in
Angiostatin in cancer treatment

subdermal and intracranial models. Consistent with the subrenal model, the growth of angiostatin-transduced glioma was inhibited by 70% relative to the control vector-transduced tumors. These data demonstrate that the retrovirus system is effective in delivering angiostatin gene for cancer therapy.

The fact that retroviral transduction in vivo is a relatively ineffective process led us to explore alternative gene-delivery systems. Replication-deficient adenoviral vectors are efficient in vivo delivery vectors capable of transducing dividing cells as well as non-dividing cells. Thus direct injections of an angiostatin-expressing adenoviral vector were attempted. We, therefore, cloned angiostatin cDNA into an adenoviral vector carrying the early CMV promoter upstream to the angiostatin-minicassette. When adenoviruses carrying angiostatin were transduced into RT2 cells, high levels of angiostatin protein were detected in the conditioned medium which was inhibitory only for endothelial cells but not for tumor cells.33 To determine the antitumor effect of the adenovirus-angiostatin, U87MG human glioma cells were implanted into the subrenal capsule of the nude mice, followed by injection of angiostatin-adenoviruses (5 × 10^9 PFU). Three weeks later, animals were sacrificed and tumors were harvested. More than 87% inhibition of tumor growth was observed in the angiostatin-adenovirus-treated tumors as compared with that in the vector-adenovirus-treated tumors.33 The vascular density was significantly reduced in the angiostatin-adenovirus-treated tumors compared with that in the control tumors. These results support a potential role of vector-mediated transduction of angiostatin DNA as a potential therapeutic strategy for the treatment of brain tumors and confirm the anti-tumor activity of angiostatin.

To explore angiostatin gene therapy in cancer treatment further, we chose a non-viral vector system of the cationic liposome-DNA to deliver angiostatin cDNA in vivo. Angiostatin cDNA was cloned into a plasmid vector containing the CMV promoter.34 Intravenous injection of cationic liposome-angiostatin cDNA complex produced a significant anti-metastatic effect on murine B16 melanoma, as determined by both the total number of lung metastases and tumors greater than 2 mm in diameter, when compared to either reporter gene-treated and untreated controls.34 The anti-tumor effect was correlated with reduction of blood vessels in tumors treated with angiostatin. These results open a possibility of angiostatin gene therapy by non-viral-mediated vectors in the treatment of cancer.

Kringle 5

Amino acid sequence alignment of individual kringle domains of human plasminogen shows that kringle 5 (K5) displays remarkable sequence identity with kringle 1 (57.5%), kringle 2 (46.25%), kringle 3 (48.75%) and kringle 4 (52.5%). Based on the primary structure similarities with other kringle domains, especially kringle 1 (highest sequence identity), K5 of plasminogen has recently been demonstrated to be a potent endothelial specific inhibitor.22 K5 obtained as a proteolytic fragment of human plasminogen displays potent inhibitory effect on bovine capillary endothelial cells with a half-maximal concentration of approximately 50 nM. Thus, K5 would appear more potent than angiostatin on inhibition of bFGF-stimulated capillary endothelial cell growth. Appropriately folded recombinant mouse K5 protein, expressed in E. coli, exhibits an inhibitory activity comparable to the proteolytic K5 fragment.22 The identification of K5 as a potent endothelial cell specific inhibitor increases our understanding of the role of plasminogen kringle 5 in inhibiting endothelial cell proliferation. Our further studies using in vivo antiangiogenic and antitumor models suggest that K5 is less potent in suppression of tumor growth, due to the short half-life of this molecule in vivo (our unpublished data).

Kringle 1-5

In the search for more potent angiostatin-related inhibitors than angiostatin, we have developed a proteolytic strategy that allows us to obtain kringle 1-5 (K1-5) fragment by digestion of plasminogen with urokinase-activated plasmin. This 55 kD proteolytic fragment was purified to homogeneity and contains the intact kringle 1-4 and most of the kringle 5 domains of plasminogen.24 K1-5 inhibited capillary endothelial cell growth in a dose-dependent manner, with a half-maximal concentration of approximately 50 pM. The inhibitory effect was endothelial cell specific and appears to be at least 50-fold more potent than that of angiostatin (Figure 2). The potent anti-endothelial effect led us to test whether K5 and angiostatin would inhibit endothelial cell growth synergistically. When proteolytic K5 and angiostatin were co-incubated with endothelial cells, a synergistic effect of endothelial cell inhibition was observed. The synergistic effect was comparable to that produced by K1-5 alone. These results suggest that angiostatin and K5 inhibit endothelial cell growth via separate pathways.

To investigate the antiangiogenic effect of K1-5 in vivo and to compare the antiangiogenic efficacy of K1-5 directly with that of angiostatin, the inhibitory effect of systemic administration of K1-5 and angiostatin on FGF-2-induced corneal neovascularization was studied. This rigorous angiogenesis assay requires systemic administration of angiostatin and K1-5 to counteract the angiogenic effect of FGF-2 which is implanted in the corneas of mice. Systemic treatment of mice with K1-5 by one subcutaneous injection daily at the concentration of 2 mg/kg significantly blocked the FGF-induced corneal neovascularization.24 In contrast, angiostatin at the same dose did not inhibit the FGF-induced corneal neo-
Figure 2. Anti-endothelial growth activity of kringle structures of angiostatin and its related fragment. Angiostatin consists of the first four kringle structures (K1-4) of plasminogen. Smaller fragments derived from angiostatin including kringle 1 (K1), kringle 2 (K2) and kringle 3 (K3) inhibit capillary endothelial cell growth in vitro. Kringle 4 (K4) does not exhibit inhibitory activity on endothelial cells. Kringle 5 (K5) which is located outside of the angiostatin region also displays potent anti-endothelial growth activity. In fact, K5 is more potent than angiostatin in suppression of endothelial cell growth. Kringle 1-5 (K1-5) is the most potent anti-endothelial fragment-derived from plasminogen. Numbers represent protein concentrations needed to reach 50% of maximal inhibition.

Table 2. Proteolytic fragments that inhibit angiogenesis.

<table>
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<tr>
<th>Source</th>
<th>M/W (kD)</th>
<th>References</th>
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<tr>
<td>Fibronectin</td>
<td>29</td>
<td>Holmandberg et al, Am J Pathol, 1985</td>
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<td>Prolactin</td>
<td>16</td>
<td>Clapp et al., Endocrinology, 1993</td>
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<td>Angiostatin</td>
<td>38</td>
<td>O'Reilly et al, Cell, 1995</td>
</tr>
<tr>
<td>Platelet factor-4*</td>
<td>7.8</td>
<td>Gupta et al., PNAS, 1995</td>
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<td>Endostatin</td>
<td>20</td>
<td>O'Reilly et al., Cell, 1997</td>
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<td>PEX</td>
<td>25</td>
<td>Brooks et al., Cell, 1998</td>
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<tr>
<td>K1-5</td>
<td>55</td>
<td>Cao et al., PNAS, 1999</td>
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* An N-terminally truncated fragment of PF-4 molecule inhibits endothelial cell growth and angiogenesis.

The role of proteolysis in inhibition of angiogenesis

Our studies suggest that proteolytic processes play essential roles in regulation of the process of angiogenesis. Of the 16 known angiogenesis inhibitors (Table 1), several are proteolytic fragments (Table 2). It appears that the generation of endogenous inhibitors in vivo from large precursor proteins with distinct functions is a recurrent theme in the inhibition of angiogenesis. In addition to angiostatin and endostatin, the 16 kD N-terminal fragment of prolactin has been characterized as an antiangiogenic domain. Similar to angiostatin and endostatin, the intact parental molecule of prolactin lacks the inhibitory activity on endothelial cells, and it is not an angiogenesis inhibitor. Platelet factor-4 is a relatively weak endothelial cell inhibitor. However, a proteolytic fragment with the N-terminally truncated sequence of PF-4 has a 50-fold greater inhibitory activity. A fibronectin fragment derived from plasmin-digestion also inhibits endothelial cell growth.

A fragment of metalloproteinase 2, which contains the C-terminal hemopexin-like domain, termed PEX, blocks angiogenesis and tumor growth.

Thus proteolytic processing plays critical dual roles in control of angiogenesis. When the process of angiogenesis begins, proteolytic degradation of the basement membrane surrounding quiescent endothelial cells is a prerequisite for endothelial cell growth in vivo. Once new blood vessels have been formed, they may require proteolytic fragments to control the over-vascularization. The molecular mechanisms underlying how protease activity is regulated in the control of angiogenesis are not known, nor do we know the substrate specificity of these proteases. However, comparison of amino acid sequences of these antiangiogenic fragments does not reveal a common cleavage site, suggesting that more than one protease participate in this process. Furthermore, one angiostatic fragment can be generated by two or more proteases. Indeed, angiostatin produced in association with tumor growth can be generated by both serine-like proteases and a metalloelastase.

Clinical applications

In conclusion, the discovery of angiogenesis inhibitors such as angiostatin and kringle 1-5 not only facilitates our understanding of the regulation of the angiogenesis process under physiological and pathological conditions, but also allows us to develop novel therapeutic strategies to interfere with blood vessel growth in diseases. The beginning of this approach becomes apparent in development and design of drugs that specifically target newly formed vascularization significantly. Thus, K1-5 displayed a more potent antiangiogenic effect than angiostatin in this in vivo model.

Proteolytic K1-5 was then used for systemic treatment of C57Bl6/J mice bearing subcutaneously implanted murine primary T241 fibrosarcomas. K1-5 at doses of 2-2.5 mg/kg once daily resulted in a significant suppression of primary tumor growth during the 20-day treatment course. At day 20 after treatment, an average of over 65% suppression of primary tumor growth was observed in the T241-treated mice. In contrast, angiostatin at the same dose did not block tumor growth significantly when compared with the growth in the PBS-treated control group. The antitumor effect correlated with reduced neovascularization. Thus, K1-5 displayed more potent antitumor efficacy than angiostatin in these animal studies and is a better candidate for cancer therapy. We should emphasize that K1-5 did not completely arrest tumor growth and K1-5-treated tumors would eventually grow to large volumes which were comparable to those of tumors treated with angiostatin or PBS.
blood vessels. In addition to the above discussed angiostatin and Kringle 1-5, monoclonal antibodies that neutralize the actions of VEGF completely block tumor growth in mice.37

Similarly, a soluble receptor for VEGF also blocks tumor growth in vivo.39 Abrogation of the functions of integrins αvβ3 or αvβ5 specifically expressed in growing endothelial cells dramatically retards tumor growth.40,41 -C-X-C- chemokines including PF-4, gro-β and IP-10 have been shown to impair tumor growth.11,42,43 Some of these endogenous angiogenesis inhibitors have already given rise to promising treatment in cancer patients. For example, interferon α-2a has successfully been used in treating life-threatening hemangioma in children.44,45

Thus, angiogenesis inhibitors seem likely to become one of several important therapeutic strategies in cancer and other vascularized diseases. Pre-clinical and clinical studies have provided several important clues for development of antiangiogenic therapy. Because several of the angiogenesis inhibitors specifically target the proliferating endothelial cell compartment, it is less likely that they will cause immune suppression, bone marrow suppression or gastrointestinal symptoms. Resistance to angiogenesis inhibitors has not been observed in animal studies. Tumors which are resistant to chemotherapy can be suppressed by angiogenesis inhibitors. Because endogenous angiogenesis inhibitors are normal constituents in the body, they are less likely to cause immune reactions. A combination of antiangiogenic therapy and cytotoxic therapy or immune therapy may become more effective because the combined treatment is directed to different compartments. For all these applications, more potent and specific angiogenesis inhibitors need to be identified and more knowledge should be gained in the characterization of the underlying molecular and cellular mechanisms.

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References


