Gene Therapy : Current Concepts

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Introduction

Gene therapy involves the efficient introduction of functional gene into the appropriate cells of the patient in order to produce sufficient amount of protein encoded by transferred gene (transgene) so as to precisely and permanently correct the disorder. Transgene can be transferred into the target cell by physical, chemical and viral vectors. New organ transplant or tissue implants, human artificial chromosome, receptor mediated delivery and virally directed enzyme prodrug therapy (VDEPT) are other advancements in the field of gene therapy. Still gene therapy is in its infancy, and current gene therapy is primarily experimental, with most human clinical trials only in the research stages. However, attempts to regulate gene expression and to select a universal donor cell might become an effective weapon in modern medicine's arsenal to help fight diseases.Gene therapy is a novel approach to treat, cure, or ultimately prevent disease by changing the expression of a person's genes. In other words, it is a novel form of drug delivery that enlists the synthetic machinery of the patient's cell to produce a therapeutic agent (1). It involves the efficient introduction of functional gene into the appropriate cells of the patient in order to produce sufficient amount of protein encoded by transferred gene (transgene) so as to precisely and permanently correct the disorder (2). There are three main strategies in gene therapy.

1. Gene addition.
2. Removal of a harmful gene by antisense nucleotide or ribozymes.
3. Control of gene expression.

Gene therapy has various potential advantages over drug therapy like:-

1. Functional gene can replace a dysfunctional gene or deficient gene.
2. Transgene can result into continuous production of a therapeutic protein that normally has a short half life.
3. Gene therapy can be focused to a specific cell type to avoid potentially toxic systemic effects.
4. Gene therapy can improve patient's compliance and decrease cost of therapy on long term bases.

Types of Gene Therapy (2,3)

Gene therapy can be targeted to somatic (body) or germ (egg and sperm) cells. In somatic gene therapy, the recipient's genome is changed, but the change is not passed along to the next generation. In germline gene therapy, the parent's egg and sperm cells are changed with the goal of passing on the changes to their offspring. Germline gene therapy is not being actively investigated, at least in larger animals and humans, although a lot of discussion is being conducted about its value and desirability. Many people falsely assume that germline gene therapy already is being done with regularity. News reports of parents selecting a genetically tested egg for implantation or choosing the sex of their unborn child may lead the public to think that gene therapy is occurring. Actually, in these cases, genetic information is being used for selection. No cell is altered or changed.

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There are two main approaches to gene therapy (4,5)**:-**

1. Ex-vivo approach where the target gene is taken out from the body and transgene is introduced into the cell and the cell is reimplanted into the human body. This approach is only applicable to the cells that are capable of reimplantation inside the human body e.g. lymphocytes, fibroblasts, myoblasts, umbilical cord blood, stem-cells, bone-marrow cells, hepatocytes etc. (Fig. 1).

2. In-vivo approach where the transgene is introduced into the target cell inside the body .To better understand how human gene therapy works, it may be helpful to review some facts about human genes and gene delivery techniques.

**Fig. 1 : Myoblasts as gene delivery tools for muscles.**
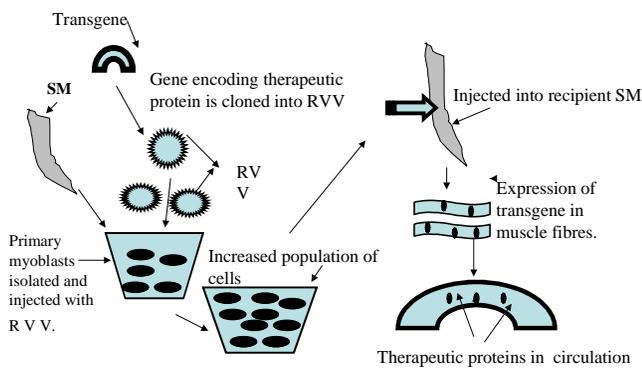
Picture showing ex-vivo approach for gene delivery using myoblasts as tartget cells and retro-virus as vector for gene delivery.

Pre-requisites for human gene therapy are (6):

1. Identification of the gene responsible for disease.
2. Cloning of the gene.
3. Identification of gene mutations.
4. Linkage of gene mutation to the pathophysiology of the disease.
5. Identification or selection of the gene transfer target cell.
6. Detection of gene expression and protein function.
7. Gene transfer efficacy and safety testing system. Transgene gene should have all the components necessary for transcription (formation of mRNA), translation (formation of therapeutic proteins) & RNA polyadenylation sequences to give stability to the transcribed RNA (Fig.2).

Fig. 2 : Retro-virus as vector for gene delivery.
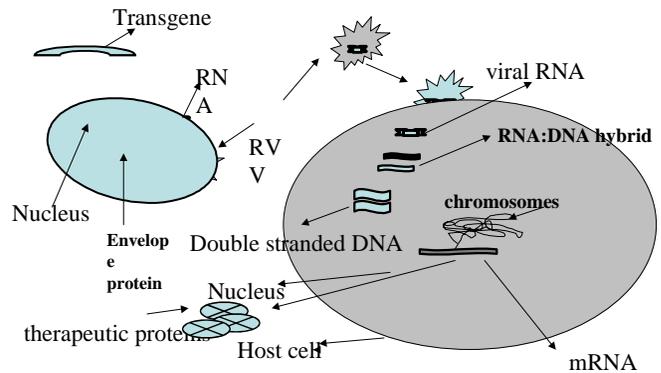
Picture showing entry of transgene inside the target cell by retrovirus vector (RVV) and conversion of modified viral RNA into DNA by reverse transcriptase enzyme, entry of the therapeutic DNA into the nucleus, followed by the process of transcription and transrelation i.e. formation of therapeutic proteins.

Methods of Gene Therapy (2,6,7)

An ideal vector should deliver gene to a specific cell type, accommodate foreign genes of sufficient size, achieve the level and duration of trans-genic expression sufficient to correct the defect and be non-immunogenic and safe. Presently we have following delivery tetechniques:-

Physical:- Parentral injections, micro-injections, aerosol, electroporation (high voltage current is passed to the target cell to produce pores on the cell surface through which transgene enters the cell) and gene guns.

Chemical:- Calcium phosphate, DEAE-dextran, liposomes and lipoplexes (for oral delivery of gene), surfactants and perflurochemical liquids for aerosol delivery of gene.

Viral vectors:- These are more promising system of gene delivery with various advantages over physical and chemical method:-

1. Gene transferred is more efficient and specific than physical and chemical method.
2. Multiple and repeated doses are required in case of physical and chemical method, whereas in case of viral vector even a single dose is sufficient.

However, viral- vectors have their own disadvantages of limited packaging capacity, unknown long term physiological effects because of co-transfer of extra-viral sequences, risk of immunological activation and

regeneration of wild type of virus. Retro-virus, adeno-virus, adeno-associated virus , lenti-virus and herpes virus are the commonly used vectors (Table1, Fig.2).

Table 1: Properties of viral vectors (2,7,)

Vector : Retro-virus

Advantages:- (1) Capacity of 8 kilobases, sufficient for most gene therapy applications; (2) Stable integration and lack of immunogenecity.

Disadvantages:- (1) Low litre; (2) Act only on the dividing cells; (3) Insertional metagenesis with risk malignancy.

Vector : Adeno-virus

Advantages:- (1) Target cell proliferation not required; (2) High titre & efficacy.

Disadvantages:- (1) Unstable integration & immunogenic; (2) Does not be repeated because of neutralizing anti-body formation.

Vector : Adeno-associated virus

Advantages:- (1) Stable integration; (2) Integrated into non-dividing cells.

Disadvantages:- (1) Small capacity of 5 kilobases; (2) Low titre.

Vector : Herpex simplex virus

Advantages:- (1) Prolonged expression of transgene because of its ability to produce latent infection

Disadvantages:- (1) Immunogenic; (2) Cytotoxic; (3) Difficult to develop.

Vector : Lenti-virus

Advantages:- (1) Acts both on dividing & non-dividing cells; (2) Stable expression.

Disadvantages:- (1) Safety concern over HIV derived virus; (2) Difficult production.

Others:- New organ transplant or tissue implants, human artificial chromosome, receptor mediated delivery and virally directed enzyme prodrug therapy (VDEPT).

New Organ transplant or tissue implants

New organs are the end result of autologous implant of genetically engineered cells within the the body new-organ transplant has been used in the treatment of lysosomal storage disease. Dr. Matapurkar and his colleagues from Maulana Azad Medical College, New Delhi have obtained US patent for a technique which enables the regeneration of damaged organ and tissues (8).

Human artificial chromosome (2)

It is a laboratory engineered chromosome that incorporates all the information necessary for its replication and independent seggregation. It forms a new chromosome in the host cell.

Receptor mediated delivery (6)

DNA is conjugated with a carrier molecule which act as a ligand to a specific receptor on the cell surface (target cell) in order to deliver the transgene to a specific cell. By this technique DNA of a considerable size can be transferred. Ligands for asialoglycoproteins receptors and transferrin receptors are used for delivery of transgene to the liver cells and ligands for polymeric immunoglobulin receptors are used to deliver transgene to human respiratory epithelium.

Virally directed enzyme prodrug therapy (VDEPT) (6)

It is based on the principle that a vector is expressed only in the specific cells (tumor cells) but not in the normal human cells e.g. herpes simplex thymidine kinase gene is introduced by retro-virus into the brain tumor cells, where it continuously increases production of thymidine kinase enzyme in the tumor cells. On administration of gancyclovir (drug), thymidine kinase enzyme activates it into its active form (gancyclovir triphosphate), which in-turn causes cell death by interfering with the DNA replication.

Therapeutic antisense and ribozymes (6,7,9)

Therapeutic antisense consists of the use of oligonucleotide sequences (15-25) that are complimentary to a gene or gene product that is desired to be inhibited and it inactivates the desired gene or gene product by forming triplex with the regulatory component of chromosomal DNA or by complexing a region of mRNA. There are several enzymes that are used to cleave foreign DNA sequence e.g. 6-methyl purine in glioma and pancreatic tumor. Fomiversen is the first antisense (21 base pairs) approved by FDA for use in human cytomegalovirus retinitis (locally intra-vitreal) for patients with immunodeficiency syndrome. Ribozymes are engineered RNA sequences containing enzyme activity capable of cleaving a specific mRNA sequence. This approach is being investigated in clinical trials in patients with HIV infection and malignancy.

Gene Regulation (10, 11)

Gene regulation theoretically means timing of gene expression and levels of the gene products to be optimized

on a case by case bases (fig.1). An ideal regulatable system should display five characters-specificity, efficiency, dose dependency, lack of immunogenicity and toxicity. A tetracycline (TC) inducible system was originally developed by Bujard and colleagues and it involves a TC transactivator (tTA), a hybrid factor composed of a bacterial tet repressor (tet R) and viral transactivator domain VP16. When bound to TC or doxycyclin tTA cannot bind to TC operator sequences and gene expression is not turned on. A variation of the system that allows expression of gene in presence of TC rather than absence is also under trial. To carry this strategy further, it is necessary to discover ways by which physiological stimuli can regulate the gene expression e.g. insulin production in response to blood glucose levels.

Universal Donor Cells (11, 12)

Limitation to the ex-vivo therapy is difficulty in targeting a large proportion of muscle tissue and the requirement that the syngenric myoblasts isolated from one patient be reinjected into the same patient to avoid rejection by immune response. Identification of muscle stem cells and encapsulation of myoblasts may abivate the requirement for a tailor made therapy, allowing allogenic cells that are invisible to the immune system to be used theoretically creating universal donor cells derived from the muscles of a single patient that could be implanted at ectopic sites in different patients for delivery of diverse products. However, the studies on bonemarrow stem cell for treatment of thalassemia and sickle cell anemia by Institute of Immuno-Haematology, Mumbai and neural stem cell studies for treatment of Alzeimer's disease by National Brain Research Centre, Delhi are under process in India.

Gene Vaccines (7, 13, 14)

Vaccination against both infectious and non-infectious diseases is possible using DNA encoded antigen. DNA vaccine has several advantages over conventional vaccines. It can stimulate both cell-mediated and humoral-immunity , lacks risk associated with attenuated pathogen and has relatively low productive cost. However, major limitations with use of DNA vaccine includes the relatively weak humoral response, risk of insertional metagenesis,

provocation of autoimmune response. Liposome encapsulated tumor associated antigen can serve as effective target for active immunotherapy against tumor. However, the results of phase II and phase III clinical trials of DNA vaccine for Malaria, AIDS and Rabies in India will become available in the next 2-3 years.

FDA and Human Gene Therapy (15)

FDA's Center for Biologics Evaluation and Research (CBER) regulates human gene therapies, which fall under the legal definition of a 'biologic'. Manufacturers of gene therapy products must test their products extensively and meet FDA requirements for safety, purity and potency before they can be sold in the United States. When a manufacturer is ready to study the gene therapy product in humans, it must obtain a special permission exemption from FDA before starting. This exemption is called an investigational new drug application or (IND). As part of the IND process, the manufacturer also must get approval from a committee of scientific and medical advisors and consumers (called an Institutional Review Board), which focuses on protecting persons who may participate in the study. Since 1989, FDA has received about 300 requests from medical researchers and manufacturers to study gene therapy and to develop gene therapy products. Presently, FDA is overseeing approximately 210 active IND gene therapy studies.

Hurdles in Gene Therapy (16-20)

The first hurdle is the gene delivery tool. Currently, the most common vectors are viruses. Viruses, while effective, introduce other problems to the body-toxicity, immune and inflammatory responses, insertional metagenesis and gene control and targeting issues. Another hurdle is understanding gene function. Of the estimated 100,000 genes, scientists know the function of a very few. Attempting gene therapy without knowing how everything works could address only some of the genes implicated in particular diseases. Likewise, genes may have more than one function. A third hurdle is multigene disorders (most genetic disorders involve more than one gene like retinopathy of prematurity). Most diseases involve the interaction of several genes and the environment like diet, exercise, smoking, and other

environmental factors. High cost associated with developing this novel technology, and regulations associated with human experimentation are also hurdles for researchers in this field.

Ethical Issues in Gene Therapy

What is normal and what is a disability or disorder? Are disabilities diseases? Do they need to be cured or prevented? Does searching for a cure demean the lives of individuals presently affected by disabilities? Is somatic gene therapy more or less ethical than germline gene therapy ? In spite of all issues human gene therapy is one of the most exciting and highly publicized areas in biomedical research.

Conclusion

The human genome project was initiated in October 1990 (21). More than 1.4 million single nucleotide polymorphisms were identified in the initial sequencing of the human genome with over 60,000 of them in the coding region of genes (22). In diseased tissues gene expression levels often differ from those observed in normal tissues, with certain genes being over or under expressed or new gene being expressed or completely absent . Gene therapy is in its infancy, and current gene therapy is primarily experimental, with most human clinical trials only in the research stages. Over time and with proper oversight, human gene therapy might become an effective weapon in modern medicine's arsenal to help fight diseases such as cancer , HIV/AIDS, diabetes, high blood pressure, coronary heart disease, peripheral vascular disease, neuro-degenerative diseases, cystic fibrosis, hemophilia A and B, and other genetic disorders (2, 6, 23). We can hope that the extensive animal studies and human clinical trials in 20th century may become a clinical reality in the first decade of 21st century.

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