Gene Therapy

Anti-Angiogenesis

Under normal conditions, the growth of new blood vessels takes place during embryogenesis, wound healing, and the menstrual cycle. Neovascularization or angiogenesis is also a component of several disease processes, including malignancy, diabetic retinopathy, and inflammatory arthritis.

Current concepts of the process of angiogenesis were suggested in the early 1970's by Judah Folkman of the Children's Hospital in Boston, who determined that tumors would not grow to more than 1 mm^3 in the absence of a blood supply and hypothesized that tumor growth could be stopped if neovascularization could be arrested.

Many cell types contribute to angiogenesis, but the endothelial cell is the final target and determinant. It appears that there are several discrete angiogenesis factors that are involved in new blood vessel development. These include secreted factors, cell surface receptors, and extracellular matrix glycoproteins. Different combinations of molecules appear to be involved, depending on the particular physiologic or pathologic condition.

Endogenously produced angiogenesis inhibitors likely have a homeostatic role. They are often secreted in conjunction with proangiogenic molecules, and they normally function to prevent uncontrolled angiogenesis. Implementation of therapeutic anti-angiogenesis strategies would have its greatest utility in situations where angiogenesis contributes to unwanted active tissue growth.

Gene therapy seeks to permit direct control over the homeostatic balance and thus to suppress angiogenesis in a controlled manner. At present, the field of anti-angiogenesis gene therapy seeks to systematically (1) overexpress angiogenesis inhibitors, (2) block the expression of angiogenesis factors, and (3) target this therapy uniquely to areas of angiogenesis.

Angiostatin and endostatin are naturally occurring protein fragments that were discovered by screening for selective endothelial cell inhibitors that do not interfere with the growth of other cells. Both molecules are attractive candidates for anti-angiogenesis gene therapy because they are peptides that are freely diffusible and could affect all vascular beds where angiogenesis is active.

In the context of gene therapy, potentially any cell could produce angiostatin or endostatin if the appropriate DNA is provided to that cell. The molecules could diffuse locally as well as via the bloodstream and interrupt unwanted angiogenesis. Early reports indicate that in animal models, transient and stable expression of angiostatin and endostatin occurs when the genes are introduced by viral vectors.

Vascular endothelial cell growth factor (VEGF) is a potent inducer of angiogenesis and has been implicated as the direct causative agent of cancer angiogenesis, proliferative retinopathy, and the cyclic neovascularization associated with the menstrual cycle. Selective VEGF antagonists are thus important in the rational design of anti-angiogenesis therapies.

VEGF induces angiogenesis by binding to specific tyrosine kinase receptors on the endothelial cell membrane, resulting in endothelial cell division, migration, and secretion of proteases. Additionally, the endothelial cell is capable of secreting a truncated form of one VEGF receptor that potentially interferes with angiogenesis by competing with normal receptor binding and activation.

My colleagues and I have determined that the truncated receptor, when introduced in genetic form, is capable of significantly slowing tumor growth and enhancing survival. It may also be useful in the treatment of proliferative retinopathy and as a nonsteroidal contraceptive. We are currently examining this molecule in combination with other therapies as a means to eradicate cancer.

Several other potent endogenous inhibitors of angiogenesis have been identified and are being investigated, including interleukin-12, tissue inhibitors of metalloproteinases, and interferon-α. Blocking the expression of angiogenic factors, particularly VEGF, suppresses angiogenesis in several small animal models and has been accomplished using ribozymes, single-chain antibodies, and antisense RNA.

Interest has shifted lately to what are called dominant negative strategies, in which minor genetic modifications are made to molecules or their receptors. The modifications are intended to have a dominant and negative effect and to prevent normal signaling.

These approaches may be quite useful in arthritis or retinopathy, where the majority of involved cells can be treated. They would be more limited in cancer, where downmodulation of VEGF in a subset of cancer cells may provide transient growth arrest at best.

The benefit of using endogenous proteins for anti-angiogenesis gene therapy is the decreased potential for immunogenicity, which is par-

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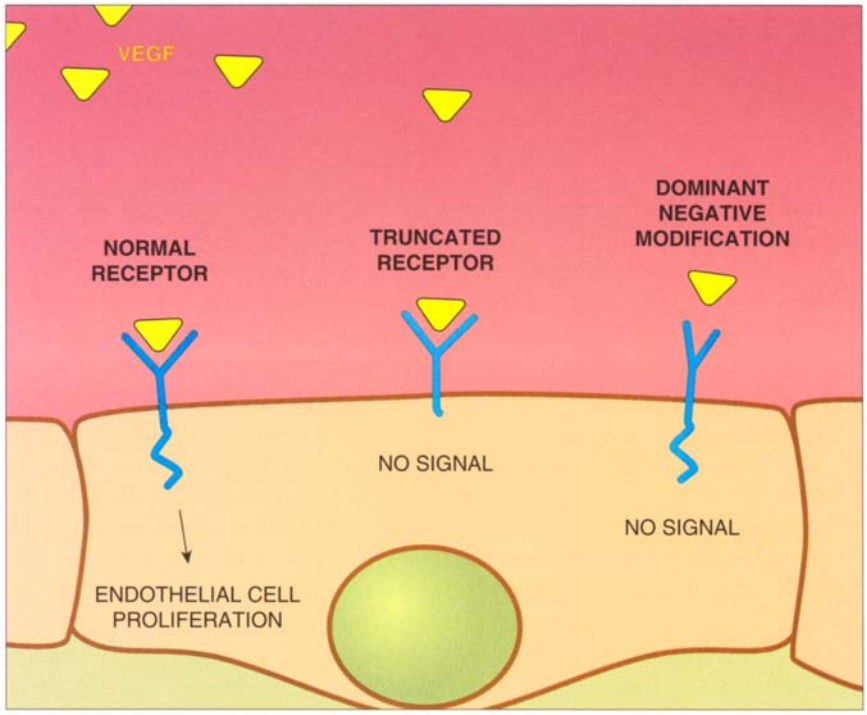

ticularly relevant because anti-angiogenic agents will need to be administered chronically. Nevertheless, anti-angiogenesis gene therapy approaches employing synthetic molecules are potentially useful. In particular, secreted single-chain antibodies that target important angiogenic mediators such as cell surface molecules and receptors are likely to be part of the gene therapy armamentarium.

Identification of proteins that are expressed predominantly by endothelial cells and preferentially in dividing endothelium is a useful step in devising targeting strategies for localized expression of potentially toxic molecules. Such peptides are found by inducing bacteriophages to express on their surfaces short amino acid sequences in every conceivable combination, thus establishing a phage library. From such a database, researchers can pick out unique sequences that bind only to endothelium, via unidentified endothelial cell surface molecules.

These peptides can be used to guide a genetic construct to its endothelial cell target with minimal binding to other cells. For gene therapy, the peptides can be fused with molecules carrying DNA or incorporated into a viral vector. Similar approaches have been applied using targeting antibodies to endothelial cell-specific receptors or growth factors and appear to be successful at achieving some restrictive targeting.

Endothelial cell-specific gene expression has been demonstrated by placing the therapeutic gene under the control of promoters that are active only in proliferating endothelial cells. This approach is most useful for the regulation of toxic genes, so that any cell could be transfected with the genetic construct but only dividing endothelial cells would be killed.

The optimal genetic vector or gene delivery system for anti-angiogenesis will depend on the particular process to be interrupted. Cancer will require chronic, persistent, and disseminated release of anti-angiogenesis molecules. The vectors for this purpose that hold the most promise are the adeno-associated virus and the hybrid adenovirus-retrovirus system. The latter is particularly attractive because it potentially can be injected as a single dose intravenously and will incorporate anti-angiogenic DNA into hepatocytes. The liver would then serve as a continuous source of anti-angiogenic protein.

For proliferative retinopathy or rheumatoid arthritis, the optimal vectors would be locally administered and stably integrated. Candidates are adeno-associated virus, the hybrid adenovirus-retrovirus system, or potentially the retrovirus vector alone. The drawback of using the retrovirus is its relatively low transfection efficiency, although it may be adequate for contained spaces such as the subretinal space or the synovial joint.

Many physicians and researchers are concerned about using angiogenesis inhibitors therapeutically because of their effect on wound repair. Studies to date suggest a minor delay in wound healing but no significant change in the tensile strength of the healed wound, probably because there are multiple convergent and possibly redundant angiogenesis signals. The issue will need to be addressed as angiogenesis inhibitors come into use.

Therapeutic expression of angiogenesis inhibitors could take the place of depot injections for contraception. Transient expression of an angiogenesis inhibitor could easily be achieved by monthly intramuscular injection of DNA in the form of a plasmid, which would not achieve significant levels of stable integration. Newer vector systems providing stable integration into host cell DNA can be controlled by administering tetracycline. Transfecting the gene for the truncated version of a VEGF receptor along with a promoter that is regulated by tetracycline would permit a patient to take tetracycline daily as a method of life-long contraception.

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