Viral gene therapy

Pablo Mancheño-Corvo and Pilar Martin-Duque


Cancer is a multigenic disorder involving mutations of both tumor suppressor genes and oncogenes. A large body of preclinical data, however, has suggested that cancer growth can be arrested or reversed by treatment with gene transfer vectors that carry a single growth inhibitory or pro-apoptotic gene or a gene that can recruit immune responses against the tumor.

Many of these gene transfer vectors are modified viruses. The ability for the delivery of therapeutic genes, made them desirable for engineering virus vector systems. The viral vectors recently in laboratory and clinical use are based on RNA and DNA viruses processing very different genomic structures and host ranges. Particular viruses have been selected as gene delivery vehicles because of their capacities to carry foreign genes and their ability to efficiently deliver these genes associated with efficient gene expression. These are the major reasons why viral vectors derived from retroviruses, adenovirus, adeno-associated virus, herpesvirus and poxvirus are employed in more than 70% of clinical gene therapy trials worldwide. Because these vector systems have unique advantages and limitations, each has applications for which it is best suited.

Retroviral vectors can permanently integrate into the genome of the infected cell, but require mitotic cell division for transduction. Adenoviral vectors can efficiently deliver genes to a wide variety of dividing and nondividing cell types, but immune elimination of infected cells often limits gene expression in vivo. Herpes simplex virus can deliver large amounts of exogenous DNA; however, cytotoxicity and maintenance of transgene expression remain as obstacles. AAV also infects many non-dividing and dividing cell types, but has a limited DNA capacity.

This review discusses current and emerging virus-based genetic engineering strategies for the delivery of therapeutic molecules or several approaches for cancer treatment.

Key words: gene therapy, virus, adenovirus, cancer.


INTRODUCTION

Most conventional drugs are proteins or small molecules that interact with them. Thus, they act at the protein level rather than at the underlying level of genes. An alternative approach is gene therapy, defined as the use of nucleic acids to repair the malfunctioning DNA sequence or to introduce a compensatory change that will restore the normal physiological functions of the cell. Gene therapy is one component of an emerging group of therapies collectively described as gene medicine. One form of gene medicine is the use of nucleic acids in the same manner as conventional drugs, though the target of the therapy is the mRNA produced by the altered gene rather than the protein. Also the use of DNA vaccines, which express antigens in the body, comes under the heading of gene medicine. All these new techniques are especially interesting for the treatment of diseases such as cancer, in which conventional drug treatment is limited.

Gene transfer technology is becoming increasingly integrated with cell therapy; a therapeutic modality based on the use of whole cells derived either from the patient or from an alternative source. Both gene and cell therapy perhaps represent the most promising of therapeutic strategies for long-term illnesses such as cancer.

Viruses have been the most commonly used vectors for gene therapy because of their high efficiency of
transduction into human cells, and about 70% of gene therapy clinical trials use viral vectors. We will review some of those vectors in this article.

GENE THERAPY

Gene therapy is a therapeutic strategy in which a patient’s cells are genetically modified in an attempt to cure a disease. There is an important distinction between somatic gene therapy, where modifications are introduced into somatic cells and are confined to the patient, and germ-line gene therapy where modifications are introduced into the cells that give rise to gametes, and can therefore be passed to subsequent generations. Only somatic gene therapy is currently permitted. While germ line gene therapy has been universally banned because of its ethical implications. Different types of somatic gene therapy approaches for cancer can be envisaged; their suitability depends on the cancer’s type.

Gene replacement therapy: the aim is to introduce a normal functioning copy of the nonfunctional target gene and then achieve the exogenous DNA to undergo recombination with the target and therefore replace it. Although this is the most straightforward way to correct genetic defects, the homologous recombination process is very inefficient in most cells.

Killing of specific cells: consists of eliminating certain population of cells, thus, it is very suitable for cancer gene therapy. The aim is to express within such cells a suicide gene whose product is toxic, or to direct the replication of viral vectors. Owing to the lethal effects of suicide genes, they must be directed to target cell types with great accuracy to avoid side effects.

GENE DELIVERY MECHANISMS

The overall strategy for gene delivery comprises the mechanism by which nucleic acids gain entry to the cell. Gene transfer mechanisms used for gene therapy are either viral or non-viral. Viral delivery, also known as transduction, involves the packaging of DNA (or in some cases RNA) into a virus particle. Gene transfer occurs by the normal viral infection route and is both efficient and cell selective. For this reason, viral delivery is the preferred strategy for in vivo gene therapy.

Viruses are pathogenic entities; therefore, steps must be taken to prevent the viral vector to cause disease. The usual method is to remove genes, which are essential for virus replication; this approach also increases the capacity of harbouring foreign DNA. The missing functions must be supplied from an alternative source. This may mean using a helper virus to make the missing gene products but in most cases a packaging line is used, i.e. a cell line that is stably transformed with the appropriated viral genes. Once the DNA is packaged, the defective virus can be isolated from the cell line. It can then infect its target cell and introduce its cargo of DNA or RNA, but it cannot replicate and cause disease symptoms.

Gene transfer ex-vivo or in-vivo

Ex vivo gene therapy involves transfer of cloned genes into cells grown in culture. Those cells that have been transformed successfully are selected, expanded by cell culture in vitro and then replaced in the patient. To avoid rejection by the immune system, the patient’s own cells (autologous) are used whenever possible. This approach is used for cells that are accessible for initial removal and that can be induced to engraft and survive for a long time after replacement. Examples include cells of the hematopoietic system, skin cells, etc.

In vivo gene transfer is the only option in tissues where the recipient cells cannot be cultured in vitro in sufficient numbers (e.g. brain cells) or where cultured cells cannot be re-implanted efficiently in patients. Tissue targeting is an important consideration. The gene transfer construct may be emplaced directly into the target tissue, or it may be injected into the general circulation but designed in some way so as to be taken up only by the desired cell type (fig. 1).

Vector integration

For achieving long-term expression it would seem desirable to integrate the foreign gene into a chromosome of the host cells, preferably a stem cell. Then, the construct is replicated whenever the host cell or its daughters divide (fig. 2).

However, integration carries certain problems and risks. Integration of most constructs occurs at random sites, and will be different in various cells of the patient. It may never be expressed, be expressed at undesirable low level, or may be expressed for a short time and then irreversibly silenced. Worse, the integration may alter expression of endogenous genes. The greatest worry is that insertion of a highly expressed construct may activate an adjacent oncogene, similar to the activation of Myc in Burkitt’s lymphoma and indeed, this is precisely what seems to have happened in two of the children successfully treated for severe combined immunodeficiency.12 Apparently, in at least one of the 108 modified T cells in each of the two children, a random retroviral insertion had activated the LMO2 oncogene. Eventually this clone outgrew all others, leading to a novel form of T-cell leukemia. It is likely that this experience will lead to a general rejection of randomly integrating vectors as tools for gene therapy.

For all these reasons, vectors that remain as extrachromosomal episomes seem likely to become the...