Gene Therapy for Antiangiogenesis

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Angiogenesis inhibitors have evolved as a new diverse class of compounds that can be used to block tumor growth (1,2). The inhibitors, which may be natural or synthetic, include protease inhibitors (i.e., tissue inhibitors of matrix metalloproteinases), tyrosine kinase inhibitors, chemokines, interleukins, and proteolytic fragments of diverse molecules (i.e., endostatin, vasostatin, canstatin, arrestin, etc.). These antiangiogenic molecules function in multiple ways, including the inhibition of endothelial cell proliferation, migration, protease activity, and tubule formation, as well as the induction of apoptosis. The antiangiogenic function of many of these molecules is well documented in vitro and in vivo, and some are currently being tested in clinical trials (http://cancertrials.nci.nih.gov). Less is known about the exact mechanism(s) of angiogenic regulation of some of the inhibitors. More than 40 endogenous inhibitors have been identified, and others may likely exist. Exactly why so many “stop signals” are present in mammals to inhibit angiogenesis is unclear, but their abundance indicates that angiogenesis must be tightly regulated in the adult.

Because of the abundance of angiogenic inhibitors, whether each works independently or in concert with other inhibitors remains to be determined. Furthermore, angiogenesis may be regulated on multiple levels because simple withdrawal of an angiogenic stimulator in vivo can reduce blood vessel formation, suggesting that angiogenic inhibitors have cell-type, as well as functional, specificity. For example, it is possible that the inhibitor(s) that function to stop new blood vessel development in maturing organs are different from those that stop new blood vessel formation in repaired wounds. Some inhibitors may function to inhibit the formation of new blood vessels, whereas others may disrupt or modify existing vessels. Angiogenesis inhibitors may also be specific for a particular tissue bed because endothelia with functional and genetic lineage differences have been identified (3).

The tumor vasculature is distinct from that in normal tissues, with altered endothelial cell morphology and a decreased number of perivascular cells that help to maintain the blood vessel integrity (2). A hallmark of many tumors is the increased production of vascular endothelial growth factor (VEGF), which appears to serve as a stabilization factor for the tumor endothelium in place of perivascular cells (4). In addition, some tumors have been found to have “channels” formed by tumor cells (5), as well as “mosaic vessels” formed by both tumor cells and endothelial cells (6). A recent study (7) has identified that the expression of a number of genes is increased in the tumor endothelium when compared with the nontumor endothelial cells. Thus, because the tumor endothelium appears to have a number of altered features, the best candidate antiangiogenic molecules for cancer therapy should be directed at differences between the tumor vasculature and normal vasculature to increase specificity and efficacy. However, many of the current antiangiogenic candidates also regulate similar activities, not only in tumor endothelium but also in normal endothelium and even in nontransformed cell types. The effectiveness of angiogenesis inhibitors in the treatment of various cancers remains to be determined. Despite the possibility of tumor endothelium being heterogeneous and tumor type specific, antiangiogenesis inhibitors have

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been used in some 22 clinical trials to date. However, a major problem has been the availability (amount and purity) and the stability of some of these molecules. Although there have been promising results in animal studies, the human trials for these protein-based inhibitors have been less successful (8). The questions of how best to deliver the inhibitor, maintain its stability and activity, and target it to the tumor vasculature have not been answered.

Gene therapy represents an interesting alternative for the effective delivery of antiangiogenic therapy, and a number of studies (9–16) have demonstrated that a gene therapy-based antiangiogenesis approach is an effective means of reducing tumor growth in animal models. Advantages of gene therapy over the direct administration of the inhibitors include the localized delivery and sustained expression of the antiangiogenic molecules, the ability to inhibit multiple angiogenic pathways with the delivery of more than one transgene, the generation of properly folded inhibitor molecules, and the potential for decreased cost (8,14).

Endostatin, a 20-kd fragment of collagen XVIII with demonstrated antiangiogenic activity, is being tested in phase I clinical trials as a protein infusion for various cancers. The gene for this protein is an ideal gene therapy candidate because the recombinant protein is difficult to produce and appears to be safe when delivered to the patient for an extended time. Endostatin has been tested in murine tumor models, with varying success, by gene therapy delivery vectors, including adenoviral vectors, adeno-associated viral vectors, in vitro transfections, polymerized plasmids, and DNA cationic liposomes (9–13,15).

In this issue of the Journal, Feldman et al. (16) present a study in which they retrovirally transferred endostatin into a transformed murine liver cell line and studied tumor growth in both subcutaneous and intraperitoneal locations. They found that endostatin gene transfer led to the expression of functional endostatin and inhibited tumor growth.

This study highlights a number of important points to consider in antiangiogenesis gene therapy. First, there is no correlation between efficacy and the level of endostatin in the serum. For example, in the study by Feldman et al. (16), there was a high degree of efficacy but no increase in serum levels of endostatin and only a modest (39 versus 11 ng/ml of protein) increase in endostatin levels in tumor lysates. By contrast, adenoviral vectors can deliver between 1 and 10 μg/mL of circulating endostatin, with generally modest effects on tumor growth (9,15). One possibility for this latter observation is that effective inhibition of endothelial activity is dependent on both the ability to deliver endostatin to the right location and the response of the endothelial cells within the local tumor microenvironment. Second, a lack of understanding of the mechanism(s) of action of endostatin has greatly hindered current efforts to improve its efficacy in vivo. In this respect, two recent studies (17,18) provide compelling data that a primary action of endostatin is the disruption of appropriate interactions between cell surface receptors and extracellular matrix proteins. Currently, with the mechanism of action of a large number of putative antiangiogenic molecules unclear, any efforts to translate these research findings to clinical therapies may be premature. Finally, the study by Feldman et al. (16) is of interest because retroviral vectors, which have been used safely in many human gene therapy trials (19), can potentially be used for the treatment of disseminated tumors of the peritoneal cavity. However, retroviral vectors only transduce dividing cells, which may limit their in vivo use. Such limitations highlight the continued need for improved gene therapy vectors to overcome the current problems associated with various gene therapy vectors, including immune response to the vector, transduction efficiency, delivery of the gene to the target tissue, and controlled gene expression by the vector.

The general area of angiogenesis modulation is an exciting one, and gene therapy directed to inhibit angiogenesis can benefit from the more advanced gene therapy efforts to promote neovascularization (8). The progress by several groups in using gene delivery for “therapeutic angiogenesis” is substantial, given the limited progress of gene therapy in other fields (8,20,21). Patients treated with VEGF and fibroblast growth factor (FGF) in phase I/II studies showed improvement in their target tissue vascular density and in their clinical symptoms (20,21). The early indications of success suggest that modulation of angiogenesis via gene therapy is reasonably safe and effective and offers considerable insight into the criteria for successful antiangiogenesis therapy. First, effective therapeutic angiogenesis is based on the considerable knowledge of how VEGF and FGF work. Second, it is likely that multiple factors will be necessary to generate vessels that are stable over time. Third, it remains unclear what is the best vector system to deliver optimal therapy. For researchers to realize the considerable advantages of antiangiogenesis gene therapy, it will be necessary to develop better vector platforms. Additional challenges for antiangiogenesis therapy are to understand how angiogenesis inhibitors function, how tumor vessels differ from normal blood vessels, and how to target tumor vessels with appropriate combination therapies.

References


