Adenoviral Gene Therapy

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ABSTRACT
As of May 2001, 532 gene therapy protocols had been approved for evaluation in clinical trials; however, only five of those had been evaluated in phase III clinical trials. Among the most commonly used vectors for the delivery of genetic material into human cells are the adenoviruses. Remarkable progress has been made with these vectors in the last decade, but some shortcomings continue to challenge investigators. The newly acquired knowledge of the adenoviral life cycle and the positive outcomes from phase II clinical trials have led to the application of vectors engineered to selectively target tumor tissue under controlled promoters. The Oncologist 2002;7:46-59

INTRODUCTION
The perfect vector system for use in gene therapy would be administered by a noninvasive route, would target only the desired cells within the target tissue, and would express a therapeutic amount of transgene products with desired regulation for a defined length of time. No single vector system is likely to be optimal for all potential gene therapy applications and, thus, in the future we will see gene therapy with adapted vector systems and gene therapy in combination with standard antitumor regimens. Adenoviruses are among the most commonly used vectors for gene therapy, second only to retroviruses. This article reviews the ongoing clinical trials of adenoviral gene therapy for the treatment of cancer and discusses the current limitations and future potential of this approach.

ADENOVIRAL VECTORS
The adenoviruses have several features that make them well suited for use in gene therapy. First, they are ubiquitous: adenoviruses have been isolated from a large number of different species, and more than 100 different serotypes have been reported, some 43 in humans. Most adults have been exposed to the adenovirus serotypes most commonly used in gene therapy (serotypes 2 and 5). Second, adenoviral vectors rapidly infect a broad range of human cells and tend to yield high levels of gene transfer compared to levels achieved with other currently available vectors. Third, adenoviral vectors have low pathogenicity in humans: they cause few and only mild symptoms associated with the common cold. Fourth, adenoviral vectors can accommodate relatively large segments of DNA (up to 7.5 kilobase pairs [kb]) and transduce these transgenes in nonproliferating cells. Fifth, the viral genome does not undergo rearrangement at a high rate, and inserted foreign genes are generally maintained without change through successive rounds of viral replication. Finally, adenoviral vectors are relatively easy to manipulate using recombinant DNA techniques.
Adenoviruses are DNA viruses. They contain about 36 kb of double-stranded DNA. After entry into the nucleus, genes from the early region 1 (E1a and E1b) are quickly transcribed (Fig. 1). During the early phase of viral replication, four noncontiguous regions of the genome are expressed (E1 to E4) (Fig. 2). They serve in part as master transcriptional regulators, starting the process of viral gene expression leading to genome replication. After the onset of DNA replication, the major late promoter drives much of the viral transcription. Viral-encoded functions can be separated into cis and trans elements. Whereas the cis genes, such as those responsible for the origin of replication or the packaging signal that condenses the DNA (protein IX, ψ), must generally be carried by the virus itself, the trans genes can be complemented or replaced by inserted “foreign” DNA.

At least three regions of the viral genome can accept insertions or substitutions of DNA to generate a helper-independent virus: a region in E1, a region in E3, and a
short region between E4 and the end of the genome. In the first-generation vectors, the E1 region was removed to make room for the therapeutic transgene and to prohibit viral replication. However, even in the absence of E1 gene products, there was low-level transcription of the remaining viral genes, resulting in early innate host cytokine transcription followed by antigen-dependent immune responses. This resulted in a reduction of the period of gene expression because of cell-mediated destruction of the transduced cells [1-3]. This strong immune response was a major drawback of early adenoviral gene therapy and might well have contributed to the decrease of gene expression in a number of adenovirus gene-transfer studies in patients. Early trials studied the effect of suppression of the immune response through concomitant administration of low-dose etoposide, but this strategy had limited success [4]. Today’s second- and third-generation adenoviral vectors have deletions of various E1, E2, and E4 genes, because viral proteins encoded by these DNA sequences were shown to induce most of the host immune response. These new vector constructs have decreased toxicity and result in prolonged gene expression in vivo [5]. However, an important limitation in the use of recombinant adenoviruses has been the difficulty in obtaining efficient gene transfer upon a second administration of virus due to formation of neutralizing antibodies. This T-helper and B cell-dependent process that develops as a result of major histocompatibility complex (MHC) class II presentation of input viral proteins cannot be prevented directly by redesigning the vectors. In contrast to retroviral vectors, however, an inhibition by circulating complement was not noted in most recent adenoviral vector constructs [6].

Adenoviral vectors allow for transmission of their genes to the host nucleus but do not insert them into the host chromosome. Therefore, there is a low probability of disturbance of vital cellular genes or processes. On the other hand, the adenoviral-vector approach limits gene therapy to treatment strategies in which only temporary protein expression is needed. Because the viral DNA eventually disappears, treatments for chronic conditions, such as cystic fibrosis, would have to be repeated at specific intervals. However, if only short-term activity of a gene is needed—for example, to arouse the immune system against cancer cells or induce apoptotic stimuli—such nonintegrating delivery vehicles are desirable.

In preclinical settings, it has been shown that adenoviral vector DNA is expressed in liver, skeletal muscle, heart, brain, lung, pancreas, and tumor tissue [7]. When adenoviral vectors are given intravenously, most of the virus accumulates in the liver. Treatment close to reproductive organs, such as treatment for prostate and cervical cancer, has been shown to be safe. Even with replication-competent adenoviral vectors, which persist longer in the target tissue and liver, no offspring have shown germline transmission [8]. Table 1 displays advantages and disadvantages of other vectors commonly used today.

**Clinical Applications of Gene Therapy with Adenoviral Vectors**

Today, adenoviral vectors are used in suicide gene therapy, in gene-based immunotherapy, in gene replacement strategies, and in approaches that combine gene therapy with chemotherapy. In addition, treatment with replication-competent adenovirus vectors are showing promising results in clinical trials.

**Suicide Gene Therapy**

The fascinating concept of suicide gene therapy (also known as prodrug therapy) refers to the delivery into tumor cells of enzymes that metabolize systemically administered nontoxic prodrugs to locally active chemotherapeutic agents. Treatment efficacy is enhanced by the death of neighboring, nontransduced cells (bystander effect). In studies in different animals, adenoviral delivery of the herpes simplex virus thymidine kinase (HSV-tk) gene, which activates the prodrug ganciclovir, has been one of the most effective approaches in treating experimental brain tumors [9-14]. Recent phase I clinical trials, however, showed substantial toxicity at high levels of vector particles due to a multifactorial cellular and humoral immune response to the first-generation vectors [15].

**Gene-Based Immunotherapy**

Because cancer patients frequently develop both humoral and cellular immune responses to tumor-associated antigens, enhancement of these reactions by gene therapy has been proposed as another potential gene therapy
Dendritic cells have emerged as the key cell type responsible for initiating and controlling cellular immune responses. They are the best equipped and most powerful antigen-presenting cells (APC) and the only cell type able to stimulate naïve T cells [16]. Various methods have been developed to “prime” dendritic cells against tumor antigens. In tumor-lysate pulsing, the dendritic cells are collected from peripheral blood and fed with whole tumor antigen proteins from lysates. The dendritic cells take up and process the proteins, then present their antigenic epitopes through the exogenous MHC class II pathway and in MHC class I molecules. Another method that proved to protect mice from tumor spread was fusion of dendritic cells with tumor cells. This enabled the tumor cell to present antigens in the manner of an APC [17].

Yet another approach to the production of patient-specific vaccines involves transducing autologous tumor cells so that they secrete GM-CSF. The clinical potential of this approach has been demonstrated in metastatic melanoma, and in May 2001, a phase II study of vaccination with adenoviral vector in patients with non-small cell lung cancer (NSCLC) was under way (Table 2).

Several issues surrounding immunostimulating gene therapies remain to be resolved. One is the need for production of autologous dendritic cells under good manufacturing conditions that are contamination free. Currently, production of autologous dendritic cells requires expensive in vitro techniques. The optimal source of dendritic cells, the optimal method of antigen loading, and the optimal source and preparation of tumor antigen remain to be determined. The optimal immunization strategy also needs to be clarified, because dendritic cells can also induce immune tolerance, a highly unwanted effect in antitumor therapy [18].

### Table 1. Pros and cons of other common vectors in current clinical trials

<table>
<thead>
<tr>
<th>Virus</th>
<th>Characteristics</th>
<th>Advantages, future potential</th>
<th>Drawbacks</th>
<th>Clinical applications</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrovirus</td>
<td>Single-strand RNA, 8 kb exogenous DNA</td>
<td>Easy to design; integration into cell DNA; chronic infection; viral envelope allows assembly with other envelope protein: “pseudotyping;” “split construct” with two retroviruses: chance of reconstitution of replication-competent virus is minimal.</td>
<td>Infects replicating cells only; potential latent disease with malignancy or immunodeficiency; infection with replication-competent virus resulted in lymphoma in primates.</td>
<td>Most clinical studies use this vector; HSV-tk suicide gene for control of GVHD in allogeneic GVHD leukemia response; gene correction in (SCID)-X1 children.</td>
<td>[89-92]</td>
</tr>
<tr>
<td>Herpes virus</td>
<td>Complex double-strand DNA virus with 152 kb genome; persist after primary infection without reactivation even in immunocompromised host.</td>
<td>Large capacity (at least 30 kb exogenous DNA); enables infection of nondividing cells; defective mutants remain silent except for transgene expression; replication-competent virus remains latent in neurons; HSV amplicon vector as alternative to replication-defective vectors.</td>
<td>Lack of experience with long-term transgene expression; vector targeting is complex since mechanism involves multiple viral proteins.</td>
<td>Successful in multiple animal models for cancer and neural tissue gene therapy; promising for intradermal application to sensory neurons; good expectation for treatment of gliomas.</td>
<td>[93-96]</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>Human parovirus requires helper virus; discovered contamination in an adenoavirus preparation; allows 5 kb exogenous DNA.</td>
<td>No disease associated; no toxicity or inflammatory response; transduction to non-dividing cells also; splitting of transgene into two vectors enables double capacity due to vector genome linkage; column chromatography might make production easier.</td>
<td>Packaging capacity low; large-scale production is labor intensive; no internalization of DNA.</td>
<td>Early evidence of gene transfer and expression in human phase I trials; promising results for gene therapy for hemophilia B.</td>
<td>[97-102]</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>RNA virus; derived from HIV-1; expanded tropism (to lymphocytes).</td>
<td>Enables infection of nondividing cells; stable long-term transgene integration; new approach with human foamy virus (replication competent) offers better transgene expression.</td>
<td>Direct transduction is more sensitive to tissue barriers than other vectors; RNA-to-DNA step with reverse transcriptase allows no introns or internal polyadenylation signals; limited expression of transgene.</td>
<td>Value in first clinical trials to be demonstrated; efficacy for gene transfer into CNS in animals with retinal photoreceptor degeneration proved.</td>
<td>[103-107]</td>
</tr>
</tbody>
</table>

Abbreviations: GVHD = graft-versus-host disease; HSV = herpes simplex virus; CNS = central nervous system
Gene Replacement and Combinations of Gene Therapy with Standard Antitumor Strategies

Roth et al. showed that the delivery of wild-type \( p53 \) to cells with \( p53 \) mutations increased their radiation sensitivity [19]. Tumor cells expressing wild-type \( p53 \) are more sensitive to chemotherapeutic agents and radiation than are cells that lack functional \( p53 \). The heightened sensitivity of cells with wild-type \( p53 \) is thought to be attributable to their propensity to undergo \( p53 \)-mediated apoptosis (programmed cell death) after insult [20–23]. Abnormality and blocking of the apoptosis pathway are often associated with resistance of cancer cells to current antitumor treatment strategies. Gene therapy has the potential to overcome this block by re-establishing apoptosis-inducing regulators (E2F-1, wild-type \( p53 \), interleukin-6 [IL-6], interferon-\( \alpha \), Bax) or by transferring genes that induce cell suicide directly (e.g., FAS-ligand, Caspase-8, tumor necrosis factor-\( \alpha \)) [24–26]. In animal models, treatment with a combination of cisplatin and adenovirus-\( p53 \) led to enhanced apoptosis and suppression of tumor growth [27]. We have found increased apoptotic cell death in breast cancer cell lines treated with a combination of adenoviral vectors with the E2F-1 transcription factor gene plus paclitaxel and doxorubicin, indicating a strong synergistic effect [28]. Chemotherapy, in return, enhances expression of transduced genes from adenoviral vectors with a wide range of promoters, and radiation improves immediate transduction efficiency and the duration of transgene expression [29].

Treatment with Replication-Competent Adenoviral Vectors

Treatment with replication-competent adenoviral vectors specific to the target tissue has been considered as a means of enhancing the transduction and expression rate of adenoviral vectors. The major advantage of these cytolytic vectors is their potential to reach widespread metastases. Delivery of replication-competent vectors in combination with incorporated suicide genes or prodrugs could reduce systemic cytotoxic effects and inflammatory responses [30]. However, the mouse systems in which replication-incompetent adenoviral vectors have been studied so far might not be adequate models for replication-competent human adenoviruses. It is not known whether treatment with these replicating vectors results in DNA integration in the host cell genome more often than with their nonreplicative counterparts [31]. The prolonged persistence and higher titers of replication-competent

### Table 2. Ongoing phase II clinical trials of gene therapy with adenoviral vectors for treatment of cancer

<table>
<thead>
<tr>
<th>Indication</th>
<th>Gene delivered</th>
<th>Action</th>
<th>Combination</th>
<th>Route of administration</th>
<th>( \text{n of pts. to date} )</th>
<th>Investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and neck cancer</td>
<td>E1b del</td>
<td>Cytolysis</td>
<td>Chemo</td>
<td>Intratumoral</td>
<td>30</td>
<td>Link</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>( p53 )</td>
<td>Gene transfer</td>
<td>Chemo</td>
<td>Intratumoral</td>
<td>n/c</td>
<td>Logothetis</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>( p53 )</td>
<td>Gene transfer</td>
<td>Chemo</td>
<td>Intratumoral</td>
<td>78</td>
<td>Brea</td>
</tr>
<tr>
<td>NSCLC</td>
<td>( p53 )</td>
<td>Gene transfer</td>
<td>Chemo</td>
<td>Intratumoral</td>
<td>n/c</td>
<td>Dobbs</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>( p53 )</td>
<td>Gene transfer</td>
<td>XRT</td>
<td>Intratumoral</td>
<td>39</td>
<td>Dreicer</td>
</tr>
<tr>
<td>NSCLC</td>
<td>( p53 )</td>
<td>Gene transfer</td>
<td>IL-2</td>
<td>Subcutaneous</td>
<td>36</td>
<td>Swisher</td>
</tr>
<tr>
<td>Melanoma</td>
<td>MART/1 + gp100</td>
<td>Vaccination</td>
<td>IL-2</td>
<td>Subcutaneous</td>
<td>36</td>
<td>Haluska</td>
</tr>
<tr>
<td>Hepatic metastases from colon cancer</td>
<td>( p53 )</td>
<td>Gene transfer</td>
<td>Chemo</td>
<td>Intrahepatic</td>
<td>n/c</td>
<td>Venook</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>HSV-tk</td>
<td>Suicide with</td>
<td>XRT</td>
<td>Intratumoral</td>
<td>50</td>
<td>Butler</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>PSA replicative virus</td>
<td>Cytolytic PSA guided</td>
<td>XRT</td>
<td>Intratumoral and intravenous</td>
<td>n/c</td>
<td>Terris</td>
</tr>
<tr>
<td>NSCLC</td>
<td>GM-CSF</td>
<td>Cytokine</td>
<td>XRT</td>
<td>Intratumoral</td>
<td>22</td>
<td>Smith II</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>Mod B-7.1</td>
<td>Immuno-stimulation</td>
<td>IL-2</td>
<td>Subcutaneous</td>
<td>n/c</td>
<td>Anonia</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>( CD 154 )</td>
<td>Immuno-stimulation</td>
<td></td>
<td>Intravenous</td>
<td>n/c</td>
<td>Wierda</td>
</tr>
</tbody>
</table>

Abbreviations: Chemo = chemotherapy; del = deleted; HSV-tk = herpes simplex virus thymidine kinase; IL-2 = interleukin-2; n/c = not communicated; NSCLC = non-small cell lung cancer; PSA = prostate-specific antigen; XRT = radiotherapy

Source: Journal of Gene Medicine website (http://www.wiley.co.uk/wileychi/genmed)
vectors and their induction of the S phase of the cell cycle (where adenovirus-associated virus and retrovirus integrate) might be indicative of a higher DNA integration rate [32, 33]. A very recent publication reports a model for replication-competent adenovirus vectors based on mouse epidermal cells [34].

**Clinical Trials of Gene Therapy with Adenoviral Vectors**

An excellent source for data on clinical trials of gene therapy is the *Journal of Gene Medicine* website at http://www.wiley.co.uk/wileychi/genmed. The site allows users to scan investigator names and results for ongoing and recent clinical trials and has hyperlinks to frequently updated sites listing adverse effects of gene therapy. Most of the data presented in this review are derived from this database.

Figure 3 shows the recent increase in the number of clinical trials based on gene therapy strategies. Over the last 3 years, the number of gene therapy trials targeting cancer has increased by about 50% each year. At the moment, there are 96 ongoing clinical trials of adenoviral gene therapy for cancer.

**Overview of Trials by Phase**

Table 3 provides details of the six completed gene therapy studies using adenoviral vectors. All these studies were phase I clinical trials designed to evaluate the toxicity of treatment. Grade III toxic effects were very rare, and there were no instances of grade IV toxic effects. Even though the number of patients entered in these trials was low, it can be concluded that the intratumoral application of up to $1 \times 10^{12}$ adenoviral particles is safe.

### Table 3. Closed phase I trials of gene therapy with adenoviral vectors for treatment of cancer

<table>
<thead>
<tr>
<th>Indication</th>
<th>Gene</th>
<th>Route of administration</th>
<th>Dose</th>
<th>n of pts</th>
<th>Principal investigator</th>
<th>Country</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer, melanoma</td>
<td>IL-2</td>
<td>Intratumoral</td>
<td>n/c</td>
<td>25</td>
<td><em>Stewart</em></td>
<td>Canada</td>
<td>1997</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>IL-2</td>
<td>Intratumoral</td>
<td>$10^8$</td>
<td>9</td>
<td><em>Tursz</em></td>
<td>France</td>
<td>1997</td>
</tr>
<tr>
<td>Central nervous system tumors</td>
<td>HSV-tk</td>
<td>Intratumoral</td>
<td>$10^{11}$ pfu</td>
<td>13</td>
<td><em>Eck</em></td>
<td>U.S.</td>
<td>1996</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>p53</td>
<td>Intratumoral</td>
<td>n/c</td>
<td>n/c</td>
<td><em>Belani</em></td>
<td>U.S.</td>
<td>n/c</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>p53</td>
<td>Intratumoral</td>
<td>$10^{12}$ vp</td>
<td>1</td>
<td><em>Belldegrun</em></td>
<td>U.S.</td>
<td>n/c</td>
</tr>
</tbody>
</table>

Abbreviations: n/c = not communicated; pfu = plaque-forming units; vp = viral particles; IL-2 = interleukin-2; HSV-tk = herpes simplex virus thymidine kinase; pts = patients  
Source: *Journal of Gene Medicine* website (http://www.wiley.co.uk/wileychi/genmed)
Adenoviral Gene Therapy

Adenoviral gene therapies have been associated with very good tolerance and minimal toxicity in most phase I clinical trials. The most common side effects with vector treatment up to 1 × 10^11 plaque-forming units (pfu) (correlating with a viral particle load about 20 times higher) administered intratumorally or subcutaneously are reported to be shivering, light fever, chills, local pain, and diarrhea. Even in the case of adenoviral load detected in the liver, only one event of hepatotoxicity and no symptoms of hepatitis have been reported. However, in phase I clinical trials in which a combination of adenoviral HSV-tk and chemotherapeutic agents was used to treat advanced malignant intracranial tumors, more serious adverse effects have been reported, such as severe headache, relapsing seizures, and transient or persistent change of mental status.

In September 1999, an 18-year-old patient with ornithine- cytosine transferase deficiency died as a direct consequence of gene therapy at the University of Pennsylvania. A first generation replication-defective adenovirus administered through the hepatic artery was used to transduce the DNA sequence of wild-type p53 into the tumor cells of NSCLC, uses a Bayesian study design allowing patients to be evaluated for the phase III study at the same time.

Adverse Events Reported to Date

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Therapeutic Outcomes Reported to Date

Ironically, the first clinical success of gene therapy against cancer occurred with a vector that did not contain a therapeutic gene. The promising results came from a phase I and a phase II clinical trial started in 1996 by Khuri that used a replication-conditional adenovirus, dl515, later called ONYX-015 [30]. This adenovirus is modified in the early regulatory protein E1b region (normally allowing the virus to bind and inactivate the host p53 gene to promote its own replication [38]). This mutated adenovirus replicates in and lyses human cells with a defective p53 pathway, including cells with loss of p14ARF (a protein that can induce apoptosis by activation of p53) function. However, the modified virus cannot replicate in cells carrying wild-type p53 and an intact p53 pathway [33, 39, 40]. As p53 is mutated in 34%-70% of adenocarcinoma cells, ONYX-015 was developed as a tumor cell-specific therapeutic agent. The standard first-line treatments of squamous cell carcinoma of the head and neck induce a response in only 30%-40% of patients, and the recurrence rates are high. The combination of intratumoral application of ONYX-015 with cisplatin and 5-fluorouracil in 30 patients caused an objective response (at least 50% reduction in tumor size) in 19 patients and a complete response in eight patients. Tumors as large as 10 cm regressed completely. None of the tumors that responded had progressed after a mean follow-up of 5 months.

In a phase I/II study, the same vector was applied in combination with fluorouracil for the treatment of unresectable primary and secondary liver tumors. Intratumoral, intravenous, and intraarterial administration of up to 3 × 10^11 pfu showed two distinct cell death patterns of tumor cells: necrosis and apoptosis. This trial demonstrated only limited clinical response [41]. In another study from New Zealand, researchers investigated the induction of cell death by ONYX-015. They, too, found that the killing of the cells demonstrated two distinct patterns: A) a rapid killing in cells transfected with adenovirus containing the E1b region, through formation of a p53-E1b complex, and B) a delayed killing (after 10 days) in p53 mutant cells or cells treated with E1b-deficient adenovirus vectors such as ONYX-015, independent of the p53 status. These results support earlier reports that questioned the dependency of induced cell
killing by ONYX-015 on the p53 status of the tumor cells [42, 43].

One reason that might explain the lack of expected treatment success in some clinical trials with ONYX-015 is the fact that the absence of the primary cellular receptor for adenovirus, coxsackie-and-adenovirus receptor (CAR), on the tumor cells restricts the oncolytic potency of a replicating adenovirus vector. The potential therapeutic advantages afforded by viral replication could be negated by poor intratumoral spread of the viral progeny due to the failure to infect non-CAR-expressing tumor cells [44].

**IMPROVEMENT OF THE ADENOVIRAL VECTOR**

The combination of adenoviral vectors with other viral vectors and the targeting of adenoviral vectors to the tumor cells with controlled expression of the therapeutic transgene have reached the preclinical stage. These improvements finally will allow us to exploit the whole potential of gene therapy.

“Gutless” or “Helper-Dependent” Adenoviral Vectors

Recently, “gutless” adenoviral vectors—vectors that are devoid of all viral-protein-coding DNA sequences—have been developed [5, 45-47]. In this helper-dependent vector system, one vector (the helper) contains all the viral genes required for replication but has a conditional gene defect in the packaging domain making it less likely that its DNA is packaged into a virion. The second vector contains only the ends of the viral genome, therapeutic gene sequences, and the normal packaging recognition signal, which allows this genome to be selectively packaged and released from cells (Fig. 4). In addition to offering further reduced toxicity and prolonged gene expression in animals, this helper-dependent system allows the introduction of up to 32 kb of foreign DNA [48-51]. Processing of gutless adenoviral vectors is currently labor intensive, but it is anticipated that the adaptation of antibody-based purification systems that use affinity chromatography can further enhance the purity of vectors and their large-scale fabrication [52-55].

**Hybrid Vector Systems**

Hybrid vector systems that combine the highly efficient infection capacity of adenoviruses with the long-term genome-integrating potential of retroviruses and adeno-associated viruses are currently being tested. Such hybrid systems showed efficacy in murine cancer models [56]. Proof of the great potential of vector engineering is the construction of a two-vector adenoviral system that causes the recipient cell, after its coinfection with both viruses, to become a retrovirus-producing cell and spread a high-titer retroviral vector to neighboring cells [57, 58].

Tissue Targeting

Adenoviruses are taken up by epithelium-derived cell types, which makes adenoviral vectors suitable for solid tumors but less so for hematologic malignancies. In addition, this natural tropism makes it impossible to control the systemic delivery of adenovirus in vivo. Until recently, the mechanism of binding and internalization of adenoviruses was not known. Studies on cell receptors revealed that the CAR plays a crucial role in the infection of human cells, many of which express this 46-kDa membrane glycoprotein [59, 60]. After anchoring at the CAR by virtue of the knob domain (the CAR ligand domain at the end of the capsid protein of the virus), the adenoviruses achieve internalization through interaction of the capsid penton protein with integrins α5β3 and αvβ5 present on the target cells [61]. Studies using cells not expressing α5 integrins indicated that the presence of these integrins is obligatory for viral integration [62]. The identification of the mechanism of adhesion and uptake of adenovirus provided important leads for retargeting adenoviral vectors to different cell receptors.

Several authors reported experimental work on retargeting adenoviral vectors to non-CAR-expressing cells by removing critical CAR/binding residues in the fiber knob and inserting new sequences that facilitate binding to alternative receptors [63-67]. Michael et al. first demonstrated that new cell-type specificities could be achieved with a retargeted adenovirus fiber [68]. Very promising is the replacement of CAR binding fiber with bispecific antibodies. In this method, one antibody is directed against a penton base target sequence on the virus, and the other antibody is directed against the novel antigen on the cell [69, 70].

The potential of retargeted adenoviral gene therapy lies not only in its systemic application but also in the potential for new clinical applications in gene transfer to hematopoietic cells. Retargeted adenoviral gene therapy enabled successful transduction of foreign DNA into hematopoietic stem cells that lack CAR [71]. This technique will allow production of immunovaccines for residual leukemias, the induction of apoptosis in selected leukemic cell populations, or the expression of apoptosis-blocking proteins in hematopoietic stem cells preventing cell death during high-dose chemotherapy.

For retargeting approaches to succeed, not only must the vector bind to a specific target cell type, but also receptor-mediated endocytosis must take place. In many cases, manipulating endocytosis could be far more complicated than altering the tropism of the vector. The currently used in vitro models may not accurately predict biology relevant to the gene delivery in situ. For example, most immortalized tumor cell lines express CAR and are, therefore, readily transduced by adenoviral vectors, but these models do not necessarily reflect what happens at the tumor site in the clinical setting.
A retrospective analysis of primary epithelial neoplasms revealed profound alteration or deficiency of CAR in about 50% of cases [72-74]. These findings might, in part, explain the inadequate efficiency of adenoviral gene therapy in clinical trials. Some investigators, therefore, concentrate on CAR-independent gene delivery by adenovectors. Other
ways to achieve internalization without CAR binding is by physical complexing of the adenoviral particles [75-77], or by using recombinant fusion protein-retargeting complexes [70, 78, 79]. Because of the diversity of adenoviruses, of which there are more than 100 serotypes, changing only the capsid of the adenoviral vector can alter the tropism of the vector to the desired cells [49, 80].

Regulation of Promoter Activity

To set off transcription of the induced transgene, this DNA sequence has to have a promoter gene in front (upstream) on the DNA strand. Very promising preclinical studies have concentrated on regulation of the promoter. One of the common promoters for adenovirus transmitted genes is the promoter for cytomegalovirus, which can start the reading of the transgene at a high rate. The use of tumor- or organ-specific promoters, such as the carcinoembryonic antigen, prostate-specific antigen, and myelin basic protein promoters, has already proven to be effective in animal models, and will soon be investigated in phase I clinical studies [81, 82]. The limitation of these promoters is their need for a specific antigen expressed by the targeted tumor or organ tissue.

The idea of using an external switch to start and stop transgene transcription is very tempting. Of particular interest is promoter control by irradiation, a technique pioneered by Weichselbaum as early as 1992. This technique allows the systemic application of vectors, which can then be activated in a specified location by external irradiation [83, 84]. Moreover, combining radiotherapy with transfer of genes that induce apoptosis, has beneficial synergistic, not just additional effects. So far, radiation-sensitive promoters have shown relatively low expression rates and are not yet applicable in clinical trials. Another promoter displaying heat-inducible qualities might overcome this shortcoming. In a preclinical mouse model, hyperthermia-mediated intratumoral expression of IL-12 without systemic toxicity has been achieved at tissue temperatures between 39°C and 42°C [85].

Other Points of Interest

At present, adenoviruses are the most effective vectors with regard to percentage of transduced tumor cells and gene expression levels. In many apoptotic-transgene delivery systems, more cells undergo apoptosis than are genetically altered because of the killing of neighboring cells through, thus far, not fully understood mechanisms (the so-called bystander effect) and, therefore, transduction of all cancer cells is not needed. Nevertheless, to effectively shrink large tumors, transduction and expression rates must be increased. The insufficient gene expression rate might explain the lack of efficacy seen in some clinical trials of gene therapy. Transcriptionally active drugs like retinoic acid and histone deacetylase inhibitor trichostatin A, a potent inhibitor of histone deacetylases, enhance adenoviral transgene expression up to sevenfold [86]. This can be explained by recent advances indicating that chromatin remodeling is important for the active transcription of expressed genes [87, 88]. Condensed chromatin, in fact, negatively influences DNA accessibility to transcription factors, and histone acetylation is required for DNA opening and active transcription.

Future Directions

Most patients treated in clinical trials of gene therapy to date have been treated in phase I studies in which the primary objectives are the delineation of safety, toxicity, and feasibility. The trials of adenoviral gene therapy have shown this approach to be generally safe and promising. However, clinical efficacy has been demonstrated only with replication-competent, tumor-cell-specific adenovirus, indicating the need for further improvement of this treatment modality. The advances in adenoviral gene therapy to date have set the stage for clinical trials with new generation adenoviral vectors that exhibit less stimulation of the host immune system and that can be selectively targeted to the desired tissue. If careful and objective trials are conducted, we will witness the expansion of the armamentarium against cancer by a versatile instrument in the very near future.

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