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Review Article

PROGRESS IN GENE THERAPY: A Review

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Abstract
Currently used therapeutic systems are undergoing major changes, in the 21st century. Gene therapy is likely to become an important therapeutic option in treatment of inherited diseases. Broadly, defined, the concept of gene therapy involves transfer of genetic material into a cell, tissue or whole organ, with the objective of curing a disease or improving the clinical status of the patient. Gene therapy carries the excitement of a cure-all for a host of diseases, the controversy surrounding the modulation of human genes, and the promise of a type of medical treatment most of us could not imagine as possible just a few years back. With its potential to eliminate and prevent hereditary diseases such as cystic fibrosis and hemophilia and its use as a possible cure for heart diseases, AIDS and cancer, gene therapy is a potential medical miracle. A key factor in e success of gene therapy is the development of delivery systems that are capable of efficient gene transfer to a variety of tissues without causing any associated pathogenic effects. Vectors based upon many different viral systems, including retroviruses, lent viruses, adenoviruses and adeno-associated viruses; currently offer the best choice for efficient gene delivery. This review outlines the major approaches used for gene delivery, the recent developments in this field of medicine.

Keywords: Gene therapy, delivery systems

Introduction

Gene therapy is a novel approach that tends to change the expression of some genes in an attempt to treat, cure or ultimately prevent diseases. Current gene therapy is primarily experiment based with a few early human clinical trials underway (1).

In the mid 1980's the focus of gene therapy was entirely on treating diseases caused by single gene defects such as hemophilias, duchenne's muscular dystrophy and sickle cell anemia. In late 1980's and early 1990's the concept of gene therapy got expanded to include number of acquired diseases. Human testing of first – generation vectors began in 1990, when a little girl named Ashanti received gene therapy treatment for SCID, a fatal inherited disease of the immune system. Strategy involved the use of a genetically modified virus as vector to carry correctly functioning gene into her immune cells. The inserted gene then programmed the cell to produced a missing enzyme (2). A number of virus based vectors were developed. However, it soon became evident that most of these vectors didn't transfer the genes efficiently and that they were not sufficiently weakened to become totally apathogenic (3). In 1995, a public debate led to consensus that gene therapy has value although many unanswered questions require continued basic research. As the field matured over the last decade, it has caught attention of biopharmaceutical industry, which has begun to sort out its own role in gene therapy. This is critical as ultimately this industry will bring gene therapy to patient populations (3).

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Human gene therapy continues to be an exciting concept for the treatment of diseases. This field of research still remains in its early stages, even though a number of studies have provided significant information about its principle strategies and approaches. Although there is no unequivocal evidence of efficacy, there have been demonstrated physiological changes that are relevant to disease process. One of the major challenges still confronting is the design of more efficient vectors.

A variety of viral and non viral possibilities are being exploited viral vectors derived from adenoviruses and adenoassociated viruses seems to have maximum promise. Specific properties of lentiviruses and retroviruses ensure there status in current gene therapy procedures. Non viral vectos based on lipids, water soluble polycations, other non condensing polymers and nano or microparticles have been proposed. Cationic polymers carrying novel targeting ligands are receiving increasing alternation. Intelligent polymers with temperaturee, pH and light sensitivities for a controlable and effective gene transfer have recently been introduced Recent advances in the preparation of lipoplexes and greater safety support the use of strategies for naked DNA transfer. The latest approach, using bacteria as vector for gene therapy or *in situ* producers of therapeutic proteins (alternative gene therapy) must undergo critical evaluation in further experiments (4). Many tools from this research are in place and the driving force will be provided by the imagination of the committed investigators. More than 300 clinical trials involving gene transfer to patients have been approved (5) and the first nucleic acid drug, an antisense oligonucleotide (formivirsen), has been approved by the United States Food and Drug Administration (FDA). Although effective gene therapy in human has continued to

Table 1: Phase I trials for somatic cell gene therapy that have been approved

GENE PRODUCT	DISEASE	TARGET CELL TYPE
Adenosine deaminase	SCID	T cell, stem cell
Tumor necrosis factor	Malignant melanoma	Tumor infiltrating lymphocytes
Interleukin-2	Advanced cancer	Tumor cells
Factor IX	Hemophilia b	Fibroblasts
LDL receptor	Hypercholesterolemia	Hepatocytes
HSV thymidine kinase	Ovarian cancer	Ovarian cancer cells
HLA-B7	Malignant melanoma	Melanoma cells
HSV thymidine kinase	AIDS	Cytotoxic T lymphocytes

be an elusive goal, recent advances lead us to hope that this goal will be realized within the next few years

Approaches Used in Gene Therapy

The defective gene of a diagnosed patient can be corrected by a number of different strategies such as Gene Replacement ; Gene Correction ; Gene Augmentation

Gene Replacement

In replacement therapy, a normal gene is inserted some where in the genome so that its product could replace that of a defective gene. This approach may be suitable for recessive disorders, which are marked by deficiency of an enzyme or other proteins. Though the gene functions in the genome providing an appropriate regulatory sequence, the approach may not be successful in treating dominant disorders associated with the production of an abnormal gene product, which interferes with the product of normal gene.

Gene Correction

Corrective gene therapy requires replacement of a mutant gene or a part of it with a normal sequence. This can be achieved by using recombinant technology. Another form of corrective therapy involves the suppression of a particular mutation by a transfer RNA that is introduced into a cell.

Gene Augmentation

Introducing a normal genetic sequence into a host genome modifies the expression of mutant gene in defective cell. The defective host gene remains unaltered.

The gene therapy recipient cells may be germline cells or somatic cells.

Germline cells: It involves modifying the genes in germ cells producing cells, which will then pass these genetic changes to the future generations as well.

Somatic Cells: As the name suggests, this therapy involves the insertion of genes into specific somatic cells like the bone marrow stem cells, fibroblasts, hepatocytes or mycocytes (6) .The genetic manipulations are not passed to the patient's offsprings. This form of gene therapy is being done at most genetic engineering laboratories throughout the world. Phase 1 trials that have been approved can be seen in table 1.

The somatic cell therapy can be carried out by one of the following approaches

Ex vivo gene therapy

This approach requires that new cells-either autologous cells or closely matched donor cells be subjected to genetic engineering for each patient. This is a costly and time-consuming strategy. Consequently, research is underway to create "universal donor" cells that have most of their immunogenic antigens stripped from the outer cellular surface so that they are immunologically benign and can be used with a range of patients.

The gene transfer protocol for ex vivo gene therapy for human subjects make use of vectors derived from mouse retroviruses. However, as retroviruses can transform normal cells into cancerous ones, it is essential that this possibility be at least diminished and preferably completely abolished.

In vivo gene therapy

Since organs like heart, brain, and lungs are less suitable for *ex vivo* gene therapy as culture of their cells and reimplantation is not feasible, the *in vivo* gene therapy comes into play. The vector, usually a retrovirus carrying the gene, is injected systemically or directly into the concerned organ. The property of the retrovirus to transduce only dividing cells, such as tumor cells, is utilized for selective delivery of the gene leaving non-dividing cells unaffected.

Antisense gene therapy

Antisense Oligonucleotide Strategy: An antisense RNA molecule is the RNA molecule having base sequence that is reverse complement of naturally occurring mRNA. It forms ds:RNA and prevents translation of the mRNA in the transformed cell. So antisense RNA (as-RNA) prevents synthesis of the product of the gene it is directed against This therapy has been designed to prevent, or at least lower, the expression of a specific gene as upon hybridization of the 2 RNA sequences-the endogenous m RNA and the exogenous as RNA, the amount of mRNA available for translation is reduced.

Methods for Gene Transfer

Genes can be introduced into cells by a number of efficient methods that can be classified as physical, chemical or biological methods (7). Before describing the classification we would first like to discuss the criteria for an ideal DNA delivery system.

Requirements for ideal DNA delivery system

It should accommodate a broad range of inserted DNA.
It must be easily produced.
It can be targeted to specific type of cells.
It should not permit replication of DNA.
It must provide long-term gene expression.
It should be non toxic and non immunogenic.
The methods for gene transfer are as follows

A. Physical Method

A number of methods utilizing various physical techniques have been developed to facilitate the transfer of foreign genes into the host cells. Some of these have been described below:

i Electroporation

A short electric pulse of specific strength creates holes in cell membrane through which foreign DNA can enter inside the cell. Discharging a capacitor across the electrodes from a specially generated electroporation chamber generates the pulse required for an efficient transfer of the DNA by electroporation.

TABLE 2: Viral vectors used for gene delivery

VECTOR	PACKAGING CAPACITY	HOST RANGE	CLINICAL TRIALS	FEATURES
AAV	Low <4 kb	Broad, infects both dividing and non-dividing cells.	+	Slow expression onset, genome integration, long term expression, inefficient large-scale virus production
Adenovirus	Medium <7.5kb	Broad, low transcription of neurons.	+	Transient expression, strong immunogenecity
Alphavirus	Medium <7.5kb	Broad, neuron and glial-cell specific strains	+	Transient, but extreme, expression levels; low immunogenecity
Herpes simplex virus	High > 30 kb	Broad, neurons, stem cells, muscle cells	-	Latent infection, long-term expression, low toxicity (mutants)
Lentivirus	Medium 8 kb	Broad, dividing and non-dividing cells	-	Genome integration, long term expression, safety concerns low titers, production inefficient
Retrovirus	Medium 8 kb	Restricted, dividing cells only	+	Genome integration, long-term expression

The generated pulse may be either a high voltage (1.5 kV) rectangular wave pulse for a short duration or a low voltage (350 V) pulse for a longer duration.

ii Microinjection

Very fine glass capillaries are used for injecting DNA directly into the nucleus of target cell.

iii Gold Bullet/ Gene gun

Microscopic gold spheres coated with genes are fired into a cell with a helium-pressurized gun. This method has been successfully used to deliver DNA *in vivo* into liver, skin, pancreas, muscle, spleen and tumors. Expression of reporter genes (e.g. firefly luciferase and β- galactosidase) or therapeutic genes (human growth hormone) have also been reported (8).

B. Chemical Methods

i Naked DNA injection

Naked DNA can be introduced into cells by using chemicals such as calcium phosphate, DEAE (diethyl amino ethyl), dextran sulphate or poly ethylene glycol (PEG).

ii Liposome mediated delivery

The foreign DNA can be incorporated into the phospholipid vesicle called liposomes, by sonication of a solution of lipids and the DNA in ether. Being amphipathic molecules, lipids form liposomes that enclose the negatively charged DNA within it.

It was found that addition of a lysosomotrophic agent like chloroquine, which raises the acidic pH of the endosomes obviates the fusing efficiency of the liposome with endosomal membrane and consequently reduces the probability of gene transfer to nucleus (9).

Proteoliposomes have been shown to transfer genes effectively. But the difficulties in their purification and characterization have limited their use. Proteoliposomes containing Sendai virus glycoproteins however could mediate the cellular entry and fusion of the liposomes with the endosomal membrane (10) .

iii Synthetic carriers

A number of synthetic transporters of DNA have been developed. One of these is the complex of spermine, a positively charged molecule, which forms lipospermine when attached to lipids. DNA being negatively charged attracts the positively charged hydrophilic portion of lipospermine to form a casing around the DNA. A large number. of lipospermine then associate together to form a second casing whose outer surface is positively charged due to spermine.

C. Biological Methods (virus mediated gene delivery)

The development of safe and efficient gene transfer vehicles is critical for the success of gene therapy. One of the most promising approaches includes the application of various viral vectors, which represent most types and families of viruses, suitable for infection of mammalian host cells. Interest in using such systems in applied settings continues to grow. Overall, there have been major improvements in all aspects of gene delivery vector development and targeting of gene expression (11).

However, before these can be used as vectors in gene therapy, critical genes encoding for pathogenic proteins and those necessary for viral replication have to be detected. The viral genes removed are:

Gag encodes core proteins/ specific glycoprotein antigens
Pol encodes reverse transcriptase
Env encodes envelope proteins

The engineered virus retains its ability to infect host cells but lacks the ability to produce viral progeny.The various virus-based vectors are adeno-associated virus, adeno virus, retrovirus, alpha virus, herpes simplex virus, lentivirus etc.

The properties of these virus based vectors and their comparison can be seen in table 2.

Some Recent Developments in Gene Therapy Research

Since the early 1990's investigators have toiled to establish the transfer of genes to human somatic cells as a possible mean of therapy. Some of the recent developments are:

Table 3: Enzyme-Prodrug Combinations for cancer gene therapy

GENE	PRODRUG
HSV thymidine kinase (HSV TK)	Gangiclovir
VSV thymidine kinase	Ara-M
Deoxycytidine kinase	Ara-C Fludarabine 2-chlorodeoxyadenosine Difluorodeoxycytidine
Cytosine deaminase	5-fluorocytidine
Nucleoside phosphorylase	MeP-dR

Cancer: It is now widely believed that cancer is a disorder of the genetic components of somatic cells. The discovery of oncogenes and tumor suppressor genes has opened up new avenues. Some of the recent approaches include

a.Enhanced Expression Of Apoptin

Apoptin is derived from chicken anemia virus (CAV) and known to induce apoptosis of tumor cells but not of normal cells. The aim of this approach has been to use increased expression of apoptin by the Myc-Max response element (MMRE) and SV40 enhancer. This strategy has been applied for in small-cell lung cancer (SCLC) gene therapy .

b.Oncogene Inactivation

Transcription of oncogenes can also be inhibited using the adenoviral gene E1A, which interferes with the transcription of erbB-2, a strategy useful in treating cancers that overexpress this oncogenic protein, such as breast and ovarian cancers (12).

Several oncogenic proteins have been identified and associated with various malignancies. A variety of strategies are under development to block expression of these oncogenic proteins in malignant cells by interfering with either transcription or translation. The most commonly applied approach has been the use of antisense strategies (13). This approach is currently undergoing clinical trials (14).

c.Cell-targeted suicide

Conversion of a prodrug to a toxic metabolite by genetically engineered tumor cells is an attractive way to create an artificial difference between normal and neoplastic tissue (15). A variety of enzymes are capable of performing such a function, and they typically kill cells by activation of a relatively non-toxic prodrug to a cytotoxic form (table 3)

Key: HSV, herpes simplex virus; VSV, vesicular stomatitis virus; Ara-C, cytosine arabinoside or cytarabine; Ara-M, 6-methylpurine-2'-deoxyriboside. The major limitation of this approach has been the requirement of delivering the prodrug-metabolizing enzyme locally at a dose sufficient to transduce most or all tumor cells without systemic dissemination (16).

Gene therapy for brain tumour: Malignant brain tumours, particularly malignant gliomas, are one of the most difficult disease to cure, gene therapy for malignant gliomas has been clinically applied since 1992 as an advanced therapeutic strategy for a breakthrough. Suicide gene therapy with retroviral or adenoviral vectors was the mainstream at first. New protocols of immune gene therapy and oncolytic therapy with replication competent viruses within the tumor cell only are increasingly being developed (17).Cancer therapy utilizing interleukin–13 receptor alpha 2 chain Cancer cells are known to express cell surface molecules such as specific antigens or cytokine receptors eg : EGFR, Fas/CD95, gp100, HER – 2/nen, IL13 R alpha 2 and MAGE. Among them interlenkin–13 rectepor (IL-13R) alpha 2 chain is expressed on certain types of cancer cells including glioblastoma, AIDS, Kaposis sarcoma and head and neck cancer. This protein is one of the receptor components for IL–13 a Th2 cell–derived pleiotropic immune regulatory cytokine. IL13R alpha 2 chain on these cancer cells can be targeted with a receptor directed cytotoxin termed IL-13 PE to induce specific cancer cell killing. However, this molecule does not mediate cytotoxicity to cells that do not express or express low levels of IL-13 alpha 2. In order to achieve a broad therapeutic window for IL-13 PE, plasmid mediated gene transfer of IL–13 R alpha 2 in cancer cells was employed in vitro and in vivo. Cancer cells transfected with IL-13 R alpha 2 demonstrated incrased binding to IL-13 and sensitivity to IL–13 PE *in vitro. In vivo* intra-tumoral gene transfer of IL13 R alpha 2 profomely enhanced antitumour activity of IL–13 PE providing complete elemination of established tumour in some xenogrphs. Further studies on such therapy are under progress.

Radiotargeted gene therapy : The radiotargeted gene therapy approach aims at localizing radionuclides at tumor sites. It involves inducing tumor cells to synthesize a membrane-expressed receptor with a high affinity for injected radiolabeled ligands. A second strategy involves transduction of the sodium iodide symporter (NIS) and free radionuclide therapy. Using the first strategy, induction of high levels of human somatostatin receptor subtype 2 expression and selective tumor uptake, imaging, or growth inhibition with radiolabeled somatostatin analogs has been achieved in human tumor xenograft models. Therapy studies have been performed on several tumor xenograft models with various radionuclides using the NIS radiotargeted gene therapy approach (18, 19, 20, 21).

Cystic Fibrosis (CF) Cystic fibrosis is a complex, multi-system disease, which is inherited in an autosomal recessive pattern affecting about 1 in 2000 caucasians (22). CF is caused due to a mutation in the cystic fibrosis transport regulator (CFTR) gene, which perturbs the salt and water composition of secretions, slows the mucocilliary clearance of airways and promotes infection resulting in progressive deterioration of lung functions. Until recently, few of the affected individuals survived beyond childhood and other than transplantation there is no permanent therapy for CF lung disease. Gene therapy provides best hope for these individuals.

Although the viability of CFTR gene transfer to the lung has been established *in vitro* and in animal models, correction of the CFTR defect in humans has not been achieved. (23). As for instance, a suitable plasmid expressing CFTR protein complexed with cationic liposomes can be successfully delivered and expressed in airway epithelia of rodents.

However, none of the viral vectors such as AAV, lentivirus, adenovirus have shown a persistent correction of the ion transport defects that occur in CF. Despite disappointing results, these studies have shown that non-viral vectors could represent viable alternative for CF. Yet another problem is the use of novel cationic lipid formulations that appear to be more inflammatory in lungs. Therefore, the amount of liposomal lipid delivered to the lung and

surfactant metabolism in pulmonary surface liquids will have to be evaluated.

Novel gene therapy approaches for AIDS: Human immunodeficiency virus (HIV)-1 continues to be a global pandemic of enormous consequence to humanity. At the heart of the virus lies its replication inside cells and the comparatively brief moments, it spends in the intracellular spaces. Against this backdrop we consider a therapeutic approach still unperfected for HIV-1 disease. Gene therapy has shown many potential targets (24).Vectors based on HIV-1, SIV, and FIV have been developed for delivery of transgenes to T cells Although ironic, perhaps the best means of delivery of genes to target cells for gene therapy of HIV-1 depends upon the use of vectors derived from HIV-1 or related retroviruses. Retroviral and lentiviral viruses have been the basis for development of improved vectors for gene transfer for the last 20 years (24)Dominant-negative effectors against HIV-1 inhibit viral replicationPost entry to the cell, the virus offers a number of enticing targets for interfering with its replication. For instance, the multimerization of Vif (the virion infectivity factor) has been shown to be essential in the life cycle of the virus. Disrupting Vif assembly is an attractive target for gene therapy and several published studies are aimed at finding peptides that in a transdominant negative manner disrupt its oligomerization. In one study, a 12-mer oligopeptide containing the aminoacid sequence PXP motif, proline-X-proline, identified through phage display, inhibited Vif oligomerization in vitro. Other peptides, derived from the Vif sequence, had a similar ability to inhibit Vif oligomerization (26, 27) Another example based on oligomerization involves the HIV-1 Vpr protein. Vpr is capable of inducing G2 cell cycle blockade. A mutation at residue 73 in Vpr results in a virus with both lower transcriptional activity and an inability of Vpr to block the cell cycle at G2. This effect is due to heterodimerization between wild-type and mutant proteins, thereby inactivating the complex. Thus, delivery of debilitated viral proteins can interfere with formation of 'normal' viral complexes and could be high on the list of therapies to be consideration (21) Intrabodies against viral or viral-related host proteins can be delivered to successfully decrease viral replication

The effectiveness of intracellular expression of recombinant antibodies or intrabodies in intracellular immunization against HIV-1 has been shown in an increasing number of studies. Targeting of Vif may have important repercussions on viral assembly and reverse transcription, as Vif is needed for efficient infectivity, viral replication, and completion of proviral DNA synthesis. Another way to interfere with Vif function is to create and express a Vif specific single-chain antibody (scFv) in the cytoplasm of T cells. The intrabodies were found to be capable of binding Vif and cells expressing anti-Vif intrabodies were more resistant to infection by several strains of HIV-1 than the cells without the intrabody. Moreover, the viral particles produced by intrabody-expressing cells were not able to complete reverse transcription in subsequently infected cells. Finally, peripheral blood mononuclear cells (PBMC) transduced with HIV-1-based vectors carrying the anti-Vif intrabody were resistant to laboratory-adapted and primary HIV strains (28). Transcription factors that specifically bind HIV-1 promoter and decrease viral transcription have been assessed In an elegant set of experiments, intracellular immunity was induced by transcriptional repression. Those experiments take advantage of the zinc-finger DNA-binding domain for the engineering of a transcription repressor of the HIV-1 LTR. The Kruppel-associated box (KRAB) repressor domain from KOX1 has been fused to zinc-fingers. One of such hybrid proteins is ZFP HIVB-KOX, shown to bind a region overlapping two Sp1 sites within the 5'LTR. The protein repressed a Tat-activated 5'LTR cellular HIV-reporter assay. HIVBA'-KOX, another protein overlapping the three Sp1 sites present in the promoter efficiently inhibited both basal and Tat-activated transcription in unstimulated and mitogen-stimulated T cells and more importantly, strongly inhibited HXB2 replication (29)

Combinations of RNA-based strategies comprising ribozymes, RNA decoys, and siRNA to efficiently block viral replication are being evaluated

RNA-based gene therapy against HIV-1 includes mainly ribozymes, RNA decoys, and siRNAs. Ribozymes, aimed at disrupting transcription regulation, can be envisioned as an efficient approach for gene therapy. Ribozymes have been engineered to ensure the proper colocalization of ribozyme and target and the high affinity of binding. Catalytic antisense RNA hybrid molecules are composed of ribozymes and stem-loops domains. Such a molecule, composed of the antisense stem-loop of the HIV-1 TAR sequence and a hairpin ribozyme, cleaved TAR-containing RNAs. The same approach was undertaken with hammerhead ribozymes also. Artificial RNA substrates containing the TARRNA stem-loop were cleaved more efficiently by the hybrid ribozyme-stem-loop antisense domain molecule than the parental hammerhead ribozyme alone. The enhancement is due to the interaction of both complementary stem-loop motifs as deletion of the TAR sequence abolishes the effect. Similar results were obtained with hammerhead- and hairpin-based catalytic antisense RNAs targeted against the HIV-1 LTR, demonstrating

that the TAR domain can be used as ribozymetargeting to TAR-containing RNAs(30). Cells have been engineered to express gp41-derived peptides on their surface to block HIV-1 entry

Clinical trials with peptides derived from gp41, such as T20, 51, 52 are underway. Other peptidic compounds such as the 18-mer T22, the 14-mer T134, and the 9-mers ALX40-4C and CGP 64222 have been identified as CXCR4 antagonists, and as such show anti-T-tropic specific HIV-1 strain activity. Peptide T, targeting the CCR5 receptor, similarly blocks M-tropic-specific strains. Other studies involved the Vif and protease proteins of HIV-1 that have been shown to interact with each other. A peptide derived from the N-terminus of protease seems to block that interaction and consequently inhibit HIV-1 replication in restrictive cells. It has yet to be seen if these and similar peptides will have antagonistic effects when expressed inside the cell. Animals already seem to have developed innate inhibitory activities against viral pathogens. For instance, Trim5a from rhesus monkeys (Trim5arh) has been identified as a species-specific restriction factor against HIV-1 in rhesus monkeys. The capsid protein is believed to be the target of the restriction, as Trim5arh seems to interact with capsid and ubiquitinate it for destruction (31,32).

The human cellular prion protein (PrPc) has been shown to have nucleic acid binding and chaperone properties similar to those of nucleo-capsid of HIV-1.

Gene Therapy for Coronary Heart Diseases : Recent progress in molecular and cellular biology has led to the development of numerous effective cardiovascular drugs. However, there are still a number of diseases for which no known effective therapy exists, such as peripheral arterial disease, ischaemic heart disease, restenosis after angioplasty, and vascular bypass graft occlusion (33). Currently, gene therapy has been emerging as strategy with great potential for the treatment of cardiovascular disease despite its limitations. The first human trial of gene therapy for cardiovascular diseases started in 1994, with an aim to treat peripheral vascular disease using vascular endothelial growth factor (VEGF). Then, many different potent angiogenic growth factors were tested in clinical trials to treat peripheral arterial disease and ischaemic heart disease. Improvement of clinical symptoms in peripheral arterial disease and ischaemic heart disease has been reported. In future, gene therapy might become a real pharmacotherapy to treat cardiovascular disease (34).

Gene therapy to treat peripheral arterial disease using therapeutic angiogenesis: Critical limb ischaemia that is estimated to develop in 500–1000 individuals per million per year is considered to be one of the most deserving target for trying gene therapy. Recently, the efficacy of therapeutic angiogenesis using vascular endothelial growth factor (VEGF) gene transfer has been reported in human patients with critical limb ischaemia (35). . In addition to intramuscular injection of naked plasmid DNA, adenoviral and liposome mediated delivery of angiogenic growth factors has also utilized in these trials

Recently some investigators have also identified hepatocyte growth factor (HGF) as a novel candidate for therapeutic angiogenesis (36). Currently, a phase III clinical trial in Japan and phase II clinical trial in USA to treat PAD are underway.

Gene therapy to treat myocardial ischaemic disease using therapeutic angiogenesis: Isner and colleagues have applied a similar idea to treat coronary artery disease using *VEGF165* gene (37). An initial trial was performed with intramuscular injection of naked plasmid encoding *VEGF* gene into ischaemic myocardium through mini-operation. They reported that the administration of *VEGF* gene resulted in a marked increase in blood flow and improved clinical symptoms without any apparent toxicity (49). In addition, a phase 1 clinical trial of direct myocardial gene transfer of naked DNA-encoding VEGF165 as sole therapy for refractory angina was reported in 30 patients with class 3 or 4 angina (38). More recently, intracoronary infusion of adenovirus encoding *FGF* gene was performed in a multicentre trial as phase I/IIa. The report documented that intracoronary infusion of *FGF* gene improved cardiac dysfunction without severe toxicity (39).

Gene therapy for restenosis after angioplasty and graft failure: Another important disease potentially amenable to gene therapy in cardiovascular disease is restenosis after angioplasty. One of the attractive possibilities of treating restenosis is to inhibit target gene expression. In particular, the application of DNA technology such as antisense strategy to regulate the transcription of disease-related genes *in vivo* has important therapeutic potential. Accordingly, inhibition of other proto-oncogenes such as *c-myc* by antisense oligodeoxynucleotidase (ODN) was also reported to inhibit neointimal formation in several animal models (40). Currently, a phase II trial using antisense c-myc to treat restenosis is underway.

Diabetes mellitus: Diabetes can be classified into type-1 or insulin dependent diabetes mellitus (IDDM) and type-2 or non-insulin dependent diabetes mellitus (NIDDM). Among the two, IDDM is an autoimmune disease, which is produced as a result of the destruction of insulin producing β-cells of the pancreas.

As the β-cells are destroyed in IDDM, any attempt to reconstitute insulin gene expression must be directed to an ectopic organ. The liver fits in as the right target, as it is the principal effector organ in maintaining blood glucose homeostasis and ketogenesis. Sustained low-level expression of the rat insulin gene was achieved by its *in vivo* administration in the liver of severely diabetic rats by using recombinant retroviral vectors. Ketoacidosis was prevented and normoglyceamia was achieved (41). A somatic gene therapy has been attempted whose results were encouraging.

Advances in neurological gene therapy : The choice of vectors, transgenes, regulatory elements, delivery approaches and the capacity to transduce appropriate target cell types, all influence the effectiveness of gene therapy for neurological diseases (42)

Transduction of microglial cells has recently been achieved through the use of specific transcriptional targeting. Using adeno-associated virus AAV2 and AAV5 and a variety of promoter sequences (derived from hCMV-MIE, CD11b, CD68 and F4/80), it was shown that the F4/80 promoter sequences were the most specific, both from AAV2 and AAV5. Microglial cells play important role in brain inflammation and immune response, and their direct and specific transduction will be of great importance for their *in vivo* engineering in models of autoimmunity, such as experimental allergic encephalomyelitis.

More recently, techniques have been developed by which the expression of small interfering RNAs (siRNAs) can inhibit specific mRNAs. Importantly, proof-of-concept experiments have been performed and have demonstrated that transfer of siRNAs can downregulate the expression of target sequences delivered simultaneously, both *in vitro* and *in vivo*. This technology has now been successfully used to achieve allele-specific inhibition of dominant mutated genes in *in vitro* models of Machado-Joseph disease/spinocerebellar ataxia type 3 (SCA3), Alzheimer's disease and dystonia.

AAV vetors were injected intramuscularly to deliver insulin like growth factor 1(IGF1) to the spinal cord by the retrograde transport of the viral vectors in a transgenic mouse model of ALS that overexpresses superoxide dismutase-1. These experiments delayed onset of motor symptoms, extended survival of spinal cord motorneurons, delayed astrogliosis and extended survival of treated transgenic animals. Injection of a lentiviral vector that is not transported retrogradely to the spinal cord but expresses IGF1 in the muscle did not improve any measure of the disease. Thus, the expression of IGF1 directly in motorneurons had a beneficial effect in an animal model of ALS, and could pave the way for the development of gene therapy for this devastating disease.

TABLE 4 : Viral vectors as vaccines[a]

VIRUS	GENOME	STRUCTURE	DC INFECTION		IMMUNIZATION	
			In vitro	In vivo	Models	Patients
Polio	+ RNA[b]	Capsid	+	+	+	?
Alphaviruses (VEEV, SFV, Sindbis)	+ RNA	Envelope	+	+	+	?
Retroviruses	+ RNA	Envelope	+	+	?	?
Lentiviruses	+ RNA	Envelope	+	+	+	?
Rhabdoviruses (VSV, rabies)	- RNA	Envelope	+	?	+	?
Influenza	- RNA	Envelope	+	?	+	?
Paramyxoviruses (Sendai, measles)	- RNA	Envelope	+	+	+	?
AAV	ssDNA	Capsid	+	?	+	?
Adenoviruses	dsDNA	Capsid	+	+	+	+
HSV	dsDNA	Envelope	+	?	+	?
Poxviruses (vaccinia, fowlpox)	DsDNA	Envelope	+	+	+	+

Progress has also attained while dealing novel approaches such as treatment of obesity through the delivery of leptin receptors or pro-opiomelanocortin to the hypothalamus, the use of a variety of antiapoptotic growth factor approaches to the treatment of ischemia, the delivery of neprilysin, an enzyme that degrades amyloid ß in brain in animal models of Alzheimer's disease, and the use of neuropeptides for the treatment of seizures or glaucoma-induced neurodegeneration. The field of neurological gene therapy been thus continuisly growing and expanding towards the implementation of clinical trials of gene therapy for brain diseases.

Haemophilias A, B

The haemophilias are inherited bleeding disorders caused by low concentrations of specific coagulation factors. The most well known are deficiencies of factor VIII (haemophilia A) and factor IX (haemophilia B), both of which show X-linked inheritance. Factor XI deficiency (originally called haemophilia C) is less common and in most cases represents mild bleeding disorder, is autosomally inherited and is particularly common in Ashkenazi Jews. The commonest inherited bleeding disorder is Von Willebrand's disease, a defect in the quantity or quality of the von Willebrand factor, present in perhaps as many as 1% of the general population (43)

Haemophilia is an ideal target for gene therapy because only a small rise in factor concentration (1-2% of normal) would achieve the goal of prophylaxis without regular infusion of concentrate and would deliver a substantial improvement in lifestyle of patients with severe haemophilia.

The ultimate gene therapy for haemophilia A and B would be direct correction of the molecular defect in the mutated gene. Such direct gene modification has been demonstrated, but for haemophilias A and B this approach remains a long way in the future. Gene therapy for haemophilia today, therefore, relies on addition of normal factor VIII or IX genes.

All human studies have been preceded by animal trials that have generally shown greater rises in factor VIII and IX than have been seen in the human trials. Thus, animal studies can only be a rough guide to human response.

Haemophilia remains a prime target for gene therapy. However, haemophilia is no longer a life-threatening disease with current therapy that is both safe and effective. A balance between the benefits and theoretical risks must be taken into account when gene-based approaches for therapy of the disease are being considered.

Gene Therapy and Vaccination

Therapeutic vaccines such as those used to combat cancer or persistent viral infections are required to reprogramme a downregulated immune system. This presents a difficult challenge for vaccine design and merits the development of novel immunization protocols. Currently, we know that mobilization of dendritic cells (DCs) to present antigens to T lymphocytes is crucial for effective immunization. Our increasing understanding of DC biology will provide most effective vaccines for priming an immune response

The development of recombinant viral vector systems for gene therapy has prompted the evaluation of their use as vaccines (table 4)

[a]Abbreviations: AAV, adeno-associated virus; ds, double stranded; HSV, herpes simplex virus; SFV, Semliki forest virus; ss, single stranded; VEEV, Venezuelan equine encephalitis virus; VSV, vesicular stomatitis virus.

[b] + RNA, positive strand RNA; - RNA, negative strand RNA.

Among all the viral vectors mentioned in table 4, lentiviral vectors are promising candidate since individuals have no pre-existing immune response to animal lentiviruses, In addition, the vectors are persistent and non-cytopathic, do not express viral proteins and can easily be potentially targeted to DCs.

Besides the above approach, DNA based vaccines are also becoming popular. They stimulate the CD4 T cells of Th-1 subset and thereby mediate cellular immune response, which is effective against pathogens. Recombinant protein vaccines on the other hand stimulate Th-2 subset of T-cells thereby eliciting a humoral response (44). DNA vaccines have been successful in protecting animals against influenza, herpes, rabies, malaria and leishmaniasis.

Recent Success & Future Prospects

Scientists have used gene therapy to halt the progression of adrenoleukodystrophy, a fatal neurodegenerative disease caused by a single defective gene, in two seven-year-old boys.(Carter,2009) Gene therapy sees early success for

neurodegenerative disease (Published online 30 October 2007 | Nature | doi:10.1038/news.2007.204). Gene therapy could remedy Parkinson's Introducing three genes corrects motor defects in monkeys (Published online 14 October 2009 | Nature | doi:10.1038/news.2009.1001).

With the explosive increase in the availability of information on human genome, several genetic disorders would become candidates for gene therapy. The field is still at its infancy and relevant.

Gene therapy's potential to revolutionalize medicine in future is exciting and preventing childhood's disease is encouraging. One day it may be possible to treat an unborn child for a genetic disease in uterus, even before symptoms appear.

Scientists are hoping the mapping of human genome will lead towards cures for many diseases and that success of current clinical trials will create new opportunities and challenges. For now, however, it's a wait-and-see situation calling for cautious optimism.

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