Gene Therapy

Transcriptional Targeting

One major hurdle of cancer gene therapy is the inability to effectively and selectively deliver the desired DNA to the target cancer cell within a patient. This hurdle has delayed the transfer of gene therapies showing clear effectiveness against human tumor cells grown on plastic dishes or in animal models to patients in everyday medical practice. This article describes the rationale of transcriptional targeting as an attempt to selectively target cancer cells and to avoid normal noncancerous cells.

Genes consist of a regulatory region and a coding region. The regulatory region or promoter region of a gene precedes the coding region and regulates the transcription of the coding region, which in turn allows for the translation into a protein.

Each cell in the human body contains the same genetic code, but the function of each cell is very different because of the selective transcription of different genes. For example, a prostate cell makes prostate-specific antigen, and a liver cell makes liver enzymes, but the converse is not true. Promoters play a critical role in regulating the different functions of each cell.

Promoters are complicated sequences of DNA that regulate what sections of the genetic code are activated in a given cell. Within a particular cell type, promoters regulate the transcription of genetic material into particular proteins that enable the cell to function. Understanding how promoters regulate normal cell function has led to the understanding that tumor cells produce unique proteins as a result of differential and specific promoter activation.

These molecular insights have allowed the design of cancer gene therapy approaches that use a unique promoter to regulate the production of a toxic protein. In a sense, these promoters can act as an Achilles heel for the particular cancer cell by allowing selective production of a toxin within the cancer cell but not in a normal cell.

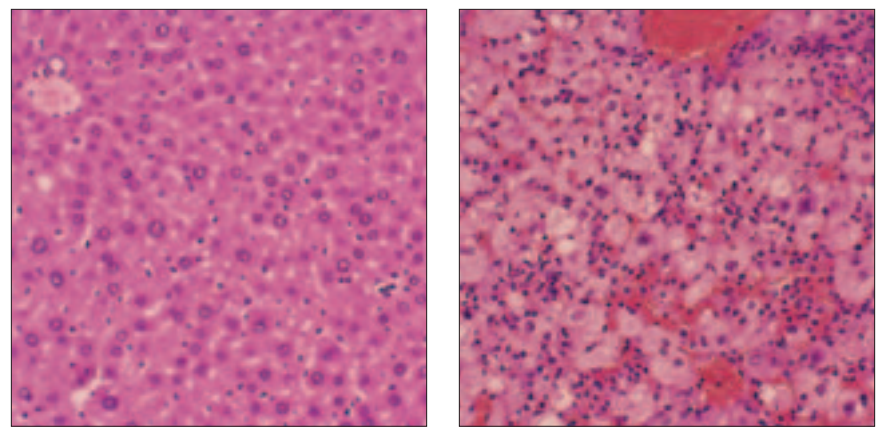
Numerous gene therapy approaches have used a recombinant adenovirus in modified form because of its ability to transfer genetic material to an infected cell (i.e., transduction efficacy). High transduction efficiency is critical for the successful development of human cancer gene therapy. The ability of the adenovirus to effectively infect and transfer its genetic material to all the cells to which it is exposed, although a benefit, also leads to some of the toxicities associated with adenoviral vectors.

Because most human cells express a receptor for the adenovirus, its infectivity is indiscriminant. Modifying the vector so that its receptor is a cell surface molecule found only on cancer cells is one approach to restricting infectivity and thus transduction. Another approach is transcriptional targeting, which can be used alone or in conjunction with altering the tropism of the adenovirus.

Transcriptional targeting can be achieved by the addition of regulatory portions of DNA that will function preferentially in the target tumor cell. Combining the high but indiscriminant transduction efficiency of the adenovirus with targeted transcriptional regulation of tumor-specific promoters allows targeted intracellular toxin expression.

For instance, prostate cancer cells at both primary and metastatic locations produce osteocalcin and prostate-specific antigen. Production of these proteins requires transcriptional activation of the defined regulatory portion of DNA (the promoter) that guides their synthesis. The same selective activation can be used to guide and transcriptionally regulate the pro-

Normal liver histology was demonstrated in mice receiving the Ad-OC-TK virus followed by ganciclovir (*left*), but mice given a universally active virus showed complete hepatic necrosis (*right*).



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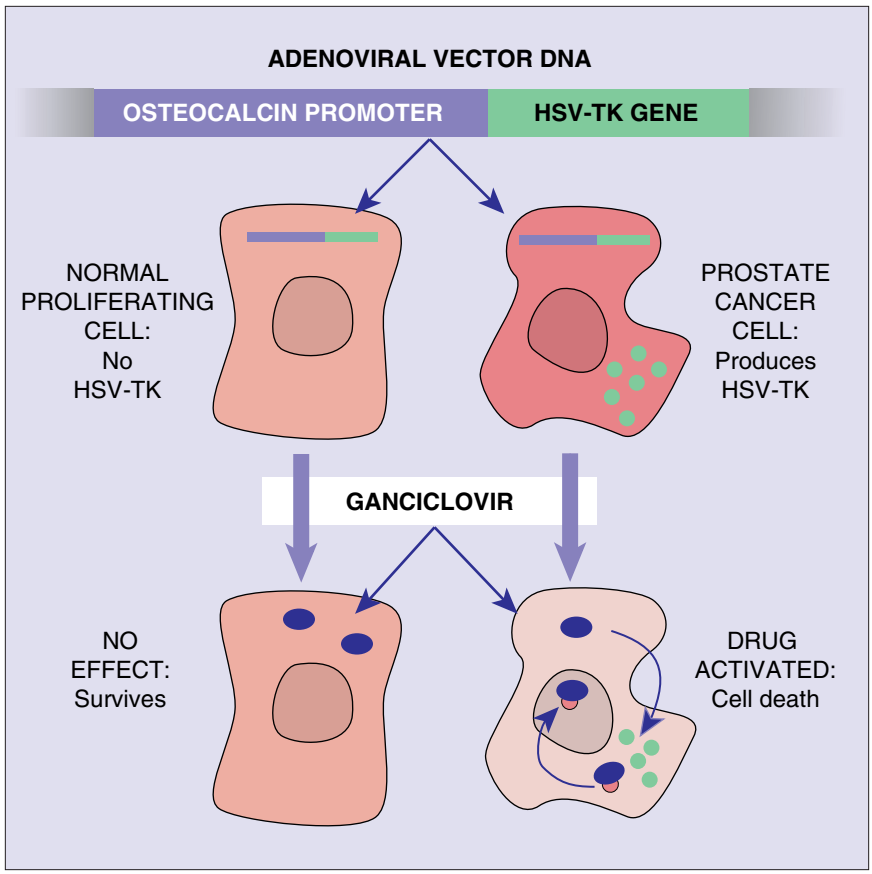

Proliferating normal cells in which the osteocalcin promoter is silent are not affected by ganciclovir because the prodrug remains inactive in the absence of HSV-TK. Prostate cancer cells produce osteocalcin, so introduction of an adenoviral vector that incorporates the HSV-TK gene and the osteocalcin promoter results in activation of ganciclovir and interruption of DNA replication.

duction of an intracellular toxin introduced by an adenoviral vector.

My colleagues and I have used this approach to successfully target prostate cancer cells growing in plastic dishes, in experimental animals, and most recently in men with metastatic and locally recurrent prostate cancer. Basically, our approach was to construct an adenovirus, Ad-OC-TK, which uses the osteocalcin promoter to achieve tumor-restricted transcription of the herpes simplex virus thymidine kinase (HSV-TK) gene.

HSV-TK converts an anti-herpetic prodrug such as ganciclovir into a guanine analogue that is incorporated into DNA in place of guanine during replication. By mimicking one of the four building blocks of DNA, the activated prodrug has the ability to block cell division. Using the osteocalcin promoter as the switch to turn on HSV-TK synthesis assures that inhibition of cellular proliferation will take place only in the prostate and not in other tissues.

To verify the safety of Ad-OC-TK, the outcome after its intravenous injection followed by ganciclovir administration was compared with injecting an Ad-CMV-TK recombinant adenovirus and then giving ganciclovir. Ad-CMV-TK contains the same HSV-TK gene but a universal CMV promoter, active in all cells infected. This comparison demonstrated the potential enhancement of the therapeutic window, with 90% mortality in the mice receiving the universal promoter and no mortality in the osteocalcin promoter group.

In the first human trial, 11 men with metastatic prostate cancer were treated with intralesional gene therapy, and the safety and biologic feasibility of this approach was confirmed. Toxicity was minimal despite evidence of systemic distribution after intralesional delivery of Ad-OC-TK. Lesion biopsies before and after treatment revealed a treatment effect and the presence of HSV-TK protein within the treated lesion.

We believe that transcriptional targeting of toxic gene expression can allow for increased safety, increased dosing, and potentially greater efficacy. Currently, we are seeking the necessary approvals to use the osteocalcin promoter to transcriptionally regulate the adenoviral replication process that will hopefully allow for increased potency of the virus while maintaining the safety profile.

THOMAS A. GARDNER

Department of Urology, Microbiology, and Immunology
Indiana University School of Medicine
Indianapolis, Indiana