Letters to editor

Gene transfer for the treatment of neoplasms

Neoplastma tedavilerinde gen transferleri

Biotechnology and gene technology are recognized by experts as invaluable and unique tools to find solutions to or improve many problems in health, agriculture and management of the environment, and are regarded as a driving economic force in the 21st century. Despite the great potential of gene therapy to become a new treatment modality in future medicine, there are still many limitations to overcome before this gene approach can pass to the stage of human trial. The foremost obstacle is the development of a safe, efficient, and efficacious vector system for in vivo gene application. Gene therapy is the transfer of deoxyribonucleic acid (DNA) into a cell to achieve the expression of a particular therapeutic protein. The ability to genetically alter a somatic cell offers intriguing therapeutic possibilities for the treatment of metabolic, infectious, and neoplastic diseases. Although clinical validation of the effectiveness of gene therapy is lacking, a variety of malignant disorders may someday be treated with gene therapy.

Methods of gene transfer: The gene that is transferred to a cell is termed the transgene. The efficient delivery of therapeutic genes and appropriate gene expression are the crucial issues for clinically relevant gene therapy. Gene transfer may be accomplished either directly in vivo or through the manipulation of cells ex vivo followed by re-instillation. A gene delivery vehicle, or vector, that may be of viral or non-viral origin, is generally used to carry the genetic material.

Viral vectors are the predominant agents used currently. Viruses have evolved the intrinsic ability to enter cells and control its machinery to support their own survival and replication and to promote expression of the transgene. Retrovirus, lentivirus, adenovirus, adeno-associated virus, poxvirus and herpesvirus are employed in more than 70% of clinical gene therapy trials. So far, viral vectors have been mainly used because of their inherently high transfection efficiency of gene. This ability made them desirable for engineering virus vector systems for the delivery of therapeutic genes. However, there are some problems to be resolved for the clinical applications, such as the pathogenicity and immunogenicity of viral vectors themselves. In most instances, a portion of the viral genome is deleted to render it replication defective to reduce pathogenicity. Many achievements have been made in vector safety, the retargeting of virus vectors and improving the expression properties by refining vector design and virus production.

Nonviral vector mediated gene transfer, compared to viral vector mediated one, is a promising tool for the safe delivery of therapeutic DNA in genetic and acquired human diseases. Nonviral methods use DNA either alone (naked plasmid) or in conjunction with liposomes. The efficiency and toxicity of the nonviral vectors used depended on the type of vector, the DNA/vector ratio, the type of cell, and the presence of serum. There is no clearly superior vector for all applications; each method has particular advantages and is suited to specific applications. Nonviral vectors, although less efficient at introducing and maintaining foreign gene expression, have the profound advantage of being non-pathogenic and non-immunogenic. Therefore, many research trials with nonviral vectors have been performed to enhance their efficiency to a level comparable to the viral vector. Liposomal formulations have been developed to improve the level of efficiency of plasmid-gene transfer. The advantage of liposomes (Lps) is that the potential toxicity of viruses is avoided. Vector administration can be repeated because of their low toxicity. Unfortunately, Lps have variable formulations, and reproducibility is troublesome. The initial enthusiasm for liposomal gene delivery in vivo has not been substantiated.

None of the available modalities is clearly superior for gene delivery since each has slightly different features and certain drawbacks. It should be readily attainable in high titers and be able to accomplish a high magnitude of transgene expression. The duration of transgene expression required varies with the clinical application. For neoplastic diseases, transient expression may suffice to stimulate an immune response or directly eradicate a tumor. In fact, an
immunogenic vector may actually serve as an adjuvant for an immune response. Second, it is often desirable for uptake of the vector to occur only in target cells (specific cellular entry-selective delivery) which is often cumbersome or impractical. Third, expression of the vector should be restricted to the target tissue. The last characteristic of an ideal vector is inducible transgene expression. Transcriptional regulatory elements that respond to endogenous factors or exogenous substances would likely provide more physiologic transgene activity and avoid the potential cellular toxicity of transgene overexpression.

**Therapeutic strategies for cancer:** There are three general approaches to the treatment of cancer by using gene transfer: immunostimulation, cytotoxicity, and gene correction. Most investigators have used immune-based strategies for human trials. The early results have not been impressive although most studies were designed to test the feasibility and safety of the techniques and vectors. Perhaps a combination of approaches in which, for example, an immunologic strategy using a cytokine gene plus the transfer of a suicide gene are used in conjunction with chemotherapy or radiation will prove most successful in cancer therapy.

Most human tumors are weakly immunogenic; therefore, methods that enhance an immune reaction (immunostimulation) are attractive. Most approaches attempt to generate a specific T-lymphocyte response. Unfortunately, in many animal models, treatment is successful only if it is administered prior to or early after tumor inoculation. One way to initiate an immune system is through the expression of cytokines in or near the tumor. In this way, the toxicity of systemic cytokine delivery may be avoided. A variety of immunostimulatory cytokines have received attention, including interleukins (IL-2,4,6,7, and 12), interferon γ (INFγ), and granulocytemacrophage colony-stimulating factor (GMCSF). The lack of immunogenicity of most tumors may be attributed in part to their inability to provide the necessary additional improvable signals to activate the immune system (antitumor immune responses) with adhesion/co-stimulatory molecules (B7-1, B7-2) have been shown to bind to receptors on T-lymphocytes. Gene transfer of tumor antigens has great potential for the treatment of neoplastic disease. A cancer vaccine is particularly attractive since the impediments of gene delivery are bypassed. A number of tumor antigens are under investigation. There are tumor-specific antigens such as the MAGE and GAGE antigens in melanoma.

Differentiation antigens exist normally and include those derived from tyrosinase, CEA, and prostate-specific antigen (PSA). Oncogene or tumor suppressor gene products may also serve as antigens. Mucins have also been targeted. The introduction of allogeneic major histocompatibility antigens into tumor cells is another strategy for inducing an antitumor response.

Several methods have been devised to accomplish direct tumor cytotoxicity using gene transfer. The most widely studied are “suicide” gene transfer and the use of replication-competent viruses. A “suicide” gene thymidine kinase gene of herpesvirus and cytosine deaminase gene are the examples of this issue. Another approach has been to enhance the effects of chemotherapy (e.g. transfer of the carboxylesterase gene). Replication-competent adenovirus and herpesvirus are being investigated and attractive agents for the treatment of tumors.

**Gene correction** involves restoring the normal genetic composition of a tumor cell. The major disadvantage of this approach is that gene transfer must occur in all or at least a high proportion of tumor cells to be therapeutic. Furthermore, the correction must be stable. In tumors with mutant p53, gene transfer of the wildtype gene has been developed and may have particular relevance for primary and secondary liver tumors. Another approach is to interfere with specific cellular messenger RNA.

In conclusion, although thousands of patients have been involved in clinical trials for gene therapy, using hundreds of different protocols, true success has been limited. A major limitation of gene therapy, especially when nonviral vectors are used, is the poor efficiency of DNA delivery to the nucleus; a crucial step to ensure ultimate expression of the therapeutic gene product. There are several difficulties in effective gene transfer that must be overcome; these include developing optimal vectors, targeting gene delivery, and regulating transgene expression. In addition, the immunogenicity of the vector and the transgene must be manipulated to avoid toxicity and to allow effective gene expression. The potential of gene therapy seems limitless. However, developed strategies and performed investigations should be regarded as preliminary, and therapeutic efficacy awaits clinical validation.

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