KAWASAKI MED J VOL 21 : NO 1•2•3•4 1-18 (1995)

Human Gene Therapy : A Medicine of the Future

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Accepted for publication on August 16, 1995

ABSTRACT. The remarkable progress which has been made in the field of gene transfer technology during the last five years clearly indicates that gene therapy hold a considerable promise for the future. The modes of gene delivery to appropriate target cells have been narrowd down and out of both viral and nonviral methods of gene transfer, adenovectors have produced the best results. Most modern gene-therapeutic approaches are now based on the introduction of functional copies of defective genes into cells and a large number of target cells and tissues are being explored for various gene therapy applications. Successful gene therapy trials in a few selected genetic disorders have shown a long-term stable expression of transfered genes. Somatic gene manipulation although has shown clear advantages over germ-line therapy but it has not yet entered into clinical scale as the better understanding of somatic cell transplantation is still inadequate. A massive international efforts are being geared toward gene therapy research but there are still many technical difficulties e.g. isolation of genes and their regulatory regions, selection of best possible gene delivery systems etc, need to be overcome. All the indications are that the gene therapy will be perhaps a clinical reality in the early part of next century but the important question is safety of patients and the efficacy of gene transduction. Ethical considerations of gene therapy can not be overlooked. Opposition undoubtedly will occur in some cases because of potential to manipulate the genetic framework of future generations but eventually the luminous promise of gene therapy, probably will outweigh the potential pitfalls.

It is now clear that genetics has an important role to play in future medicine.[1] Increased understanding of molecular basis of human diseases has led to a number of potential gene-based therapies for various medical and surgical disorders. Recent advances in the DNA recombinant technology have produced the means for defining the role of specific gene products in the pathogenesis of human diseases. As a result of that bases of several human diseases have been cloned deciphered and a whole series of genes of clinical interest have either been or sequenced. The ability to characterize the disease in such molecular terms have already led to more precise and efficient clinical interventions in patients suffering from severe combined immunodeficiency disease (SCID).[2,3] Similarly, in a recent gene therapy protocol, the engraftment of hepatocytes transduced with LDL receptors in a patient with familial hypercholestrolemia have indicated that the treatment reduced the cholestrol levels.[4]

In the United State, at least 15 gene therapy trials are now in progress and almost 300 patients are recieving the treatment. More than 100 clinical protocols have been approved. It now appears that the ultimate goal of treatment of genetic diseases is repair of genetic defect i.e. GENE THERAPY : which means introduction of normal genes into the cells of at least one body organ to remedy or cure a single-gene defect. There are now very large number of human diseases that represent potential targets for this kind of manipulation and approximately 5000 human diseases have been classified as genetic.[5] Most modern gene-therapeutic approaches are now based on the introduction of functional copies of defective genes into cells. They really represent gene augmentation rather than correction. These approaches are therefore most applicable for dominant diseases in the cases where a dominant phenotype can be overridden by expression of the functional copy of a wild-type gene. Despite all successes in gene manipulation considerable difficulties still lie ahead as once introduced in cells, genes may not function at all, do so poorly or function at a slow rate. Moreover, inserting a single gene into human DNA, even if the gene could be precisely mapped to the correct chromosome, may still upset the neighboring genes. Several types of transfer vehicles are being tried for gene delivery system, these have some problems e.g. the viruses that now being tried to "carry" genes into cells have themselves became incorporated into the genes of recipient animals. We already know that insertion of viral genes can cause new genetic disorders (such as limb defects) and it could be of no surprise if cancer was another possible consequence. Therefore the major focus of all present trials of gene therapy is to find more efficient delivery to appropriate target cells, in vivo, as well as, in vitro, to establish gene therapy as an effective modality for common genetic disorders. Although as said above, there are still many technical difficulties to overcome, it is not far away that gene therapy will play an increasing role in clinical medicine and it will not be restricted to the management of single-gene defects but will have broad applications both in the genetics as well as in all areas of medicine. In this concise review, we will present recent advances in human gene therapy and its future prospects in medicine which might have its new name "molecular medicine" in the 21st century.

THE GENES

The gene is a piece in a piece of DNA at a locus on a chromosome and it encodes for a specific protein or several related proteins. The analysis of functional genes have shown that their coding sequences (exons) are joined by sequences of unknown function (introns) or intervening sequences (IVS) (Fig 1). The number and size of introns, which are often considerably longer than exons, varies from gene to gene. The DNA sequences extending on either side of specific locus are called flanking regions. The introns, the 5' end and 3' flanking regions and exons are transcribed into nuclear mRNA precursors.

Fig 1. Gene Structure

At the 5' end of the gene, there is a specific triplet (ATG) (initiation code) and at the 3' end, there is a termination (stop) codon (TAA, TAG, TGA) controlling protein synthesis. Genes also have blocks of sequences in their 5' flanking regions. The first one is about 20-30 bp upstream from the RNA initiation or CAP site called the ATA box, the second, called the CCAAT box, located about 70-90 bp upstream from the beginning of the gene.

GENE TRANSFER DELIVERY SYSTEM

The insertion of DNA into cells is perhaps not difficult but what happen after that is a matter of concern for many physicians and molecular biologists. To achieve a longterm stable expression of transferred gene, a large number of target cells and tissues such as peripheral blood lymphocytes, homeopathic stem cells, fibroblasts, hepatocytes, airway epithelial cells, skeletal muscle myoblasts and tumor cells are currently being explored for various gene therapy applications.[6-13] Apart from that the availability of a suitable disease model, it is important to determine whether a particular cell or tissue is a desirable target for gene therapy. Current research on gene therapy focuses on diseases of bone marrow and blood including sickle cell anemia, thalassemias and adenosine deaminase deficiency, each of which is a single-gene defect disorder. The genes involved are all expressed in marrow cells, which are relatively easy to manipulate.

The present need of gene therapy is "safe transfer techniques" and few people are likely to have serious objections whether the mode is somatic gene therapy or germ-line therapy. The differeces between the two is, that somatic (body) gene therapy requires the introduction of a normal recombinant gene into the somatic cells of a person with a heredity disorder to reconstitute specific gene products and their functions (Fig 2). In contrast, germ-line therapy would change the genetic make up of gamete-forming cells (sperm and ova) or of developing embryo. Embryonic gene transfer, in fact, likely to pose

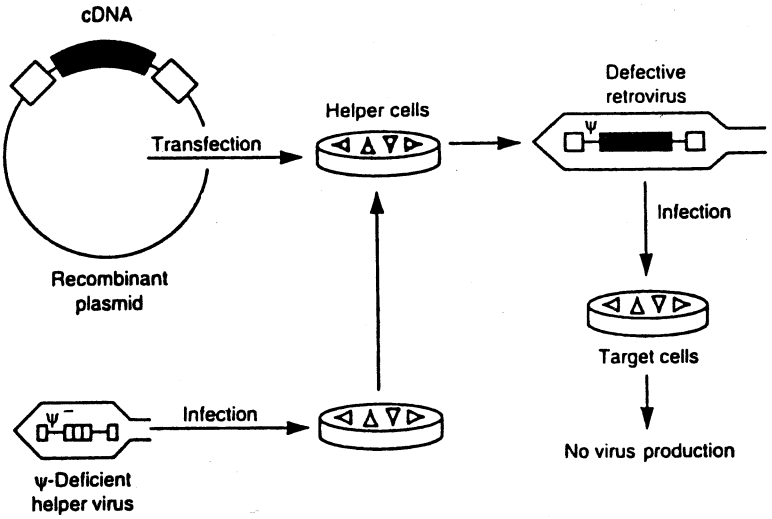

Fig 2. Strategies for retoviral vector mediated somatic gene therapy

less technical difficulties than somatic gene therapy as a gene that is successfully transferred or micro-injected into a single-embryo subsequently would be cloned into all of somatic cells and germ cells during embryogenesis.

MODES OF GENE DELIVERY

The choice of gene transfer method is critical to the successfulness of gene-therapy. The fact of the matter is that any gene transfer method should have a potent to transduce a high percentage of cells in the target tissues, thereby leading to high level, long term expression of the introduced gene. A large variety of both viral and non-viral methods of gene transfer (Table 1) have been developed. Non-viral transfer includes chemical or physical method such as transfection by calcium phosphate precipitation, electroporation, direct DNA injection, liposomal transfer or receptor mediated delivery.[14–17] The possibility of transferring and maintaining DNA by the use of extrachromosomal elements is also under evaluation.

TABLE 1. Methods Of Gene Transfer

Viral	Nonviral
Retrovirus	Direct injection of DNA
Adenovirus	Receptor mediated endocytosis of
Adeno-associated virus (AAV)	DNA (ligand-DNA conjugates).
Herpes Virus (HVS)	Liposomes (Lipofection)
Vaccina Virus	Ca PO_4 precipitation
	Artificial chromosome

Naked DNA is taken up inefficiently by cultured cells after it is precipitated in a calcium phosphate solution. There is also low expression of gene products after direct injection of naked plasmid DNA into skeletal muscle. However, since most gene-therapies will require more efficient techniques, other avenues have been developed to varying degrees. These include now receptor mediated endocytosis of DNA complexes to ligand, and viruses. Much efforts have so far have focused on a vector system to deliver the repaired gene into target marrow cells. The likely candidates are altered retroviruses which have complicated structure and life cycle (Fig 3). Their oncogenes not only are deleted but these altered viruses also lack genes coding for proteins (gag, pol, env) necessary to package new viruses and therefore can only undergo one round of infection. This would prevent the viruses from reinfecting when the manipulated cells are replaced in the body and since the red blood cells are thought to rise from a small population of stem cell clones, achieving nearly 100% infection of stem cells in the marrow sample may prove critical to successful therapy.

RETROVIRUSES

The retrovirus is a member of class of RNA viruses that propagates by conversion of the RNA into DNA by enzyme reverse transcriptase. These are adapted by evolution for the efficient delivery of their genome into cells, with integration into the host genome and a high level of expression of their internal

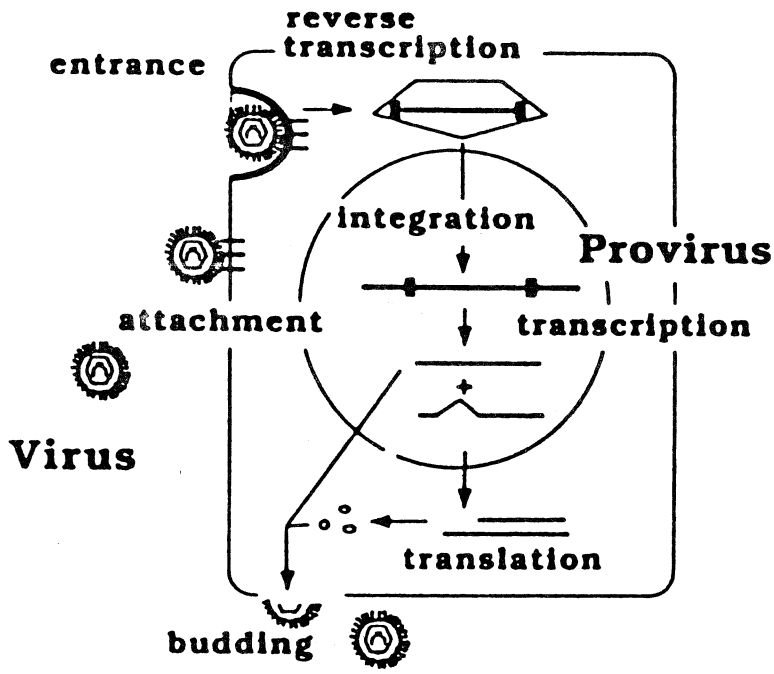

Fig 3. Structure and life cycle of a virus

sequences. After integration viral sequences transcribe full-length and splice RNAs. The spliced RNAs are translated into internal structural proteins of virion core and transcriptase or packaged into virion particles as new genomic RNA. The subsequent assembly and budding of virion particle from infected cells in non-lytic process.

Retroviuses have useful properties for exploitation[19] as vector, because (a) they cover a wide host range including avian mammalian and other animals (b) infection does not lead to cell death as infected cells produce virus over an indefinite period (c) viral gene expression is driven by strong promoters-these can be harnessed to foreign genes, and (d) in the case of murine mammary tumor virus, the promoter function can be switched off.

Retroviruses used for gene therapy are based on the Moloney leukemia virus (MuLV)[20] and are similar to the HIV virion in structure, containing long terminal repeat sequences at each end (LTR's), and coding regions for reverse transcriptase (gag), and viral capsid proteins (pol, env). The viral sequences can be removed from the RNA and replaced with foreign genes of interest, provided a packaging cell can encode the necessary proteins to make the viral capsid (Fig 4). The additional inclusions of a selectable markers such as neomycin phosphotransferase (Neo) allows selection of packaging cell clones which have incorporated the provirus DNA.[21] A packaging signal allows the foreign RNA to be packaged into the viral particles which bud from the cell membrane, however, since the mature viral vector is missing gag, pol, env regions, infection of target cells is a single event. The therapeutic vector RNA is converted into DNA and is integrated at random into the target cell, following which, all progeny will contain the integrated therapeutic gene.

Retroviruses are thus potentially an excellent vector choice for situations requiring long-term stable expression of a foreign gene. However, since they only infect dividing cells, these are difficult to produce in high titer, and are intrinsically unstable. Therefore much attention is being focused on other viral vectors.

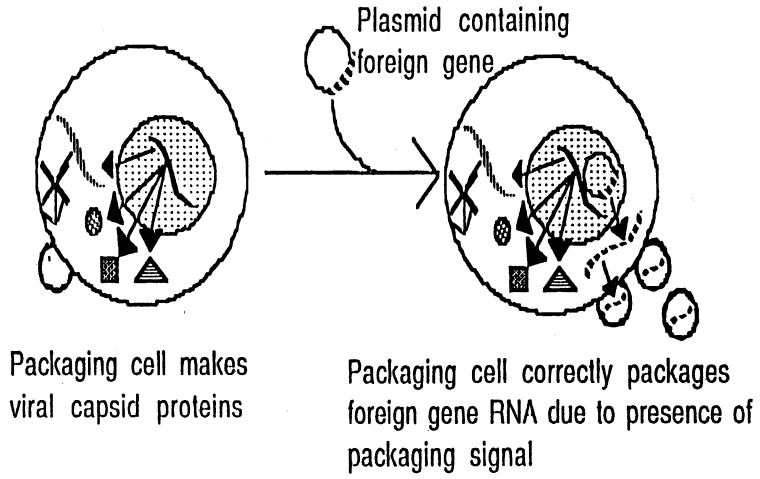

Fig 4. Retoviral packaging cell line

Retroviral mediated gene transfer is now clinical reality and most commonly used vectors are those derived from murine retroviruses.[22–24] From the murine retroviruses from which current retroviral vectors are derived, successful completion of viral life cycle with integration[25] into the host genome requires dividing cells. This is not true for HIV, which is able to infect nondividing cells such as macrophages. Thus, conventional retroviral vectors are unsuitable for gene transfer into postmitotic cells such as neurons and are not ideal for infection of tissues such as those in normal airway epithelium cells that have a low mitotic index. However, they are well suited for rapidly dividing cells since the transferred DNA is integrated into the host cell genome. Consequently, the progeny of originally infected cells will also carry the retrovirus.

ADENO-ASSOCIATED VIRUS

The adeno-associated virus (AAV) vectors share the property of retroviral vectors integrating into host chromosomes, preferentially into long arm of chromosome 19.[26] The AVA vector is a small double-stranded DNA virus of the parvovirus family and can only accommodate about 4.7 kb of DNA inserts. Further, it must be "rescued" by wild type adenovirus, yielding certain challenges to produce pure clinical stocks.[27]

ADENOVIRUSES

These are group of viruses responsible for respiratory infections like common cold, viral conjunctivitis, intestinal infections and latent infections which may cause some swelling of tonsils, adenoids and other glandular tissues

especially in children. Adenoviruses are double-stranded DNA viruses with a 36 kb genome. The Ela and Elb genes are deleted from the virus to render the virus replication-defective and to make room for inserted genes. Thus far, vectors with a capacity of about 7 kb have been developed. An advantage of adenoviruses for human gene therapy trial is that they have a long safety record, having been used in live virus vaccines for many years without problem.[28,29]

Adenoviral vectors are particularly attractive as agents for gene transfer into cells of the airway[30] because of their tropism for this tissue, but they are also being applied to variety of other cell types such as hepatocytes and neurons. Unlike retroviruses, adenoviruses can infect nondividing cells. Introduced DNA seems to remain episomal and so may not be transferred to daughter cells in tissues that undergo cellular turnover. This is not a problem for neurons but may be for cells of airway. Because it does not integrate into the host genome, the possibility that introduced vector DNA will be lost, is of a significant concern that has not been well studied.

A variety of other questions remain with regard to the use of adenoviruses as vectors for gene transfer. The duration and level of expression of genes introduced by adenoviral vectors have not been adequately investigated. Possible problems include shutoff of inserted gene promoters and concern about viral cytotoxic effects e.g.. it is known that the viral penton protein causes toxic effects in cells, but it is not known whether these affects will be significant in replication-defective viruses used for gene therapy. In fact, it is not entirely clear that currently used vectors are completely replication-defective. Adenoviruses have an endosomolytic activity that may also have deleterious effects on cellular metabolism. However, this activity is being used to enhance the efficiency of receptor-mediated gene transfer.

HERPESVIRUS

Herpesvirus is a large DNA virus (100-250 kb) that can be used to deliver genes and the potential of these viruses as gene vectors lies in their ability to carry large foreign DNA inserts and their ability to establish life long latent infections in which the virus genome exists as a stable episome with no apparent affect on the host cell. Because of this quality herpesvirus-based vectors may provide a unique strategy for persistence of foreign DNA in the cells of the central nervous system.[31] Many different herpesviruses may have potential as gene delivery vehicles but todate only Herpes simplex virus (HSV), a virus which naturally establishes a silent, latent infections of neurons in both humans and animals has been tried.

VACCINA VIRUS

This is another large DNA virus that can be utilized to deliver genes into mammalian cells. Pox virus-based vectors have number of advantages and because of their large size they can infect all cell types. Unfortunately, infection of cells with the wild-type virus leads to cell death, as such the use of vaccina as long term delivery is limited.[32,33]

CANDIDATES DISEASES FOR GENE THERAPY:

After considering the various routes of gene transfer delivery systems, the next obvious question is: which diseases are good candidate for gene therapy and what should be their selection criteria. Obviously the selection should take into the consideration the burden and abundance of disorder in a given community. It also should include those diseases for which the available therapy can not stop the progression of the disorder thus leading to an early mortality and morbidity in affected subjects. Recessive disorders caused by the mutations of both normal copies of genes perhaps can meet the above criteria and the introduction of just one functioning gene can correct the problem. Treatment of autosomal dominant diseases, in which the presence of one abnormal copy of a gene that produces disease, for the time being will remain unapproachable, until all recent techniques of recombinant DNA technology are further improved or perfected for either "turning off" or "removing" defective genes. It requires that the affected genes must be cloned first and the normal genes should be able to code for an enzyme containing a single protein strand. Finally, the gene therapy should be able to improve or correct the patient's condition by treatment of target cells that can be removed from the body and returned safely. This also requires that at the time of treatment, the patient should not have an irreversible damage, otherwise it will not be possible to measure an improvement.

Based upon above considerations, the most obvious human genetic diseases which can be candidate for potential gene therapy are those in which a single gene does not function. The Table 2 lists selected single gene-defects and these include hemopoeitic conditions such as thalassemias, and various forms of immuno deficiency, as well as disorders such as phenylketouria, familial hypercholestrolemia and alpha 1-antitrypsin deficiency, each affecting proteins made in liver. In case of many single-gene defects, altered DNA sequences have been now cloned into bacterial plasmids. These can be now used to identify whether or not a person is homozygous for the disease or carrier. In this category, sickle cell anemia and thalassemias in which globin genes are defective, growth hormones, some of the collagen disorders and Lesch-Nyhan syndrome in which there is an absence of the purine synthesis enzyme hypxanthine phosphoribosyl transferase, can be considered. Treatment function is also being explored in lymphocyte and stem cells of patients with ADA deficiency, in hepatocytes of patients with LDL receptors defects and in fibroblasts engineered to produce factor 1X for hemophilia B patients. Cancer and infectious diseases are also target for these new modalities. Tumor infiltrating lymphocytes are being given to genes that will cause them to overproduce cytokines and melanoma cells are being treated to produce foreign HLA antigens and thus becoming targets for immune rejection. The first gene ransfer in patients which was approved was actually designed to mark infused tumor-infiltrating lymphocytes with a DNA tag that would not alter cellular physiology, but allowed the investigators to monitor the fate of these cells when given to the patient. Intrestingly this transfer was not for the treatment of single-gene defect disorder, but rather to evaluate the immunotherapy of melanoma in five patients, through the use of tumor infiltrating lymphocytes.[34]

TABLE 2. Single Gene Defects

Disorders	MIM#	Chromosomal Mapping	Global+ Frequency	Inheritance
Adenosine deaminase deficiency	102700	20q 13.11	Rare	AR
Alpha 1-anti-trypsin deficiency*	107400	14q 32.1	1/3000-1/20,000	AR
Cystic Fibrosis*	219700	7q 31.3-q 32	1/2000 Rare in Asians	AR
Duchenne/Becker Muscular Dystrophy*	310200	Xq 21.2	1/3000-5000 males	X-
Familial Hypercholestrolemia*	143890	19p 13.2-p 13.1	1/500 hetrozygotes	AD
Fragile X Syndrome*	309550	Xq 27.3	1/1500M 1/2000-3000	X
G6PD deficiency*	305900	Xq 28	Most common	X
Hemophilia*				
A	306700	Xq 28	1/10,000males	X
B	306900	Xq 27.1-27.2		
Huntington Disease*	143100	4pter-p 16.3	4-8/100,000	AD
Myotonic Dystrophy*	160900	19cen-q 182	1/10,000-1000	AD
Neurofibromatosis type 1*	162200	17q 11.2	1/3000-5000	AD
Osteogenesis Imperfecta	120150	17q 21.31-q 22.05	1/15,000	AD
Phenylketonuria*	261600	12q 24.1	1/5000-200,000	AR
Retinoblastoma*	180200	13q 14.1-q 14.2	1/14,000	AD
Sickle cell anemia*	141900	11p 15.5	1/100-400	AR
Tay-Sach disease	272800	15q 23-24	1/3000	AR
Thalassemia*				
alpha	141800	16p 13.3	most common	AR
beta	141900	11p 15.5		
Wilm's Tumor	194070	11p 13	1/10,000	AD

AR=autosomal recessive AD=autosomal dominant X=x-linked
* prenatal diagnosis is currently feasible
+in affected populations

RECENT CLINICAL TRIALS AND FUTURE PROSPECTS OF GENE THERAPY IN SOME SELECTED GENETIC DISORDERS

Adenosine Deaminase Deficiency

The first transfer of gene for the correction of a genetic defect was

performed in patient with severe combined immunodeficiency (SCID) caused by adenosine deaminase (ADA) deficiency,[35,36] an abnormal recessive disease which entirely results from abnormalities in lymphocytes. In this disease, deficiency of ADA, an enzyme necessary for the production of DNA, results deoxyadenosine (a toxic intermediate) accumulation in lymphocytes, which has numerous affects that would impair DNA replication and cell division. This results in a profound failure of both cell-mediated (T-cell) and humoral (B-cell) immunity, making ADA deficiency one of the many causes of SCID.

Satisfactory results were obtained using retroviral constructs that contained the ADA and dominant selectable marker neo gene. Peripheral blood lymphocytes from these patients, who were being treated with intravenous injection of purified ADA enzyme, were isolated and infected with vectors. The transfected cells were then returned to patients, who also continued to receive the purified enzyme ADA. The transfer of a functional human ADA gene into immortalizes T-cells from patient with ADA deficiency although has resulted in an increased expression of transduced T-cells but this approach was not able to permanently cure the defect (because of shortened life span of two months of the lymphocytes). The repeated use of the procedure was therefore necessary and also for monitoring the safety of the procedure. So far the data reported on all the 10 children with ADA deficiency is of preliminary nature as most the children have been engrafted for one year or so.[2] Transfer of human DNA gene has also been performed efficiently into murine hemopoetic stem cells. Based on the initial data on gene transfer studies into T-cells and hemopoetic stem cells, which have been performed in the last few years, no apparent side affects were observed.

Cystic Fibrosis

Cystic fibrosis (CF) is a genetic defect with an autosomal recessive pattern of inheritance. In white population, about 1 in 25 individuals carries recessive mutation on one chromosome. The gene is less common seen in other races. The CF gene is located on the long arm of chromosome 7, region q 31.03-q32. Although the gene itself remarkably large, spanning about 250 kb of DNA, the protein coding sequences (exons) present are only about 2.5% of the genome. The CF gene was isolated in 1989[37] and the protein encoded by gene is known as cystic fibrosis transmembrane conductance regulator (CFTR), containing 1480 amino acids. The CF is caused by dysfunction of cAMP regulated chloride channel located in the epithelial membrane of airway. An increase in cAMP level within the cells causes increased secretion through CFTR channel into lumen. Loss of function of this channel leads to abnormal salt and water transport which causes a variety of effects including increased accumulation of mucous in lungs and GI tract.

A number of vector systems both viral and nonviral have been proposed for gene therapy and of all these, adenovectors have produced the best results as seen by the clinical trials which have taken so far. Virus-mediated gene transfer was first used in 1990, to successfully correct the CF defect in cultured cell lines derived from CF patients.[38,39] The studies represented a clinical step toward effective treatment of this lethal disorder. The first report of using a CF adenovector was by Zabner *et al*[40] who showed that a single dose of Ad (CFTR) to the nasal epithelium of 3 CF patients corrected the defective Cl^-

channel with no sinus inflammatory reaction or evidence of viral replication. Recently the clinical trials of liposome mediated CFTR delivery to nasal epithelium of CF patients have successfully been completed and these have shown no adverse treatment-related side effects. These results are very encouraging, but lack of toxicity of liposome route is counterbalanced by it apparent low efficiency of gene transfer.

Cancer

Molecular genetic approaches to the study of cancer are based on the hypothesis that specific changes in DNA of a cell results in its transformation and the eventual development of neoplasia. In that sense, cancer is a genetic disease in which a parent cell carrying a mutated gene multiplies, and its progeny, which also carries the mutation, clonally expand to form a neoplasm. Thus cancer can be studied by the genetic methods, and specific genes can be identified that carry mutations. These mutations can be small point mutations or may involve DNA rearrangements or large deletions and insertions of DNA. Regardless of the type of cancer, whether it is carcinoma, sarcoma, leukemia, lymphoma or other, studies which have appeared in the last decade have confirmed that the abnormalities are in the DNA,[42] and this demands and entirely new approach for the treatment of cancer. There are now variety of gene delivery systems under development[43] and the murine viral vectors, the most commonly being used in clinical trials, require a proliferating target cell to stably integrate and express the vector genes. Adenovectors are now a days more desirable compared to retovectors. This is because of their ability to produce high titer and efficient gene transfer into target cells regardless of the state of cellular proliferation, however these vectors do not stably insert their genes into the genome of the target cell.[44] The nonviral gene transfer systems that include unconjugated DNA (naked DNA), DNA conjugates and liposomes are therefore being tried for gene transfer.[17] These nonviral methods appear to be non-toxic, non-integrating vector delivery system. A number of exvivo gene transfer approaches to cancer gene therapy are also directed toward directly enhancing tumor immunogeneicity[45] in which poor gene deliveries and low titers are of less concern since in vitro gene transfer is generally quite efficient in tumor cells. However the ability to induce systemic immunity against a malignancy has been complicated by the debilitated state of the patient and lack of full understanding of optimal gene combination for initiation of a potent systemic anti-tumor response. The ex vivo method involves the protection of tissues against the toxic effects of systemic chemotherapy. Insertion of resistance genes into hemopoetic stem cells may allow the administration of higher doses of chemotherapy and decreased marrow toxicity.

The concept of genetic modification of a patients own tumor cell for use as a cancer therapeutic was received with some skepticism when it was shown[46] that the implantation of IL-4 gene containing plasmacytoma cells into syngenic mice resulted in transient tumor growth followed by complete tumor rejection. This led to the insertion of a variety of genes (e.g. cytokines, cell surface proteins) into a number of malignancies.[47] In the United Sates. There are now 23 protocols which have been approved for ex vivo manipulation. These are primarily focused on the treatment of melanoma, colorectal cancer and renal carcinoma (Table 3) based on the observations that these are amenable

TABLE 3. Protocols Approved For Human Cytokine Gene Therapy Trials For Cancer In USA

Type	Tisseues	Genes
Brain tumors	Tumor cells	HS-tk*
Brain tumors	Tumor cells	antisense IGF-1
Brain tumors	Hemopoetic stem cells	MDR-1
Breast cancer	Fibroblasts	IL-4
Breast cancer	Hemopoetic stem cells	MDR-1
Colorectal cancer	Tumor cells	IL-2 or TNF
Colorectal cancer	Fibroblasts	IL-2 or IL-4
Colorectal cancer	Tumor cells	HLA-B7+ 2-microglobulin*
Malignant melanoma	T-cells	TNF
Malignant melanoma	Tumor cells	TNF, IL-2, IL-4
Malignant melanoma	Fibroblasts	IL-4
Malignant melanoma	Tumor cells	-interferon*
Malignant melanoma	Tumor cells	B7 co-stimulatory molecule
Malignant melanoma	Tumor cells	HLA-B7+ 2-microglobulin*
Neuroblastoma	Tumor cells	IL-2 or -interferon
Non-small cell lung cancer	Tumor cells	antisense K-ras or WT p53*
Ovarian cancer	Hemopoetic stem cells	MDR-1
Ovarian cancer	Tumor cells	HS-tk
Renal cell carcinoma	Tumor cells	IL-2, TNF or GM-CSF
Renal cell carcinoma	Tumor cells	HLA-B7+ 2-microglobulin*
Renal cell carcinoma	Fibroblasts	IL-4
Small cell lung cancer	Tumor cells	IL-2
Solid tumors	Tumor cells	HLA-B7+ 2-microglobulin*

*in vivo gene transfer protocols

to immunologic manipulations than other solid tumors.[48–52] All the studies carried carried so far direct that for cancer therapy, the most common approach is to modify tumor cells with a cytokine gene or other immunotherapy gene such as HLA encoding gene, then return the modified cells to the patient as vaccine. Ongoing clinical trials now include modifications of tumor cells to express IL-2, IL-4, GM-CSF and HLA-B7. Another approach is to make immunologic effector cells such as tumor infiltrating lymphocytes more toxic by secretion of tumor necrosis factor. A protocol to render human bone marrow cell resistant to chemotherapy based on transduction of DMR gene has also been approved by the NIH, Beheads MD, USA. For each of these approaches, these is a reasonable evidence that some results might be achieved based on pre-clinical models. However, it remains to be determined whether treatment of

a patient with a measurable tumor is likely to be effective and at this early stage, the important question in every mind is the safety of the patient and efficacy of gene transduction.

Hemophilias

Hemophilia A (classical) or hemophilia B (Christmas disease) results from defects in the coagulation factor Ⅷ and factor Ⅸ genes located on the long arm of X chromosome at position q 28 and q 27 respectively. Analysis of DNA from patients with both disorders have shown that these are very heterogeneous group of genetic diseases including partial or complete gene deletion, point mutation and insertion of DNA within the factor Ⅷ ro Ⅸ genes.[53]

Gene therapy for both types of hemophilias is now in the developmental stage as the genes for both factors have been cloned and vector constructs containing hemophilia genes have been developed for gene transfer system. Expression of human factor Ⅷ into vitro has been reported in several cases of primary cells and cell lines. The diversity of cell types and of vectors is great. Since the discovery that fibroblasts could produce active factor Ⅸ,[54] there has been many other reports of successful expression in cells as diverse as endothelial cells, myoblasts and keratinocytes as well as lymphocytes. Retroviral vectors used in the study were based on the Moloney murine leukemia virus and the factor Ⅸ was secreted ex vivo at between 0.01-4.6 ug/ 10^6 cells/24hr. Fibroblast appear to secrete the highest yield of factor Ⅸ ex vivo.

Gene therapy now offers the prospect of a cure for the disease and in late 1991, clinical trials were initiated in China on two related siblings with moderately severe hemophilia B using autologous fibroblasts transduced with factor Ⅸ retrovirus.[55] The results showed that both the plasma factor Ⅸ antigen and clotting activity gradually improved, accompanying clinical improvement in bleeding in the recipient. Gene therapy for hemophilia A patients is now receiving an increased attention, because of the significantly decreased amounts of protein required for hemostais compared to hemophilia B, but nufortunately, the shorter half-life of factor Ⅷ and its inefficient secretion due to retention within the endoplasmic reticulum negates the advantages.

HIV Infection

The concept of gene therapy for infectious diseases is relatively new and a number of different stages in the epithelial life cycle are being studied as targets for gene therapy. This is due to the fact that infection pathogens which integrates their genomes into host cell chromosome, and which transmit their progeny to daughter cells, may be considered genetic disease. Since the major route of transmission is still cell free rather than "vertical" and the deleterious genetic material becomes part of the human genome, thus there is a chance that virus infections such as HIV, HTLV-1 and hepatitis B can be tackled by a genetic approach to treatment.[56] Several gene therapy protocols for AIDS have been approved in USA and the new vectors HIV and adenoassocaited virus (AAV) are receiving considerable attention for gene transfer. A major advantage of the HIV based vectors is that in common with other lentiviruses,

these will integrate their into nondividing cells. Theoretically, treating HIV infection with gene therapy is formidable (as deficiency is located to specific tissue). But the difficulty stems from the nature of HIV disease, as the viral infection leads to the destruction of $CD4^+$ lymphocytes, which are essential for maintaining protective immunity.

Hemoglobinopathies

Thalassemias are a major public health problem in many world populations. Thalassemic children, many of whom can be kept alive through regular blood transfusion and effective chelation therapy, grow and develop normally only to die in the second or third decade. It has been goal of several major laboratories to introduce a beta globin gene into hemopoetic stem cells of patients with β-thalassemia syndrome and although a great deal is known about the molecular defects in thalassemias, it seems unlikely that it will be possible to insert normal globin genes in the immediate future. The globin genes are seems to be under extremely tight regulation and inorder to achieve a significant amelioration of disease phenotype, it would require at least 20-50% expression of a transduced gene with respect endogenous level[57] to correct beta thalassemia and sickle cell anemia. Therefore, therapeutic options for the diseases are limited to allogenic bone marrow transplant (BMT) but it has also a limited applicability being available to only those patients who have a HLA matched donor.[58,59]

FUTURE DIRECTIONS OF GENE THERAPY AND COMMON CONCERNS

The recent advances in DNA recombinant technology have made it clear that we can now isolate, clone and modify almost every gene. The concepts and modes of gene delivery system, in particular, those involving retroviral and adenoviral vectors, have progressed well. But unfortunately concerns are still there about the safety of these viruses and the efficacy with which they can be prevented from replication. The gene therapy trials which have been carried so far included both non-therapeutic cell marking studies aimed simply to determine the fate of genetically marked cells after grafting into patients and some had specific therapeutic goals whose results are now being interpreted with extreme caution. On the basis of available informations on the gene transfer studies, we can say that in the near future, the genetic correction by site-specific targeting of many disease-related genes may become clinical reality. Unfortunately there is still long way to go, even though we know that once isolated, a gene can be introduced into living cells and shown to produce its products.

There is now massive international effort that is being directed at gene therapy research but there are still many technical difficulties to overcome such as (a) isolation of genes and their major regulatory regions (b) amount of gene product required for product (c) purifying population into which genes can be inserted (d) inserting the genes and restoring the transfected cells to the patients (e) and finally, ensuring the safety of the recipient and monitoring the associated risks.

There is now general agreement, as opposed to germ-line therapy, somatic genetic manipulation for the purpose of ameliorating disease should be

pursued,[4] as the major advantage offered by somatic gene therapy is that this approach would circumvent or avoid, in certain instances the technical problems which are relevant to organ transfer. Yet it should be clear that somatic gene therapy has not yet really entered into clinical scale sufficient to permit definite conclusions regarding its potentials for human benefits. A better understanding of somatic cell transplantation is essential e.g. if hemopoetic stem cells can be expanded in number in culture in culture without diminution of their ability to repopulate the hemopoetic system, such development would revolutionize the disease treatment. Until now gene therapy has focused on expensive ex-vivo approaches for rare diseases, including cancer and SCID but it should be remembered that this method of gene-engineered cells that are produced in ex vivo is still an intermediate approach. Exact localization of gene defects on its chromosomes, together with appropriate regulation of the gene and ability to introduce the gene into nondividing cells is important. Much is still to be learned about how inserted genes function and how these can upset the activities of other genes. We already know that in one type of cancer, malignant retinoblastoma a change in one gene sparked the uncontrolled cancer-causing activity of another gene. Similarly experiments in rats showed that gene for producing growth hormones functioned excessively and a giant size rat resulted. Ethical considerations and human sufferings from incorect gene therapy can not be overlooked and it important that all the gene therapy protocols should clearly state, why the disease is good candidate for gene therapy and what makes you think that the therapeutic gene will be inserted where it belongs in the patient's cell and it will be expressed usefully in the patient with minimal side effect.[60]

In conclusion the recent advances in gene therapy have thrilled those who are involved in health care delivery system and has brought hope for many patients who are suffering from the genetics diseases. All the signs are there that the gene therapy will be a clinical reality in the 21st century, but the safety of inserted genes into a cell is matter of concern and it will remain with us in the future as GOD has not given us an absolute knowledge to probe into the nature.

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