Hurdles and Hopes for Cancer Treatment

Kelly K. Hunt and Stephan A. Vorburger

For decades, gene transfer techniques have been used in the laboratory setting to introduce altered or foreign genes into cells in order to produce a specific, desired outcome. Since the 1980s, "gene therapy" has been studied as a possible means of modifying the genetic program of an organism to treat a specific disease. The results of clinical gene therapy trials began to appear in the 1990s, and today gene therapy is being actively explored as treatment for a wide variety of diseases (*1*). Treating genetic diseases often requires the replacement of a missing gene in cells that otherwise have a normal gene makeup.

In the laboratory, gene transfer is a powerful tool that can be readily adapted to meet the needs of an individual investigator. In the clinical arena, however, gene therapy is more complex and requires consideration of the interplay between the disease and the patient, along with any other conventional treatments being administered. Here we review the current limitations and future potential of gene therapy for the treatment of cancer.

Basic Challenges

One of the difficulties in advancing gene therapy technology from the laboratory to the clinic is that the perfect vector system has yet to be created. In clinical gene therapy, the ideal vector would be administered through a noninvasive route, transducing only the desired cells within the target tissue. This vector would then allow for expression of therapeutic amounts of the transgene product with desired regulation for a defined length of time. By definition, gene therapy should be able to replace, augment, or block gene expression toward a specific therapeutic goal. For the treatment of cancer, which often occurs as a result of multiple genetic changes, gene therapy may involve the replacement of missing tumor suppressor genes and/or the blocking of oncogenes or proinflammatory genes. No single vector system can meet all the strict criteria for each situation and be optimal for all potential gene therapy applications.

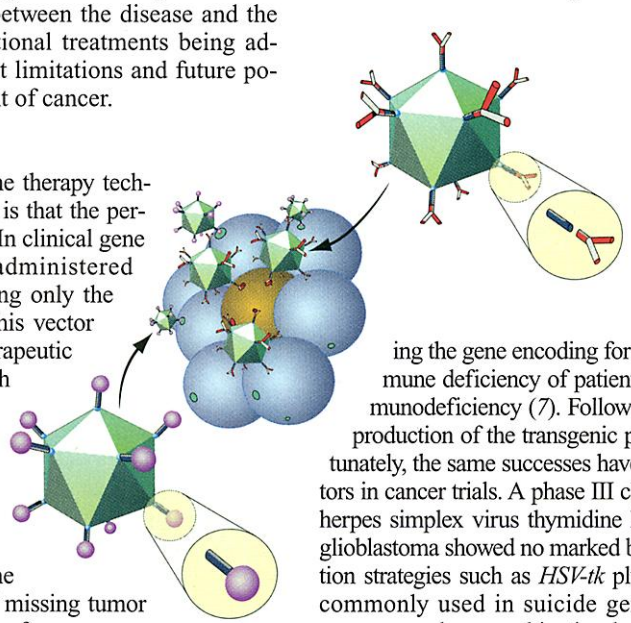
Another major restriction in treating cancer with gene therapy is the limitations in specifically targeting tumor cells, especially cells that have metastasized into the systemic circulation. Areas of continuing investigation in cancer gene therapy are (i) development of vectors to treat systemic disease and prolong gene expression, (ii) specific targeting of vectors to achieve tumor-selective binding, and (iii) clarification of the role of gene therapy among standard therapies for cancer.

Limitations of Current Viral Vector Systems

Viruses were first used in cancer therapy by inoculating a patient with either live or attenuated viruses to generate antitumor responses (*2*, *3*). Later, an increased understanding of the viral life cycle led to the use of genetically altered viruses that were replication-defective. These viruses could infect host cells and use their machinery to produce a desired gene product without subsequent viral replication. Viruses have been used extensively for gene delivery because of the efficiency with which they transfer their genetic material into cells. Adenoviruses and retroviruses are among the most frequently chosen vector systems; however, clinical successes with these vectors have been modest, and specific limitations of their use have been identified (*4*–*6*).

Retroviral vectors. Though relatively easy to design the integration of transgenetic material into the cellular DNA with these vectors, retroviruses infect only replicating cells and have relatively low transduction efficiency (measured as the percentage of cells expressing the transgene).

For clinical application, retroviruses are probably best suited for diseases that require lifetime production of a specific gene that has been lost because of a hereditary disorder. An excellent example of this approach was recently described by Hacein-Bey-Abina *et al.*, who demonstrated that ex vivo gene therapy with a retroviral vector containing the gene encoding for the common γ chain corrected the immune deficiency of patients with X-linked severe combined immunodeficiency (*7*). Follow-up observation showed that sustained production of the transgenic protein lasted up to 30 months. Unfortunately, the same successes have not been realized with retroviral vectors in cancer trials. A phase III clinical trial of retroviral delivery of the herpes simplex virus thymidine kinase (*HSV-tk*) gene to patients with glioblastoma showed no marked benefit (*8*). Enzyme-prodrug combination strategies such as *HSV-tk* plus the prodrug ganciclovir have been commonly used in suicide gene therapy approaches. This same enzyme-prodrug combination has been used with adenovirus vectors with more promising results.



Targeting of an adenoviral vector. Usually adenoviruses use the knob domain (rounded purple structure) to bind to the CAR, which is expressed on most normal epithelial cells (blue). By replacing the CAR domain with an antibody (red y-shaped structure) to PSA (orange) on the adenovirus fiber knob, the viral vector can be selectively targeted against tumor cells.

Adenoviral vectors. The adenoviruses efficiently infect many human cell types and yield high levels of transgene expression compared with other currently available vectors. They also have low pathogenicity in humans, causing few symptoms that are usually only mild symptoms associated with the common cold. Third, adenoviral vectors can accommodate relatively large segments of foreign DNA and are comparatively easy to manipulate using recombinant DNA techniques.

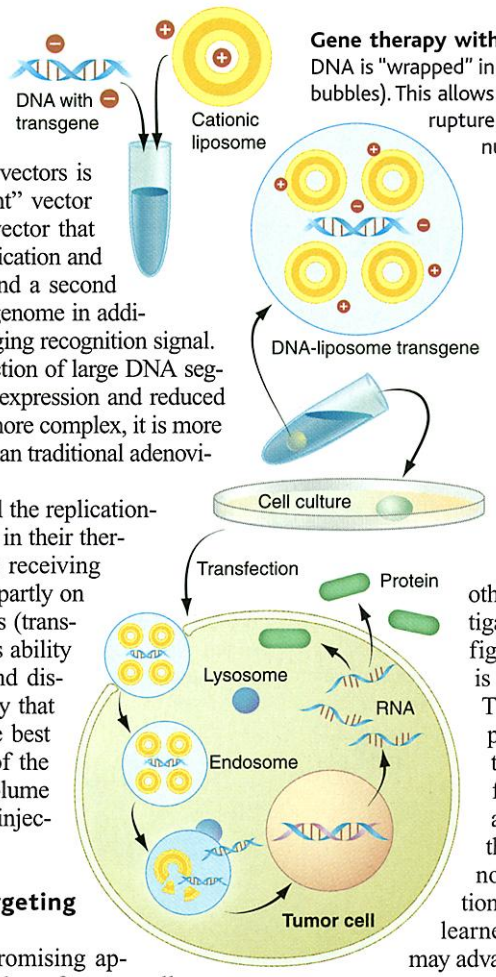
One of the drawbacks in the clinical application of adenoviruses is that the transgene is transported to the host nucleus but is not inserted into the host chromosome. Gene expression is only temporary, and therefore repeated administration of the adenoviral vector is required for continued transgene expression. In addition, the remaining viral genes are transcribed at a low level, resulting in early innate host cytokine transcription followed by additional immune responses. This, in addition to the formation of neutralizing anti-

The authors are in the Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA. E-mail: khunt@mdanderson.org

bodies, may contribute to a shorter duration of gene expression.

Newer-generation adenoviral vectors have been designed to address many of these shortcomings. One method of improving gene expression with adenoviral vectors is to use the "gutless" or "helper-dependent" vector system. This requires two vectors: one vector that contains the viral genes important for replication and has a defect in the packaging domain, and a second vector that contains the ends of the viral genome in addition to the therapeutic gene and the packaging recognition signal. This vector system allows for the introduction of large DNA segments (up to 32 kb) with improved gene expression and reduced toxicity. But because the system is much more complex, it is more labor-intensive and thus more expensive than traditional adenoviral vector–based gene therapy.

Replication-defective viral vectors. All the replication-defective viral vector systems are limited in their therapeutic efficacy by the number of cells receiving the transgene. This number is dependent partly on the uptake of the virus by individual cells (transduction efficiency) but also on the virus's ability to deliver the vector to a solid tumor and disperse it to individual cells. It seems likely that replication-defective viral vectors will be best for situations in which direct injection of the tumor is easily accomplished and the volume of tumor is small enough that repeated injections may not be needed.



Gene therapy with liposomes. The therapeutic transgene containing DNA is "wrapped" in a nonviral system such as cationic liposomes (yellow bubbles). This allows uptake of the DNA into the cell by endosomes. After rupture of the endosomes, DNA is freed and can enter the nucleus (orange), where the therapeutic transgene is transcribed into corrected proteins (green).

Improving Efficiency of Tumor Targeting and Transduction

Oncotropic viruses. One of the most promising approaches in terms of increasing the number of tumor cells transduced is the use of replication-competent viruses. Some naturally occurring viruses appear to selectively replicate within malignant cells and lyse them more efficiently than with nonmalignant cells. The replication-competent viruses include autonomously replicating parvoviruses, human reovirus, and vesicular stomatitis virus. Another example is the Newcastle disease virus, which has been shown to inhibit tumor growth in mice bearing human tumor xenografts.

Ongoing work includes manipulation of engineered oncotropic viruses, including conditionally replicating adenoviruses (CRADs). CRADs are adenoviruses that have been modified to replicate in human cells and lyse them if a specific genetic defect is present in the cells (*9*). An example is the ONYX-015 viral vector, which has shown promising results against cancer in phase I and II clinical trials alone and in combination with chemotherapeutic agents. The ONYX-015 adenovirus has been modified in the E1B region, which participates in the binding and inactivation of *p53* in cells infected with adenovirus. Adenoviral vectors that lack this E1B region cannot efficiently replicate because the p53 in the host cell induces an antiviral defense. However, in tumor cells that have mutations in the *p53* pathway ($p14^{ARF}$ or *p53*), this host defense cannot be activated, and the vector is allowed to replicate. Thus, ONYX-015 replicates only in cells with mutated *p53* or $p14^{ARF}$ and not in normal cells with wild-type *p53*.

Adenoviruses with tumor-specific promoters. Tissue retargeting or the use of tumor-specific promoters can also improve uptake of viral vectors. It has now become clear that the rate-limiting step for adenoviral infection is the binding to the primary receptor, the coxsackie-adenovirus receptor (CAR). Adenoviruses anchor at the CAR through the knob domain and are then internalized through interaction with integrins on the target cell surface. Tumor cell transduction can be greatly improved through modification of the knob domain by removing CAR-binding residues and inserting sequences that target alternative receptors. A tumor-specific approach would use conditionally replicating adenoviruses that have modifications in the knob domain containing an antibody to a tumor-associated antigen or a tumor-specific promoter (see binding figure, previous page).

Nonviral gene delivery systems. Liposomes and other nonviral delivery systems are also under investigation for use in cancer gene therapy (see liposome figure, left). At present, the efficacy of these systems is limited largely by poor transduction efficiencies. Though the opportunity for systemic delivery with predictable pharmacodynamics is attractive, long-term gene expression is not possible, and the need for repeated delivery greatly increases the costs associated with manufacture of the vehicle and the therapeutic transgene. Liposomal formulations are now common drug delivery systems for conventional drugs in medicine, and it is hoped that lessons learned from the use of liposomes in other diseases may advance liposome use for cancer gene therapy.

Integration and Future Potential

The goal of cancer therapy is to eradicate not only the primary tumor but also any systemic metastases that may reside in the body's organs and tissues. Direct injection of vectors containing therapeutic genes may result in regression of the tumor at the injection site but is unlikely to affect tumor cells at distant sites (*10*). Gene therapy for cancer may be most successful when combined with standard antitumor therapies, such as chemotherapy and radiation therapy. When used in combination with standard therapies, gene therapy may significantly enhance current treatment strategies.

The use of gene therapy for the treatment of cancer has many limitations but almost endless possibilities. As the technology advances, it will become possible to customize therapy on the basis of the histologic type of cancer and the genetic background of the specific tumor. Vector systems and therapeutic genes tailored for local or systemic delivery—either alone or in combination with conventional therapies—will add to our armamentarium of tools to treat cancer.

References and Notes

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11. The authors are grateful to S. Swisher for his critical reading of the manuscript and to S. Deming for her editorial assistance.