Gene Therapy

Vectors for Cancer Gene Therapy

Gene therapy was initially conceived as an approach for inherited genetic disorders. There are several thousand diseases for which replacement of a single deficient gene would be expected to rectify the pathophysiologic consequences of the deficiency state. The first human clinical trials of gene therapy were directed toward adenosine deaminase deficiency, which is an example of a single-gene disorder.

Against this background, it may seem ironic that almost half of the human gene therapy trials approved so far are directed against cancer. There is, however, a certain logic behind this statistic. Cancer is far more common than all of the single-gene defects added together, and the specific genetic lesions that cause neoplastic transformation are being defined to an increasingly greater extent.

Neoplastic diseases arise from an accumulation of genetic lesions, not just one, and these lesions are acquired rather than inherited. Cancer is properly considered a genetic disease in which the lesions accumulate over the life of the patient instead of being acquired at birth, and gene therapy makes sense for any disease that results from genetic lesions.

Another important factor driving the effort to apply gene therapy to cancer is the ineffectiveness of existing therapeutic approaches. For many of the major epithelial malignancies, including cancers of the lung, breast, colon, and pancreas, little survival advantage has accrued from the large number of experimental therapies tried over the past several decades.

Two main types of genetic lesions are associated with alterations in cell growth regulatory mechanisms. The first is overexpression or dysregulation of certain genes. Several dozen such "dominant oncogene" mutations have been defined, and in some instances, more than 95% of tumors of a particular histologic type can be shown to harbor such mutations, demonstrating their central importance to the process of neoplastic transformation.

Loss of function of growth-inhibiting genes can also result in the aberrant pattern of growth control that typifies neoplastic transformation. These are the "tumor suppressor" genes, and here again there is a sizable list of known mutations and associations with certain cancers.

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The expression of a gene can be inhibited or even abrogated by any of several elegant strategies that function at various levels of gene expression. For example, the so-called antisense method uses short, single-strand nucleic acid segments (oligonucleotides) that hybridize to target mRNA sequences and destabilize the mRNA. Ribozymes are oligonucleotides that also possess catalytic activity and act by cleaving their targets. Both strategies are in clinical trials for carcinoma of the lung and for leukemias. Another method is to inhibit the function of the protein product of a dominant oncogene by inducing the intracellular expression of an antibody.

Replacement of deficient tumor suppressor genes has been shown experimentally to revert the associated malignant phenotype in a number of instances. The results of a phase I clinical trial of *p53* gene therapy in lung cancer were reported in the September issue of *Nature Medicine* by Jack A. Roth and associates of the University of Texas M. D. Anderson Cancer Center.

When gene therapy was first discussed as an approach to inherited deficiency diseases, it was understood that certain practical goals had to be realized before human trials could be considered. First, methods had to exist for delivering therapeutic genes specifically to target cells. Second, the delivered genes had to be expressed at an appropriate level for therapeutic effect. Third, delivery and expression of therapeutic genes had to be safe for the target cell and, by extension, for the patient.

The same criteria are relevant to cancer gene therapy as well, but their relative importance is different. Precise regulation of the expression of a delivered gene may be crucial for correction of a metabolic abnormality, but for the eradication of tumor cells, this consideration is not likely to be of overriding importance. The issue of safety is also much more important in the context of long-term correction than in short-term cancer therapy.

In any case, the ultimate criterion of effective gene therapy is delivery of the therapeutic gene to the appropriate cells. Delivery vehicles, or "vectors," can transfer genes in two ways. The ex vivo approach isolates target cells outside the body, introduces the therapeutic gene, and returns the genetically modified cells to the patient. The in vivo approach delivers genes to target cells within the body.

Ex vivo gene therapy has practical advantages. The ability to ge-

netically manipulate target cells ex vivo generally allows more efficient gene transfer than the in vivo approach, and maintaining the modified cells ex vivo for a time before reimplanting them assures a certain level of safety.

In the case of cancer, ex vivo genetic modification of cells and their subsequent reimplantation, the "cancer vaccine" concept, is intended to provide immunologic help. Modified tumor cells that are better able to express cytokines or accessory molecules should function like vaccines by stimulating an antitumor immune response. This is at present the most common approach to human cancer gene therapy.

For a therapeutic gene to eradicate a tumor by altering the function of a dominant oncogene or a tumor suppressor gene, it must be delivered directly to the tumor in situ. Candidate therapeutic genes may have untoward side effects in normal cells, so the requirements for effective vectors are stringent. Moreover, the requirements are different for a localized mass or for a tumor confined to an anatomic compartment than for a disseminated metastatic cancer.

When neoplastic cells are wholly or largely confined to a single tissue such as the central nervous system or the ovary, in situ gene delivery should produce high local vector concentrations and thus relatively more efficient tumor cell transduction. The containment function provided by the anatomic compartment itself improves vector localization and discourages dissemination, with its possible consequence of adversely affecting normal tissue.

For these reasons, most cancer gene therapy strategies proposed to date have been directed at localized or regional tumors and not at metastatic disease. To achieve transduction of widely disseminated tumor cells, a vector would have to be efficient and specific, and it would also have to be administered intravenously. Targeted, tumor cell-specific delivery is an absolute requirement in this instance.

One promising concept is to use a DNA-ligand complex to deliver therapeutic genes via normal pathways. Recognition of the ligand component by its cognate receptor is followed by internalization of the receptor, ligand, and DNA. Vectors of this type would potentially allow specific gene delivery to target cells by virtue of specific receptor populations characterizing these cells.

Ligand-DNA complexes have indeed achieved cell-specific gene delivery to a variety of cell types in vitro. Unfortunately, these vehicles exhibit a lower gene transfer efficacy in vivo, apparently because they are destabilized by host serum factors. Possibly for the same reason, other nonviral vectors in general achieve exceedingly low gene transfer efficacies.

Adenoviral vectors are relatively more efficient in vivo and have been found to deliver genes effectively to lung, bladder, and liver. A variety of local and regional epithelial malignancies can therefore be approached. In addition, animal studies have shown that recombinant adenovirus can achieve high levels of in vivo gene transfer after intravenous administration. Of the available vector systems, adenoviral vectors appear to be able to accomplish the most effective in vivo gene delivery and have been the most widely employed for local and regional tumors.

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This vector system, however, cannot be used to deliver therapeutic genes to metastatic cancer cells, because the cell binding characteristics of the parent virus are promiscuous. Systemic adminstration would be expected to cause widespread transduction of healthy cells, not just tumor cells. Alteration of the vector's tropism so that the recombinant virion recognizes tumor targets exclusively would in theory result in a delivery system combining efficacy with specificity.

In this scheme, a virion would be required to recognize and bind to a cell surface receptor that it does not normally see. Further, after it binds to such a non-native receptor, the virion would need to retain its efficient cell entry characteristics, which are the reason for its use in gene delivery to begin with.

Several strategies have been employed to modify the tropism of recombinant retroviral vectors and achieve cell-specific targeting, but retroviruses lack the requisite high efficiency in vivo delivery characteristics. Still, it seems logical that some of the methods used for retroviral retargeting should apply to adenoviral vectors as well. My laboratory and others are at work on this task.

The impetus to build the "targetable injectable" vector system on a viral foundation is based on the inescapable fact that virus-based vectors are simply the most efficient gene transfer agents currently available. Millennia of evolutionary pressure have produced a highly developed gene delivery mechanism that cannot be matched by synthetic vector systems, which offer theoretical safety advantages but are otherwise far from ideal. The continuing development of "vector science" will allow the full potential of gene therapy to be realized for cancer.

DAVID T. CURIEL

Director, Gene Therapy Program
University of Alabama at Birmingham

"Gene Therapy," intended to explore issues and advances in the field, appears occasionally in SCIENCE & MEDICINE.