REVIEW

Gene Therapy Strategies for Tumor Antiangiogenesis

Hwai-Loong Kong, Ronald G. Crystal*

Based on the concept that solid tumors cannot grow without the generation of new blood vessels, there is growing interest in the use of antiangiogenesis agents to inhibit tumor growth. This review summarizes the concepts of using gene transfer vectors to provide high concentration of antiangiogenic proteins within an organ. While there are many challenges that must be met before antiangiogenesis can be used to effectively treat human tumors, gene transfer strategies have the potential to provide sustained, high, local concentrations of antiangiogenic mediators specifically targeted to organs containing tumors, minimizing systemic toxicity. Antiangiogenesis gene therapy strategies will most likely be effective in a state of low tumor burden, where this “genetic tourniquet” can provide trans (i.e., acting in the extracellular milieu as opposed to within tumor cells) suppression of the growth of endothelial cells in the milieu of micrometastases. [J Natl Cancer Inst 1998;90:273–86]

Angiogenesis, a complex process that includes the activation, proliferation, and migration of endothelial cells, disruption of vascular basement membranes, formation of vascular tubes and networks, and linkage to the pre-existing vascular networks, is a critical process required by solid tumors to support their growth (1–6). This concept is supported by a large body of evidence demonstrating that, once solid tumors grow to more than 1–2 mm³, further growth must be preceded by neovascularization of the tumor (7). There is also increasing evidence to support the concept that metastasis from solid tumors is facilitated by angiogenesis of the primary tumor (8–10). Importantly, there are extensive data showing that solid tumors express genes coding for angiogenic mediators; i.e., part of the malignant phenotype is the ability of the tumor cells to elicit angiogenesis in the local milieu and thus to direct the production of the new blood vessels they require to grow (11–18).

With the knowledge that solid tumors cannot progress without the generation of new blood vessels, a major effort has been focused on understanding the biology of angiogenesis, the identification of mediators that normally dampen angiogenesis, and the evaluation of strategies that employ antiangiogenesis agents to inhibit the growth of tumors (17,19–25). As these studies have progressed, the requirements for successful antiangiogenesis therapy of solid tumors have been defined. To be useful in cancer therapy, the antiangiogenesis agent must have a biologic half-life sufficient to counter the proangiogenesis phenotype of the tumor, it must act broadly across different tumor types, and it cannot be promiscuous (i.e., it cannot significantly interfere with physiologic angiogenesis).

Based on these considerations, in addition to the choice of the therapeutic molecule, the major challenge of developing a successful antiangiogenesis therapy is to deliver the antiangiogenesis agent to the tumor in sufficient quantities for a sufficient period to suppress neovascularization within the tumor but not to adversely affect normal physiologic processes. Since many of these requirements may be achieved by use of gene transfer vectors, the focus of this review is to summarize the evolving concepts of antiangiogenesis gene therapy strategies to treat solid tumors.

Why Use Gene Therapy to Deliver Antiangiogenesis Agents?

There are a variety of approaches not involving gene therapy that are under evaluation for their ability to suppress tumor neovascularization; they include recombinant proteins, monoclonal antibodies, and small molecules, many of which are already in clinical trials (Table 1). To date, all of these strategies utilize systemic administration of the therapeutic agent. Gene therapy can potentially expand the horizons of tumor antiangiogenesis therapy by virtue of its ability to produce the therapeutic agent in high concentrations in a local area for a sustained period, either external to or within the target cells. Gene therapy can also be designed to deliver the therapeutic agent systemically, but this strategy has no major advantages over conventional administration of recombinant proteins other than the ability of gene therapy to enable the therapeutic protein to be available for a prolonged period with only a single administration. Throughout this review, it should be kept in mind that gene therapy is a new therapeutic paradigm, and there are many challenges that will have to be overcome before antiangiogenesis gene therapy strategies can be used to effectively treat human cancers. In this context, many of the parameters for successful local delivery of gene products have not been optimized, nor have the methodologies to provide sustained and/or regulatable

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expression as required. These challenges will be discussed in more detail later in the review.

**General Concepts of Gene Therapy**

Gene therapy describes the process of transferring DNA or RNA to modify the genetic repertoire of target cells for therapeutic purposes (26–28). In conceptualizing the application of gene therapy to suppress tumor neovascularization, it is useful to view gene therapy as a sophisticated drug delivery system, where the antiangiogenic molecule expressed from the transferred gene is designed to function within the target cell or in the local milieu. In this regard, one advantage of gene therapy over the administration of therapeutic proteins is that the gene transfer can be designed to result in a local production of the gene product, thereby confining the therapeutic effect to only the organ of interest. If designed appropriately, the locally produced antiangiogenic gene product will accumulate within the organ of interest, leading to a high regional concentration and a correspondingly low systemic level of the gene product, thus minimizing unwanted systemic toxicity of the gene products. Theoretically, another appealing feature of gene transfer to deliver an antiangiogenesis agent is that the production of the gene product can be sustained, as long as the transduced cells are not eliminated by the body and the function of the expression cassette is not suppressed. In essence, the transduced cells serve as local “slow-release preparations,” allowing the maintenance of the therapy over time. The duration of gene expression depends on a variety of factors, including the choice of the method of gene transfer, the promoter, the transgene, the target cell, and the response of the host’s innate and adaptive immune systems to the process of gene transfer and the genetically modified cells.

The process of gene transfer is generally accomplished with the use of gene carriers, referred to as “vectors” (26–28). The purpose of the vector is to transfer the therapeutic gene to the cell and to ensure that it efficiently translocates to its site of function, usually the nucleus (26–28). Typically, the gene to be transferred is in the form of a complementary DNA (cDNA) coding for the therapeutic protein and is contained in an “expression cassette” that includes a constitutive or regulatable promoter driving the cDNA (26–29).

Broadly, gene transfer vectors are classified as either nonviral or viral (27,30–37). Nonviral methods of gene transfer usually contain the expression cassette in the form of a plasmid that is on the surface of, or contained within, the gene transfer vector. The most common nonviral strategy is to use cationic liposomes as the carrier, with the plasmid linked by charge interactions to the liposome surface (35,38). Other vector systems in the nonviral category include neutral and anionic liposomes and nano-particles (39,40). In the “gene gun” approach, the plasmid is on the surface of gold microspheres that are propelled into the target cells (41,42). One practical advantage of the nonviral vectors is that, theoretically, there is no limitation on the size of the expression cassette to be transferred. Unlike the vigorous host responses to some viral vectors, most nonviral vectors have limited numbers of antigenic epitopes for the adaptive immune system to recognize, resulting in little or no host response to the

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**Table 1. Strategies for antiangiogenesis therapy other than gene therapy**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Specific agent*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutralization or sequestration of angiogenesis mediators</td>
<td>Chimeric soluble Flt-1 IgG heavy-chain protein</td>
<td>(152)</td>
</tr>
<tr>
<td></td>
<td>Anti-VEGF monoclonal antibody</td>
<td>(92,153–157)</td>
</tr>
<tr>
<td></td>
<td>Anti-bFGF monoclonal antibody</td>
<td>(158)</td>
</tr>
<tr>
<td></td>
<td>Anti-angiogenin monoclonal antibody</td>
<td>(159)</td>
</tr>
<tr>
<td></td>
<td>Suramin†</td>
<td>(160,161)</td>
</tr>
<tr>
<td></td>
<td>Sulfated polysaccharide-peptidoglycan complex (tecogalan; DS-4152)†</td>
<td>(162)</td>
</tr>
<tr>
<td></td>
<td>Pentosan sulfate</td>
<td>(163)</td>
</tr>
<tr>
<td>Interference with normal function of receptors for angiogenic mediators</td>
<td>Chimeric soluble Flt-1 IgG heavy-chain protein</td>
<td>(152)</td>
</tr>
<tr>
<td></td>
<td>Specific inhibitors of receptor tyrosine kinases†</td>
<td>(164)</td>
</tr>
<tr>
<td></td>
<td>Specific inhibitors of Flk-1 receptor</td>
<td>(165)</td>
</tr>
<tr>
<td>Inhibition of functions of basement membrane and extracellular matrix</td>
<td>Anti-αvβ3 integrin monoclonal antibody</td>
<td>(222)</td>
</tr>
<tr>
<td></td>
<td>Batimastat (BB-94)† and Marimastat (BB-2516)†</td>
<td>(166)</td>
</tr>
<tr>
<td></td>
<td>Angiostatic steroids</td>
<td>(167)</td>
</tr>
<tr>
<td>Direct endothelial cell injury (vascular targeting)</td>
<td>VEGF-diphtheria toxin conjugates</td>
<td>(168)</td>
</tr>
<tr>
<td></td>
<td>Antibody-directed vascular targeting of tissue factor to induce vascular thrombosis</td>
<td>(169)</td>
</tr>
<tr>
<td>Unknown mechanisms of action on endothelial cells</td>
<td>AGM-1470 (TNP-470)†</td>
<td>(170–174)</td>
</tr>
<tr>
<td></td>
<td>Interferon alfa</td>
<td>(175–177)</td>
</tr>
<tr>
<td></td>
<td>Interferon beta</td>
<td>(175,176)</td>
</tr>
<tr>
<td></td>
<td>Angiostatin</td>
<td>(119,131,132)</td>
</tr>
<tr>
<td></td>
<td>Endostatin</td>
<td>(120)</td>
</tr>
<tr>
<td></td>
<td>Interleukin 12†</td>
<td>(178–180)</td>
</tr>
<tr>
<td></td>
<td>Carboxyaminotriazole†</td>
<td>(181,223)</td>
</tr>
<tr>
<td></td>
<td>Recombinant platelet factor 4†</td>
<td>(127,128)</td>
</tr>
<tr>
<td></td>
<td>Retinoids</td>
<td>(183)</td>
</tr>
<tr>
<td></td>
<td>Tamoxifen</td>
<td>(184)</td>
</tr>
<tr>
<td></td>
<td>D-Penicillamine</td>
<td>(185)</td>
</tr>
<tr>
<td></td>
<td>Nitric oxide synthase inhibitor</td>
<td>(186)</td>
</tr>
<tr>
<td></td>
<td>Thalidomide†</td>
<td>(187)</td>
</tr>
</tbody>
</table>

*IgG = immunoglobulin G; VEGF = vascular endothelial growth factor; bFGF = basic fibroblast growth factor.
†Currently in clinical trials.
administration of the vector. The disadvantage of the nonviral methods is the low efficiency of gene transfer and transient expression achieved by the transfer of plasmids, particularly with \textit{in vivo} applications (26–28,31,37). In contrast, viral vectors often achieve high levels of gene transfer and expression whether they are administered \textit{in vitro} or \textit{in vivo}. Viral vectors capitalize on the ability of the viruses to efficiently enter cells through specific receptors, and the mechanisms inherent in the virus structure that facilitate effective transfer of their genome to a site in the cell where the host cell machinery will transcribe and translate the expression cassette (26,27,32). To effectively utilize a virus as a gene transfer vector, sufficient viral genes are removed to permit insertion of the expression cassette and to render the virus \textquote{replication deficient} and nontoxic (27,32). Examples of viral vectors in clinical use include retrovirus, adenovirus, and adeno-associated virus (26–28,30,32,33). Retrovirus vectors are most useful in transferring genes \textit{in vitro} (i.e., for \textit{ex vivo} gene transfer strategies, \textit{see below}), but they are limited by the requirement for the target cell to be proliferating in order for the expression cassette to be successfully transferred and the propensity for promoter shutdown, which can limit the duration of expression (32). Adenovirus vectors are currently the \textquote{gold standard} vector for \textit{in vivo} gene transfer in that they are easy to produce in large amounts with high-purity, transfer genes to almost all cell types (quiescent or proliferating and regardless of the state of differentiation of the cell), and the resulting expression of the transgene is robust (27,30,32,33,36). However, adenovirus vectors do not integrate into the target cell genome, and the expression is transient, lasting for days to weeks. In this regard, one challenge in using adenovirus vectors is that they often elicit a vigorous host humoral and/or cellular immune response that limits the effectiveness of gene transfer following re-administration of the vector and eliminates the target cell expressing low levels of viral genes (43–46). Adeno-associated virus vectors seem to work well in delivering the expression cassette to tissues like muscle and brain with consequent long-term expression but not to other organs and are limited by the size of the expression cassette that they can transfer (27,47–52). Like other viral vectors, adenov-associated virus vectors elicit humoral immunity against the capsid proteins that may limit effective readministration of the vector.

Gene therapy can be carried out \textit{ex vivo} or \textit{in vivo} (26–28). Both strategies have been used in experimental models of antiangiogenesis of solid tumors (53–59). \textit{Ex vivo} gene transfer requires the target cells to be isolated and purified, and the gene transfer is carried out in the laboratory. For antiangiogenesis therapy, the genetically modified cells are then transferred back to the recipient in the region of tumor growth. \textit{Ex vivo} gene transfer permits maximum control of the cell target, but this approach can be used in only a limited number of cell targets, is inherently cumbersome and expensive, and has the potential to permit biologic contaminants to be transferred into the target cells. In contrast, \textit{in vivo} gene transfer capitalizes on properties of the vectors to transfer the expression cassette into the cells of specific organs \textit{in situ}. From a practical standpoint, \textit{in vivo} gene transfer is more convenient, sterile, and feasible. One limitation of \textit{in vivo} gene transfer is the lack of certainty that the specific cell populations of interest are being transduced. This problem is less relevant if the gene product is a secreted protein that acts on the target cells \textit{in trans} (i.e., acts in the extracellular milieu as opposed to within tumor cells). Such is the case with the use of gene transfer strategies for antiangiogenesis therapy of solid tumors.

**Advantages of Gene Transfer Strategies for Antiangiogenesis Therapy**

Theoretically, there are several reasons why gene therapy should be considered as a strategy to suppress the neovascularization of solid tumors.

First, gene therapy can reduce the risk of promiscuous antiangiogenesis inherent in the systemic administration of an antiangiogenesis agent. While the expression of genes that promote angiogenesis is a phenotype of solid tumors, it is also a normal physiologic process in organs such as the ovaries and the uterus and in organs following injury or hypoxia secondary to vascular occlusion (60–70). In addition, some angiogenic mediators normally serve physiologic functions other than promoting angiogenesis. For example, vascular endothelial growth factor (VEGF) expressed by megakaryocyte precursors and CD34+ stem cells likely helps to maintain normal bone marrow microvascular endothelial function other than promoting new blood vessel formation (71,72), and basic fibroblast growth factor (bFGF) serves to modulate the proliferation of mesenchymal cells (5,13,16). VEGF, the most specific of the known proangiogenic mediators, can function to attract monocytes and to suppress maturation of dendritic cells, suggesting it has a role beyond that attributable to new blood vessel formation (73,74). In this context, systemic administration of antiangiogenesis drugs may lead to undesirable suppression of physiologic functions. Indeed, a recent report by Klauber et al. (75) demonstrated that systemic administration of AGM-1470 (a synthetic analogue of fumagillin, a fungal product that inhibits endothelial proliferation \textit{in vitro} and angiogenesis \textit{in vivo}) to nonpregnant mice inhibited endometrial maturation and corpus luteum formation; moreover, in pregnant mice, it led to a complete failure of embryonic growth. In contrast to systemic delivery of agents like AGM-1470, the delivery of genes encoding antiangiogenic proteins to specific target organs results in a localized expression of the therapeutic proteins only in those organs; i.e., the end result is a restriction of the antiangiogenic effects to only the organs of interest. The sites of gene delivery, and hence the site of therapeutic antiangiogenesis, can be defined by an appropriate choice of the gene vectors. For example, intravenous administration of recombinant adenovirus vector delivers more than 90% of the transgene to the liver (76–79), a strategy that might be used to deliver an antiangiogenesis therapy to the liver for the treatment of hepatoma and liver metastases from colorectal cancers.

Second, gene transfer can lead to a local accumulation of the antiangiogenesis protein. Systemic treatment with antiangiogenesis drugs cannot lead to a high regional drug concentration without also increasing the systemic drug level and hence the risk of systemic toxic effects. By producing the therapeutic protein only within the organs to which gene transfer has occurred, gene therapy can lead to a high regional concentration of the antiangiogenesis protein and a correspondingly low systemic level. This improved therapeutic profile has the potential to per-
mit the use of lower doses of antiangiogenesis gene therapy to achieve successful local–regional tumor suppression.

Third, some anticancer gene therapy strategies exhibit a phenomenon referred to as the “bystander” effect, where the therapeutic effect is exhibited in cells in the local milieu of the cells to which a gene has been transferred. For example, when the Escherichia coli cytosome deaminase gene is transferred to colon carcinoma cells in vitro and the cells are exposed to the “prodrug” 5-flourouracil (5-FU), the cytosome deaminase converts the 5-FU to the chemotherapeutic agent fluorouracil (5-FU). Not only will the 5-FU function within the cell to which the cytosome deaminase gene is transferred, but it also diffuses out of the cell, providing a therapeutic effect to “bystander” cells in the local region (76–78). In some examples of the bystander effect, the mechanism is not apparent, and it may be mediated by immune responses to tumor cells injured by the primary therapy (79,80).

The concept of the bystander effect is important for antiangiogenesis antitumor gene therapy strategies, because it is not possible with current technology to transfer genes to all target cells relevant to the growth of the tumor. For example, liposome-mediated transfer of the p53 gene to experimental mammary tumors led to less than 5% tumor cell transfection but was associated with a 60% reduction in the number of blood vessels in the treated tumors (81).

Fourth, gene therapy should provide a sustained local antiangiogenesis effect if expression of the gene is persistent. Based on the current understanding of the mechanism of action of antiangiogenesis agents, suppression of neovascularization of solid tumors is cytostatic rather than cytotoxic for the tumor. Thus, a prolonged biologic half-life of the therapeutic agent is crucial to successful antiangiogenesis therapy, since a sustained antiangiogenic effect is likely necessary to achieve long-term suppression of tumor growth (20,24,25). With the use of antiangiogenesis proteins, it is often necessary to use prolonged infusions or complicated treatment schedules to maintain an adequate systemic and tissue level of the drug (57). Gene transfer strategies, in contrast, have the potential for a sustained release of the antiangiogenesis protein in a regional fashion to counter the proangiogenesis phenotype of the tumor. The duration of expression of the therapeutic protein following gene transfer depends on a variety of factors, including the vector used to transfer the gene, the biology of the cells to which the antiangiogenesis gene has been transferred, whether the transgene has been integrated into the host cell genome, the rate of clearance of the transduced cells, and the ability of the expression cassette to continue to function over time. For example, retrovirus-mediated ex vivo gene transfer results in integration of the expression cassette into the target cell; thus, if the cell remains viable in vivo, this strategy has the potential to provide long-term local delivery of an antiangiogenesis gene (26,32). In contrast, adenovirus-mediated in vivo gene transfer results in a much higher local concentration of the transgene for days to weeks, but it may be associated with immune-mediated clearance of the transduced cells, requiring intermittent therapy to achieve a persistent antiangiogenic effect (30,32,33,36).

Finally, appropriately designed gene therapy for antiangiogenesis could be titrated according to the clinical state of the patient. Successful antiangiogenesis strategies should be regulatable. With the use of an inducible promoter to drive the expression of an antiangiogenesis transgene, the basal level of therapeutic antiangiogenesis could be up-regulated by the exogenous administration of agents that induce the promoter, permitting transcriptional up-regulation of antiangiogenesis gene expression if the patient has a tumor relapse following an initial response to the antiangiogenesis gene therapy.

**Biological of Tumor Angiogenesis Relevant to Antiangiogenesis Gene Therapy**

Like many other potential molecular targets for antitumor therapy, antiangiogenesis gene therapy capitalizes on the concept that the proangiogenesis processes in a tumor are more exaggerated in intensity and localized to a limited anatomic site than are normal physiologic proangiogenic processes. In that context, the rate and extent of angiogenesis within an organ represent a balance of local proangiogenic and antiangiogenic functions; thus, a shift to an angiogenic phenotype can result from an enhancement of proangiogenic mechanisms and/or suppression of the opposing antiangiogenesis processes. Our current understanding of the proangiogenic malignant phenotype is that it is primarily the result of tumor cells overexpressing genes coding for angiogenic mediators (15,18).

**Production of Angiogenic Mediators by Tumor Cells**

At least 15 different proteins have been shown to have angiogenic properties (Table 2). Two of these mediators, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF; also called FGF2), appear to be the most important relevant to the proangiogenic phenotype of solid tumors. Importantly, both VEGF and bFGF are expressed by many tumors (15), and both have been shown to be angiogenic in vivo (15). The roles, if any, of the other proangiogenic mediators (e.g., angiogenin, epidermal growth factor, fibroblast growth factors other than bFGF, granulocyte colony-stimulating factor, hepatocyte growth factor, insulin-like growth factor-I, interleukin 8, placental growth factor, platelet-derived endothelial cell growth factor, transforming growth factor-α, transforming growth factor-β, and tumor necrosis factor-α) in tumor angiogenesis are unknown (Table 2). However, like other cytokine networks, there are likely complex interrelationships among these mediators, and they should all be kept in mind in designing antiangiogenesis strategies, particularly in those strategies that are directed specifically to inhibit one proangiogenic mediator. Despite this theoretical caveat, several mediator-specific, antiangiogenesis gene therapy strategies have been shown to be effective in experimental models of therapy for solid tumors (see below).

**Vascular endothelial growth factor.** VEGF is a 34- to 46-kd, homodimeric glycoprotein normally produced during embryogenesis and during adult life in the pituitary gland, hypothalamus, choroid plexus, renal glomeruli, adrenal cortex, cardiac myocytes, prostate epithelium, semen, endometrium, lung alveolar epithelial cells, and the corpus luteum of the ovaries (2,60,63,66,82–86). There are four known VEGF isoforms (VEGF 121, 165, 189, and 206; the numbers refer to the number of amino acids in each), all derived from the single VEGF gene through alternative splicing (84,86–89). Most solid tumors overexpress the VEGF gene, with the 165 and 121 isoforms being...
### Table 2. Angiogenic mediators produced by tumor cells

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Structure*</th>
<th>Effects on endothelial cells</th>
<th>Other effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiogen</td>
<td>14.1 kd</td>
<td>Angiogenesis in vivo only; no effects in vitro; likely acts through stimulating the release of other factors</td>
<td>Weak ribonucleolytic activity required for neovascularization</td>
<td>(188,189)</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>6 kd</td>
<td>Proliferation and migration</td>
<td>Varied</td>
<td></td>
</tr>
<tr>
<td>Estrogen</td>
<td>Steroid</td>
<td>Proliferation, migration, and tube formation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroblast growth factors (FGFs), acidic GGF (aFGF-1) and basic GGF (bFGF-2)</td>
<td>16.4- to 18-kd polypeptide; heparin-binding; absence of signal peptide; stored in extracellular matrix</td>
<td>Proliferation, migration, and tube formation</td>
<td>Induced proteases and plasminogen activator; mitogenic for a wide variety of cell types derived from mesoderm and neuroectoderm; neurotrophic action</td>
<td>(111,113,116)</td>
</tr>
<tr>
<td>Granulocyte colony-stimulating factor</td>
<td>18 kd</td>
<td>Proliferation and migration</td>
<td>Increases number and function of circulating granulocytes</td>
<td>(193)</td>
</tr>
<tr>
<td>Hepatocyte growth factor (also called scatter factor)</td>
<td>92 kd</td>
<td>Proliferation, migration, and tube formation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor-I (also called somatomedin C)</td>
<td>7.6 kd</td>
<td>Migration and tube formation</td>
<td></td>
<td>(194,195)</td>
</tr>
<tr>
<td>Interleukin 8</td>
<td>40 kd</td>
<td>Proliferation and migration</td>
<td>Attraction of neutrophils</td>
<td>(197,198)</td>
</tr>
<tr>
<td>Leukotrienes C4 and D4</td>
<td>Lipids</td>
<td>Migration</td>
<td></td>
<td>(199)</td>
</tr>
<tr>
<td>Placental growth factor</td>
<td>25 kd; 2 isoforms</td>
<td>Migration; stimulates endothelial cell DNA synthesis but not proliferation</td>
<td></td>
<td>(200)</td>
</tr>
<tr>
<td>Platelet-derived endothelial cell growth factor (also called thymidine phosphorylase)</td>
<td>45 kd; absence of signal peptide; acidic; main source is platelets</td>
<td></td>
<td>Amplies activity of FGFs on endothelial cells</td>
<td>(201–204)</td>
</tr>
<tr>
<td>Platelet-derived growth factor</td>
<td>3 isoforms of dimer</td>
<td>Proliferation and migration</td>
<td>Mitogenic for fibroblasts, smooth cells, and glial cells</td>
<td>(205)</td>
</tr>
<tr>
<td>Prostaglandin E1 and E2</td>
<td>Prostaglandins</td>
<td>Proliferation</td>
<td>Suppression of inflammation</td>
<td>(206)</td>
</tr>
<tr>
<td>Transforming growth factor-α</td>
<td>5.5 kd; 40% homology to epidermal growth factor</td>
<td>Proliferation</td>
<td>Autocrine growth factor for tumors; inhibits gastric acid secretion</td>
<td>(191)</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>25-kd homodimer; 3 isoforms</td>
<td>Stimulates tube formation; also inhibits endothelial cell proliferation in vitro</td>
<td>Enhances extracellular matrix production; chemotactic for monocytes</td>
<td>(207,208)</td>
</tr>
<tr>
<td>Tumor necrosis factor-α (also called cachectin)</td>
<td>17 kd; 28% homology to lymphotxin (tumor necrosis factor-β)</td>
<td>Migration; also inhibits endothelial cell proliferation in vitro</td>
<td>Induces inflammatory response and capillary leakiness; direct antitumor and antiviral properties; induces bFGF secretion; chemotactic for monocytes</td>
<td>(209,210)</td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF; also called vascular permeability factor)</td>
<td>34- to 46-kd homodimer; 4 isoforms; some isoforms heparin-binding; homology with platelet-derived growth factor and placental growth factor</td>
<td>Proliferation, migration, and tube formation; vascular permeability; endothelial cell survival</td>
<td>Attracts monocytes; inhibits maturation of dendritic cell precursors</td>
<td>(83,84,86)</td>
</tr>
</tbody>
</table>

*kd = kilodaltons.

Most common (90–95). The mechanism of the overexpression is not totally clear, but it may result from hypoxia within the tumor, stimulating hypoxia-responsive enhancer in the 5’ region flanking the VEGF gene (96,97). VEGF activates endothelial cells via interaction with Flt-1 and/or Flk-1/KDR, high-affinity, membrane-spanning receptor tyrosine kinases found almost exclusively on endothelial cells (86,98–108). This selective distribution of the cognate receptors ensures that the action of VEGF is confined to endothelial cells, in contrast to the more widespread distribution of the receptors for all other proangiogenic mediators (83,84,94). VEGF is overexpressed in most human tumors and tumor cell lines, including lymphoma, sarcoma, meningioma, glioblastoma multiforme, adenocarcinoma, melanoma, gastric cancer, renal cell carcinoma, colon cancer, ovarian cancer, and hepatoma (90–95). In addition to causing endothelial cells to proliferate and form neovascularization within the tumor, VEGF causes an increase in vessel permeability that has been implicated in the pathogenesis of malignant ascites and pressure symptoms from solid tumors (5,109,110).

**Basic fibroblast growth factor.** bFGF belongs to a family of structurally related polypeptide mitogens with nine members (5,11,111). It is a cationic 18-kd polypeptide with 155 amino acid residues, although higher molecular weight forms with larger N-terminal extensions have been identified (112,113). bFGF is one of the most widely distributed growth factors in the body and is expressed in different tumor types, including bladder cancer, glioma, hepatoma, gastrointestinal cancer, breast cancer, renal cell carcinoma, and thyroid cancer (12,112,114,115). The protein is multifunctional. It promotes mitogenesis, chemotaxis, and migration of endothelial cells; it stimulates endothelial cells to produce collagenase that degrades basement membrane; and it induces proliferation and differentiation in a wide range of cell types derived from the mesoderm and neuroectoderm (5,13,16,116). bFGF also exhibits neurotrophic action, promoting the survival and differentiation of neurons in the nervous system. Biosynthetic studies indicate that bFGF can be secreted despite the absence of a consensus signal peptide in the open reading frame of the bFGF gene, although the mechanism of...
Endogenous Inhibitors of Angiogenesis

The ability of the proangiogenic mediators to stimulate new blood vessel growth is normally counteracted by endogenous inhibitors of angiogenesis (Table 3). Most of these inhibitors are secreted in their active forms, but some require extracellular proteolysis of proteins unrelated to angiogenesis to release the biologically active antiangiogenic moieties (119,120). Endogenous antiangiogenic mediators may be secreted either by the tumor cells or by other stromal cells within or surrounding the tumor, such as the fibroblasts and pericytes (121). The major natural antiangiogenesis inhibitors include thrombospondin-1 (TSP-1), platelet factor 4 (PF4), soluble flt-1, angiostatin, and endostatin.

Thrombospondin-1. TSP-1 is a heparin-binding, 450-kd homotrimeric glycoprotein found in the alpha granules in platelets and is constitutively secreted by fibroblasts, endothelial cells, glomerular mesangial cells, smooth muscle cells, monocytes, and macrophages (121,122). TSP-1 inhibits endothelial cell proliferation in vitro and angiogenesis in vivo (5,11,73,121,122). It is found in plasma and, like bFGF, is also stored in the extracellular matrix (5,11,121). TSP-1 has been shown to modulate platelet aggregation, wound healing, protease activity, and other cellular functions (121). It is interesting that TSP-1 expression is under the control of p53 such that mutation or loss of p53 is associated with a decrease in TSP-1 expression (15,123). TSP-1 is one of the natural inhibitors of angiogenesis; for some tumors, TSP-1 is down-regulated when tumors begin generating their own vasculature (123,124). Consistent with this observation, transfection of TSP-1 cDNA has been shown to inhibit tumor growth and metastatic potential (59).

Platelet factor 4. PF4 is a heparin-binding, 28-kd protein that exists as a tetramer. Like TSP-1, PF4 is found within the alpha granules of platelets (125–128). Unlike platelet-derived endothelial cell growth factor, a proangiogenic mediator secreted by platelets, PF4 is a potent antiangiogenesis factor (125,126). It thus appears that a balance of proangiogenic and antiangiogenic platelet-secreted proteins may influence new blood vessel growth (11). In this context, an excess of PF4, in the form of locally administered recombinant protein, has been shown to inhibit tumor growth (128).

Soluble Flt-1. Flt-1 is a high-affinity, VEGF tyrosine kinase cell surface receptor found almost exclusively on endothelial cells (94,101,107). Alternative splicing of Flt-1 pre-messenger RNA (mRNA) generates two distinct products, one encoding the full-length membrane-spanning receptor and a second encoding a soluble form (sFlt) that is made up of only six of the seven immunoglobulin sequences in the extracellular domain without the transmembrane and the intracellular domains (129,130). Although both species bind to VEGF with similar affinity, binding

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Structure*</th>
<th>Source</th>
<th>Antiangiogenesis mechanism of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiostatin, 1,25-Dihydroxy-vitamin D₃</td>
<td>38 kd, 417 d</td>
<td>Plasminogen, Skin</td>
<td>Inhibits endothelial cell proliferation, alters RNA synthesis</td>
<td>(119,131,132)</td>
</tr>
<tr>
<td>Endostatin</td>
<td>18 kd</td>
<td>Collagen XVIII</td>
<td>Inhibits endothelial cell migration; inhibits lymphocyte-induced</td>
<td>(120)</td>
</tr>
<tr>
<td>Interferon α</td>
<td>8- to 20-kd glycoproteins</td>
<td>Mononuclear phagocytes; lymphocytes</td>
<td>Inhibits endothelial cell migration and proliferation; inhibits lymphocyte-induced angiogenesis</td>
<td>(175,176)</td>
</tr>
<tr>
<td>Interferon β</td>
<td>23-kd glycoproteins</td>
<td>Fibroblasts; epithelial cells</td>
<td>Suggested to be cytotoxic to proliferating endothelial cells</td>
<td>(175,176)</td>
</tr>
<tr>
<td>Interferon γ</td>
<td>20- to 25-kd glycoproteins</td>
<td>T cells; natural killer cells</td>
<td>Inhibits endothelial cell differentiation in capillary tube formation</td>
<td>(212)</td>
</tr>
<tr>
<td>Interferon gamma-inducible protein IP-10</td>
<td>C-X-C chemokine that lacks ELR motif†</td>
<td>Bone, mononuclear phagocytes, and liver</td>
<td>Induction of interferon gamma</td>
<td>(216)</td>
</tr>
<tr>
<td>Interleukin 1α and β</td>
<td>17 kd, 2 isoforms</td>
<td>Mononuclear phagocytes, and liver</td>
<td>Inhibits endothelial cell migration in capillary tube formation</td>
<td>(213–215)</td>
</tr>
<tr>
<td>Interleukin 12</td>
<td>75-kd glycoprotein, heterodimer</td>
<td>Mononuclear phagocytes, B cells, and tissue mast cells</td>
<td>Induction of interferon gamma</td>
<td>(178–180)</td>
</tr>
<tr>
<td>2-Methoxyestradiol</td>
<td>302 d</td>
<td>Endogenous estrogen</td>
<td>Inhibits endothelial cell migration; inhibits urokinase plasminogen activator</td>
<td>(224)</td>
</tr>
<tr>
<td>Platelet factor 4</td>
<td>28 kd, heparin-binding</td>
<td>Platelets</td>
<td>Down-regulates acidic fibroblast growth factor receptors; inhibits collagenase activity</td>
<td>(125,126)</td>
</tr>
<tr>
<td>Prolactin, 16-kd* fragment</td>
<td>16 kd, N-terminal fragment</td>
<td>Prolactin</td>
<td>Inhibits endothelial cell DNA synthesis</td>
<td>(217,218)</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>43 kd, arginine-rich</td>
<td>Sperm</td>
<td>Binds to heparin; requires proliferating/remodeling vessels</td>
<td>(183)</td>
</tr>
<tr>
<td>Thrombospondin-1 and thrombospondin-2</td>
<td>300 d, 450-kd trimeric glycoprotein</td>
<td>Platelets and fibroblasts</td>
<td>Transcriptional regulator</td>
<td>(121,122,219)</td>
</tr>
<tr>
<td>Tissue inhibitor of metalloproteinase-1 and -2</td>
<td>8.5 and 21 kd</td>
<td>Cartilage</td>
<td>Blocks collagen synthesis and deposition</td>
<td>(121,221)</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>25 kd, 3 isoforms</td>
<td>Platelets, bone, placenta, kidney, and tumor cells</td>
<td>Blocks endothelial cell motility</td>
<td>(207,208)</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>17 kd</td>
<td>Macrophages</td>
<td>Inhibits fibroblast growth factor-induced endothelial cell proliferation</td>
<td>(209,210)</td>
</tr>
</tbody>
</table>

*kd = kilodaltons; d = daltons.
†C-X-C cytokines have four highly conserved cysteine residues, with the first two separated by one nonconserved amino acid residue. ELR = Glu–Leu–Arg.
to the sFlt form does not lead to signal transduction because it is not cell associated and lacks the intracellular tyrosine kinase domains (129,130). It has been postulated that, under homeostatic conditions, endogenous sFlt sequesters excess VEGF and/or inactivates endogenous Flt-1 or Flk-1/KDR receptors through heterodimerization, thereby serving as a feedback mechanism to prevent uncontrolled angiogenesis (129).

Angiostatin and endostatin. Both angiostatin and endostatin are two secreted proteins that may play a role in maintaining the quiescent state of normal endothelial cells. Angiostatin is a 38-kd protein whose sequence is identical to that of the first four kringle structures of plasminogen (58,119,131,132). Angiostatin is cleaved by elastase from plasminogen (119). The two molecules have different biologic functions: Neither plasminogen nor plasmin inhibits angiogenesis, and angiostatin has no anticoagulant activity (119). Angiostatin acts specifically on endothelial cells without affecting tumor cells directly, and administration of angiostatin to tumor-bearing mice leads to an inhibition of angiogenesis and an increased apoptotic rate in the tumor cells, resulting in a state of tumor dormancy (119). Analogous to angiostatin, endostatin is an 18-kd protein that is cleaved enzymatically from collagen XVIII (120). It has antiangiogenesis activity similar to that of angiostatin (120).

Regulators of Angiogenesis Function In Trans

Since endothelial cells are the primary structural units of blood vessels, the signals that initiate angiogenesis do so by interacting with receptors on endothelium (86,98–108). Inherent in the strategy of using gene therapy to suppress angiogenesis of tumors is that the positive and negative regulators of angiogenesis act on the endothelial cells in trans; i.e., the endothelial cells themselves are functioning normally in response to signals external to the endothelium. Thus, while the aim of antiangiogenesis gene therapy is to suppress the growth of endothelial cells and/or to prevent the endothelial cells from forming vascular networks, the therapeutic antiangiogenesis gene does not have to be transferred to the endothelial cells themselves. This is important for strategizing antiangiogenesis gene therapy, because it eliminates the challenge of having to deliver the antiangiogenesis gene to a specific cell type; i.e., delivering and expressing the antiangiogenesis gene in cells in the local environment of the tumor should be sufficient to provide effective antiangiogenesis therapy.

These considerations provide the biologic basis for the use of gene transfer strategies to achieve regional antiangiogenesis; i.e., manipulation of the extracellular milieu of the endothelial cells should be sufficient to alter their biologic state. Thus, in vivo transfer of the antiangiogenesis gene to normal and malignant cells within the target organ should result in a secretion of the therapeutic protein into the extracellular milieu by both cell populations. This common extracellular pool of secreted antiangiogenesis factors can then act on the endothelial cells in trans to dampen angiogenesis. The net result is therapeutic antiangiogenesis without the need to transduce every cell in the organ or the need to target any cell population specifically. This advantage is important because the current gene transfer technology is limited by the inability to deliver the therapeutic gene to every target cell in vivo (77).

Table 4. Gene therapy antiangiogenesis strategies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Delivery system</th>
<th>Experimental model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombospondin-1</td>
<td>Calcium phosphate transfection</td>
<td>Ex vivo transfection of breast cancer cells, followed by implantation into nude mice</td>
<td>(59)</td>
</tr>
<tr>
<td>Vascular endothelial growth factor antisense</td>
<td>Phosphorothioate oligonucleotides</td>
<td>Primary glioblastoma model in nude mice</td>
<td>(53)</td>
</tr>
<tr>
<td>Dominant-negative Flk-1 mutant receptor</td>
<td>Retrovirus vector</td>
<td>Various primary tumor models in mice</td>
<td>(55,56)</td>
</tr>
<tr>
<td>Soluble platelet factor 4</td>
<td>Adenovirus and retrovirus vectors</td>
<td>Primary glioma models in nude mice</td>
<td>(57)</td>
</tr>
<tr>
<td>Soluble Flt-1 receptor</td>
<td>Adenovirus vector</td>
<td>Primary and metastatic tumor models in mice</td>
<td>(54)</td>
</tr>
<tr>
<td>Angiostatin</td>
<td>Adenovirus and retrovirus vectors</td>
<td>Primary glioma models in nude mice</td>
<td>(58)</td>
</tr>
</tbody>
</table>

Antiangiogenesis With the Use of Gene Therapy

Various proof-of-principle experimental animal studies suggest that gene therapy may be an effective means to deliver antiangiogenesis therapy to solid tumors (Table 4). These antiangiogenesis gene therapy strategies can be categorized into those that suppress the proangiogenic signal and those that augment the inhibition of angiogenesis.

Suppression of the Proangiogenic Signal

The proangiogenic signal can be suppressed by decreasing the amounts of the angiogenic mediator available to induce tumor neovascularization or by interfering with the process of the angiogenic mediator signaling within the endothelial cell.

Inhibition of Gene Expression of Angiogenic Mediators

This strategy capitalizes on the ability of gene therapy to alter the genetic repertoire of target cells—in this case, the tumor cells overexpressing specific angiogenic mediators. The fundamental approach is to transfer antisense sequences or ribozymes that will deplete mRNA coding for the angiogenic mediator. The proof-of-principle study demonstrating that such an approach is feasible capitalized on the knowledge that VEGF is a potent angiogenic mediator secreted by many tumor types. By using an antisense construct against VEGF, Cheng et al. (53) showed that calcium phosphate transfection of glioblastoma cells with this oligonucleotide led to a decrease in VEGF mRNA and protein production as well as a reduced tumorigenicity in nude mice following implantation of the transduced cells. One challenge to this approach is that antisense functions as a cis effect only. Thus, for this antisense gene therapy to work in vivo, a large percentage of cells needs to be inhibited.

Interfering With Signaling of the Endothelium

This approach is based on the concept that interfering with the normal function of receptors for angiogenic mediators should potently disrupt the angiogenesis cascade. To evaluate this concept, Millauer et al. (56) constructed a dominant-
negative Flk-1 mutant VEGF receptor encoded by a retrovirus vector. The mutant lacks the intracellular domain but retains the extracellular and the transmembrane domain; as such, the mutant receptor remains cell associated but dysfunctional. By infecting endothelial cells with the retrovirus vector coding for the mutant receptor, heterodimerization occurs between the mutant receptor and the full-length, native Flk-1 receptor on endothelial cell membrane. Unlike the native homodimeric Flk-1 receptor, the heterodimer was unable to bring about signal transduction and endothelial cell activation. As a proof of principle, a combined ex vivo and in vivo strategy was used, where an ectopic packaging cell line producing a retrovirus vector coding for the mutant receptor was co-implanted with glioblastoma cells in nude mice; the result was suppression of tumor growth compared with findings in control animals (56). It is interesting that intratumoral administration of the retroviral supernatant also suppressed growth of primary glioblastomas. Although the dominant-negative Flk-1 receptor in this example functions in a cis-acting strategy, requiring gene transfer to all endothelial cells in the milieu of the tumor, dominant-negative receptor strategies can be developed where the mutant receptor is soluble and thus could function in a trans “bystander” fashion.

We have developed an in vivo antiangiogenesis gene therapy strategy that functions to sequester VEGF in the tumor and/or functionally inactivates the VEGF high-affinity receptors Flt-1 and Flk-1/KDR (54). To accomplish this, an adenovirus vector (AdSflt) was designed to deliver a cDNA coding for a truncated form of the Flt-1 VEGF receptor that lacked the intracellular domain, the transmembrane domain, and part of the extracellular domain. The product expressed by the vector (sFlt) is a diffusible, soluble receptor molecule that can bind to VEGF molecules with high affinity. The sFlt molecule also binds with high affinity to the endogenous Flk-1 and Flk-1/KDR VEGF receptors, forming an inactive heterodimeric receptor unable to trigger the endothelial cell to proliferate. It is not clear whether VEGF sequestration and/or receptor heterodimerization is the dominant in vivo mechanism that prevents VEGF secreted by tumor cells from triggering endothelial cells to initiate the angiogenic cascade. Irrespective of the responsible mechanism, when the AdSflt vector was administered in vivo to mice bearing primary or metastatic tumors that arose from syngeneic colon carcinoma cells, substantial tumor suppression was observed, and the treated animals had a statistically significant survival advantage. Importantly, the therapeutic effect was found to be regional, i.e., confined to only the organs in which gene transfer had occurred.

Augmentation of Inhibition of Angiogenesis

Since angiogenesis is the net result of a dynamic balance between the proangiogenic and antiangiogenic factors in the extracellular microenvironment of the tumor, increasing the local concentrations of endogenous inhibitors of angiogenesis should shift the balance between angiogenesis and antiangiogenesis in favor of the latter. To evaluate this concept, Tanaka et al. (57) cloned the cDNA sequence encoding the signal peptide from the monocyte chemoattractant protein-1 in-frame to the cDNA encoding human PF4, an endogenous inhibitor of angiogenesis. With the use of adenovirus as well as retrovirus vectors, cells transfected with the genetically engineered soluble PF4 cDNA secreted a biologically active form of PF4 that induced greater than 40% inhibition of DNA synthesis in endothelial cells. Importantly, the growth of gliomas in nude mice could be suppressed following intratumoral administration of the vectors; the treated tumors appeared hypovascular, and the treated animals had a survival advantage over the control animals (57).

In another study evaluating the concept of increasing the local concentration of inhibitors of angiogenesis (59), the TSP-1 cDNA was transfected into breast carcinoma cells, followed by their injection into the mammary fat pads of nude mice. The resulting tumors were found to be smaller and to have a lower metastatic potential than the naive (i.e., untransfected) tumors.

Challenges to Successful Antiangiogenesis Gene Therapy

Although the gene therapy approach to antiangiogenesis therapy for solid tumors is still in its infancy, the preliminary data developed to date suggest gene therapy should live up to its theoretical potential of providing high, local concentrations of the therapeutic molecule, while avoiding the potential toxicity of systemic administration. However, there are several challenges that will have to be overcome before antiangiogenesis gene therapy becomes a useful strategy to treat human tumors. Some of these challenges are specific for gene therapy per se; others are generic challenges for antiangiogenesis.

Gene Therapy as a Delivery System

It is apparent from experimental animal studies and from the early clinical studies of systemic antiangiogenesis therapy that, for antiangiogenesis therapy to be effective in treating tumors, the antiangiogenesis effects must be maintained for a long time (20,25). If we assume that remaining tumor cells have the potential to express proangiogenic mediators, interruption of antiangiogenesis therapy has the potential risk of tipping the angiogenesis balance in favor of proangiogenesis, allowing the tumor to emerge from its dormancy. A successful gene therapy for antiangiogenesis should therefore have a sustained effect. This is an important challenge for current gene transfer vectors, which either inherently provide only transient expression (e.g., nonviral vectors) or elicit host responses that conspire to eliminate the genetically modified cells (e.g., adenovirus vectors). There are various solutions to the challenge of maintaining persistent expression of a transgene following gene transfer. They include the following: 1) designing vectors to be more efficient in entering the target cell and transferring genes to the nucleus, 2) permanently incorporating the transgene into the target cell genome, 3) designing the vector to be stealthy with regard to detection by the hosts’ innate and adaptive immune systems, 4) using pharmacologic agents to suppress host responses to the vectors, 5) designing the transgene to code for an antiangiogenic protein that has a longer biologic half-life in the target organ, and 6) using promoters that are resistant to shutdown by the host cell. An alternative strategy to prolong the effect of antiangiogenesis gene therapy strategies is to combine delivery of an antiangiogenesis gene product with gene therapy-based immunotherapy and/or gene therapy-based prodrug chemotherapy strategy (76–78,133–144). Although there are risks to this approach in that the other antitumor gene strategy may eliminate the cells expressing the antiangiogenesis genes, the initial tumor reduction
brought about by an antiangiogenesis treatment might be maintained by chronic tumor suppression from antitumor genes that function to suppress tumor growth by other mechanisms. In this context, gene therapy-based immunotherapy may be the best choice for combined therapy, in that it would provide tumor-specific suppression, while the gene therapy-based antiangiogenesis therapy would attack the tumor by a very different mechanism, independent of the cell site of the antiangiogenesis genes.

While the local production of a therapeutic antiangiogenesis protein is an inherent advantage of gene therapy in that it limits the risk of promiscuous systemic antiangiogenesis, such a strategy suffers from an inability to treat widespread metastases. Thus, one challenge of future antiangiogenesis gene therapy is to target the vector or its transgene product to tumor-associated vessels, permitting systemic treatment of disseminated tumors. Such a goal may be achieved when more is known about the phenotypic differences between normal vessels and those that are induced by and support growing tumors. In this regard, there are emerging data suggesting that tumor neovascularization behaves differently from its normal counterpart in that, although the tumor neovasculature is composed of normal cells, its architecture is abnormal. For example, tumor blood vessels are leaky and aberrantly arranged, with unusual fan and spiral motifs, forming right angles and arteriovenous shunts (147–150). Up-regulation of \( \alpha_5\beta_3 \) integrins is also a feature of new blood vessels in tumors, a biologic process believed to be critical for the survival and differentiation of vascular cells undergoing angiogenesis (146). Since adenovirus vectors use \( \alpha_5\beta_3 \) integrins as an internalization signal, it may be possible to capitalize on this feature to design adenovirus vectors specific for active angiogenesis, such as occurs in growing tumors (147–150). Another possible target to achieve tumor vessel specificity is E-selectin (151).

**General Issues for Antiangiogenesis Therapy**

One of the challenges to success of antiangiogenesis gene therapy is to ensure that the strategy will be applicable to a broad range of tumor types, regardless of their profiles of angiogenic mediators. A successful strategy will have to counteract the proangiogenic phenotype induced by VEGF, bFGF, and likely other angiogenic mediators. It may therefore be more rational to target genes that express products that function to interrupt processes downstream in the angiogenesis cascade. In this regard, gene therapy approaches that target the common signaling cascades in the end organ of angiogenesis, i.e., the endothelial cells, rather than specific angiogenic mediators or their receptors may be more universally effective.

**Conclusions**

With a better appreciation of the critical and universal dependence of tumor progression on neovascularization, it is rational to hypothesize that suppression of this rate-limiting step could suppress the growth of a wide range of tumor types. As the cellular and molecular events that underlie tumor angiogenesis become better defined, rational strategies can be derived to apply this molecular tourniquet. An ideal antiangiogenesis strategy should be targeted to only the organs that contain the tumors and should not interfere with normal angiogenesis; it must achieve a high ratio of regional-to-systemic concentrations to minimize systemic toxicity; it must have a biologic half-life sufficient to counter the proangiogenesis phenotype of the tumor; and its antiangiogenesis effects should be regulatable. Gene transfer strategies potentially satisfy many of these requirements. Critical to the success of antiangiogenesis gene transfer is the fact that endothelial cells are activated or suppressed in trans, depending on the composition of the extracellular milieu. In this context, gene therapy for angiogenesis does not have to transduce all or any specific populations of cells in the target organs to achieve a high, local concentration of the antiangiogenesis proteins.

Antiangiogenesis treatment is likely to be most effective in a low tumor burden state. In such a setting, therapeutic antiangiogenesis can be expected to prolong the state of tumor dormancy by suppressing micrometastases that remain despite successful treatment of the primary tumors. One appropriate clinical approach to using antiangiogenesis therapy in cancer is to combine it with conventional therapy to reduce the initial tumor burden, followed by its use in an adjuvant setting to prolong disease-free survival.

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Notes

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