

PLENARY LECTURES

Prospects for Gene Therapy for Hematological and Neoplastic Disorders

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During the past decade, important progress has been made in research on gene therapy for various human diseases.^(1,2) However, gene therapy should still be viewed as an experimental procedure as, to date, few patients have directly benefited from it. In this presentation, I will summarize the different vectors that are being used to deliver genes into cells, their application to hematologic and malignant disorders, and some of the problems that need to be addressed.

1. Vectors for Gene Delivery

Vectors for gene delivery can be divided into viral and non-viral types. Among the viral vectors, the most commonly used ones in gene therapy are the retroviral vectors. Other vectors undergoing testing include adenovirus and adeno-associated virus. For delivery of genes to the nervous system, the herpes virus is being advocated but is at present highly experimental. A modified human immunodeficiency virus (HIV) has also been proposed as a vector for gene delivery.

A. Retrovirus Vectors

The commonly used retrovirus vectors are based on the Moloney murine leukemia virus. For gene transfer purposes, two types of retroviruses are used: the ecotropic virus, which infects murine cells but not non-murine cells, and the amphotropic virus, which infects both murine and non-murine cells, including human cells. Most gene therapy protocols, therefore, utilize the amphotropic virus. Retroviral vectors are made in packaging cell lines that are derived either from murine cells such as NIH3T3 cells or from human cells such as 293, a human embryonic kidney cell line.⁽³⁾ Whereas most protocols so far utilize packaging cells derived from murine cells, recent studies suggest that viruses produced by murine cells are subjected to rapid inactivation by human complement.⁽⁴⁾ Therefore, it may be advantageous to make packaging cell lines out of human cells. A packaging cell line is constructed by integrating into its chromosome genes that encode the gag, pol and envelope proteins of the retrovirus. These genes could be driven by the LTR or by CMV promoters. If they are driven by the LTR, the packaging signal (ψ) of the virus is deleted. When a ψ^+ vector containing the gene to be inserted is transfected or infected into the packaging cell line, a virus carrying that gene will be produced to infect target cells.

A requirement for gene expression introduced by retrovirus is that the provirus must be integrated into the cell chromosome. To achieve integration, cell division is necessary as the virus can only cross the nuclear membrane during cell division. This requirement poses a hindrance to retroviral transduction of hematopoietic stem cells since division of stem cells for self-renewal appears to be infrequent. Thus, although retroviral transduction of hematopoietic stem cells has been demonstrated in mice, it has not been as successful in higher animals including humans.^(5,6) Since HIV can integrate into the

genome of non-dividing cells, vectors derived from this viral sequence are being explored for gene transfer.⁽⁷⁾

Because amphotropic viruses lack tissue specificity and have tropism for many cell types, most human experiments using retrovirus vectors are performed *ex vivo*. Tissues such as bone marrow or cancer cells are taken out of the subjects, cultured *in vitro*, transduced by viral vectors, and then replaced into the subjects. This process is tedious and may pose problems with transduction of hematopoietic stem cells, as stimulation of cell division with cytokines may direct the stem cells toward differentiation pathways. When applied to cancer cells, only those tumors that can be cultured readily, such as melanoma cells and renal carcinoma cells, can be treated in this way. Primary breast cancer cells, for example, are difficult to culture and can rarely be so treated. Hence, design of tissue-specific vectors may overcome these difficulties.^(8,9)

Great care must be made in the preparation of retrovirus vectors in order to minimize the potential for recombinatory events that lead to the production of replication competent viruses (RCR), as RCR viruses have produced lymphoma in monkeys.⁽¹⁰⁾ There are also potential risks associated with retrovirus integration, although none have been observed so far in humans, where retroviruses have been used in over 300 cases. Theoretically, integration could disrupt and abolish the function of an important gene, such as a tumor suppressor gene or a gene on the male X chromosome. Alternatively, since the LTR is a powerful enhancer, it may activate some oncogene. Although the LTR is a strong enhancer, it may eventually be inactivated *in vivo*, and gene silencing has been observed in long-term retroviral transduction experiments in animals.

B. Adenovirus Vectors

The adenovirus, about 36kb in length, can be made into replication-incompetent viral vectors by deletion of certain viral genes. Usually the E1 gene, which regulates other viral functions, is removed together with the E3 gene,⁽¹¹⁾ and newer vectors have been devised in which more sequences are removed and their gene functions supplied *in trans*.⁽¹²⁾ The E1 and E3 gene deleted virus can accommodate about 7kb of DNA sequence, while the newer vectors may accommodate more DNA. However, the titer of newer vectors may be lower than the first ones, which can reach as high as 10¹² plaque forming units (pfu). The high titer enables adenovirus vectors to be very efficient in transducing cells.

Unlike retroviral vectors, adenovirus vectors enter both dividing and non-dividing cells. The virus remains extrachromosomal and is not integrated into the cell chromosome. Hence, the vector may be lost on subsequent cell divisions and expression of the transduced gene lasts only for a defined period. Because a natural site of infection of adenovirus is the respiratory tract, adenoviral vectors have been used for gene transfer in cystic fibrosis.^(13,14) Adenovirus has also been tested as a vector to deliver genes into muscles to produce clotting factors and hormones.⁽¹⁵⁾ Because these vectors can be made in high titers, they have been used to transduce tumor cells to deliver agents that inhibit tumor growth.^(16,17)

Adenovirus vectors can be cytopathic and immunogenic. In preliminary trials of gene transfer in cystic fibrosis, high doses of the adenovirus vector were found to cause pulmonary inflammation in humans.⁽¹³⁾ Also, animal experiments have shown that the

second administration is usually ineffective as a result of antibodies produced against the virus.⁽¹⁸⁾ Experiments are ongoing to see if repeated administrations are possible using different strains of adenovirus to prepare vectors. Alternatively, the co-administration of an immunosuppressive agent with the adenovirus vector may allow repeated administration.⁽¹⁹⁾

C. Adeno-Associated Virus

Adeno-associated virus (AAV) is a small DNA virus that belongs to the parvovirus family, with a genome of 4.7kb. A great attraction of AAV is that, although most humans have been exposed to it, it is not known to cause any human disease. Hence, it may prove to be a completely harmless vector. To produce a vector, the viral genes *rep* and *cap* are deleted and their functions supplied *in trans*. Only the inverted terminal repeats are needed in the vector, which can accommodate about 4.5kb of gene.⁽²⁰⁾ The limited size is a disadvantage with some diseases such as cystic fibrosis because the functional CFTR cDNA is larger than 4.5kb. AAV is also prepared in the presence of adenovirus; hence, it is necessary to free the vectors of adenovirus by various means.

There was initially great enthusiasm for using the AAV vector to transduce hematopoietic stem cells since it was thought that the virus integrates into the chromosome 19q of non-dividing cells.⁽²¹⁾ However, it is now known that although the wild type AAV may preferentially be integrated into chromosome 19q without cell division, the recombinant virus, which lacks the *rep* gene, remains extrachromosomal and integrates infrequently without preference for chromosome 19.

AAV vectors can deliver genes into the central nervous system and are potentially useful for treating conditions such as Parkinson's disease. They can also introduce genes into muscles to produce hormones and clotting factors efficiently.

D. Non-Viral Vectors

Although naked DNA can introduce genes into cells *in vitro*, its efficiency is low *in vivo*. By coupling the DNA with a ligand such as asialoglycoprotein, preferential introduction of genes into liver cells has been demonstrated.⁽²²⁾ However, conjugated DNA-protein complex transduces cells quite inefficiently *in vivo*. To increase the efficiency in transduction, DNA has been coupled with cationic lipids, as liposomes have been shown to be non-toxic in mice, rabbits, and non-human primates. Again, although *in vitro* liposome transduction of cells are quite efficient, it is less so *in vivo*, as shown in human experiments where liposomes were used to introduce the cystic fibrosis gene into the nasal epithelium.⁽²³⁾

2. Gene Transfer into Hematopoietic Cells

Most protocols for introducing genes into hematopoietic cells are performed *ex vivo*. The first disease so treated was severe combined immunodeficiency (SCID) due to adenosine deaminase (ADA) deficiency.⁽²⁴⁾ Other immunodeficiency disorders in which the genes have been isolated could potentially be targeted, such as agammaglobulinemia, chronic granulomatous disease, and Wiskott-Aldrich syndrome. The long-term effectiveness of ADA gene therapy is not yet clear, as the two patients who have been so

treated are still on peg-ADA treatment. The three newborns who received their own cord blood cells retrovirally transduced with the ADA gene are being followed. There is an advantage in treating conditions such as ADA deficiency, as the T lymphocytes that have received a functional gene have a survival advantage. Other inherited disorders in which gene therapy is contemplated or in progress are the liposomal storage disorders such as Gaucher disease and Hurler disease. In these diseases, it is not known how extensive the gene replacement has to be before clinical effects are seen. Recently, the gene responsible for Fanconi anemia has been cloned. Gene therapy of this disorder is being planned.⁽²⁵⁾

Theoretically, sickle cell anemia and thalassemia appear to be good targets for gene therapy, but in reality, major problems need to be surmounted. Unlike treatment in enzymopathies, the transduced globin gene has to be expressed at a high level before the therapy is useful in patients. This may be achieved by including in the vectors the locus control region (LCR), which is responsible for the erythroid-specific and high-level expression of globin genes. However, the first attempts in incorporating the LCR or part of it into retrovirus vectors showed that the LCR imparts instability in retroviral vectors. Mutagenesis of the LCR and of some sequences in the globin gene could allow the vector to remain stable and the globin gene to maintain its function.^(26,27) The long-term expression of such vectors *in vivo* has yet to be determined. AAV has also been used to transfer the LCR and globin genes into hematopoietic cells. Since AAV vectors infrequently integrate into the cell chromosome, gene expression is expected to be transient.

Major efforts are being devoted to research in gene therapy for HIV infection.⁽²⁸⁾ Anti-HIV genes are transduced into hematopoietic cells either to kill the virus or prevent viral infection. These agents include dominant negative mutants to inhibit viral replication, antisense RNAs to abolish vital viral functions, RNA decoys to tie up important *trans* acting factors essential for viral replication, ribozymes to cleave the viral RNA, or intracellular antibodies to destroy important viral proteins. Since HIV undergoes frequent mutations, the long-term effectiveness of these approaches *in vivo* is not yet known.

3. Gene Therapy in Cancer

Retrovirus, adenovirus, and AAV vectors are being tested experimentally to treat cancer, utilizing several approaches to kill cancer cells. Early studies biopsied the tumors, cultured and expanded the tumor infiltrating lymphocytes (TIL) and then reinjected the lymphocytes into the patients. Genes such as tumor necrosis factor were also introduced into these lymphocytes in order to enhance the killing of tumors.⁽²⁹⁾ Other strategies to kill tumor cells are the introduction of a tumor suppression gene or anti-oncogene, the metabolic killing of the tumor cells, and the stimulation of autoimmunity against tumors.

For tumors that are due to mutations in the p53 gene, introduction of wild type or p53 are being tested. Also, antisense constructs directed against cMyc are being evaluated. Since these strategies require the transduction of the therapeutic genes into every cancer cell, it is doubtful that such efficiency can be achieved with the present vector techniques.

A second approach to gene therapy of cancer is the use of “pro-drugs,” the most common one employing the herpes simplex thymidine kinase (TK) gene.^(16,17) Herpes TK converts ganciclovir to the triphosphate form efficiently while the mammalian TK enzyme does not. Ganciclovir triphosphate is incorporated into the DNA, stops DNA synthesis, prevents cell division, and promotes cell death. Thus, inserting the herpes TK gene into cancer cells followed by administration of ganciclovir will kill cancer cells. Even cancer cells that are not transduced may be killed, as ganciclovir triphosphate can diffuse through gap junctions into neighboring cells. Hence, not every tumor cell needs to be transduced; it has been estimated in some tumors that as low as 10% tumor cell transduction can spread cell death to other tumor cells. Because killing occurs during DNA replication, it is an attractive strategy for treating cancer arising from organs such as the brain or liver, where the normal cells do not or infrequently divide.

Genetic modification of tumor cells to enhance immunity against tumor cells is another focus of intense research.⁽²⁸⁾ Cytokines such as IL-2 and GM-CSF have been shown in animals to promote autoimmunity against tumor cells. Costimulator molecules such as B-7 may also promote an immune reaction against the tumors. These agents serve as vaccines for antibody production against tumors and have been successful in animals. Their effects in humans have yet to be determined.

An indirect approach in treating cancer by gene therapy is to create hematopoietic cells that are resistant to chemotherapy in order to allow high-dose administration of a chemotherapeutic agent. One gene that is undergoing clinical trial is the multiple drug-resistant gene (MDR1), which functions by transporting drugs out of cells.^(30,31) The gene is introduced into bone marrow cells in patients undergoing autologous bone marrow transplantation, and the patients are then treated with high doses of taxol. Such protocols are being tested in patients with ovarian cancer where taxol is often used as one of the chemotherapeutic agents.

4. Conclusion

Although expectations for gene therapy in the treatment of diseases may have been too high, real progress has been made in many areas. Its application in certain genetic diseases may well be successful in the future. *In vivo* introduction of functional genes may be useful for the long-term delivery of drugs or hormones. For the treatment of cancer, gene therapy may or may not be curative but it could add to the armament of surgery, radiation therapy, and chemotherapy in prolonging and improving the quality of patients' lives.

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