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Review Article

Gene therapy for primary immunodeficiency diseases: where are we now?

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Abstract

In 1950s, double-stranded deoxyribonucleic acid (DNA) had been identified as the biochemical basis of heredity and 'gene' had been shown to be a segment of DNA. Accordingly, vast majority of hereditary disorders involve changes, i.e. mutations in specific genes. Can medical treatment leading to genetic cure of these hereditary disorders possible? The answer is 'yes' as the initial thoughts of 'Gene Therapy' have been transformed into reality in recent times. Gene therapy is the application of various technologies including 'Recombinant DNA Technology' for introduction of a relevant functional gene, i.e. exogenous DNA, into a cell to achieve therapeutic effects for genetic disorders. Since 1990, gene therapy has become standard treatment for a number of primary immunodeficiency diseases (PIDs) such as adenosine deaminase deficiency form of severe combined immunodeficiency (ADA-SCID), SCID-X1, Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD) and others. As we eagerly wait until the results of ongoing further clinical trials are available, updated accounts of the status of 'Gene Therapy' for selective PIDs are presented in this review article.

Key words: Gene therapy, genetic disease, immune defficiency.

Introduction

In 1865, Gregor Mendel established a quantitative analysis of breeding experiments between various pea strains and provided a statistical basis of his laws of inheritance. A Danish botanist, Johannsen, subsequently coined the term 'gene' for the hereditary factors, i.e. units of heridity. Since then the sciences of 'Genetics' and 'Medical Genetics, have evolved and DNA has been identified as the biochemical basis of heredity.[1,2] A gene, segment of DNA consisting of regulatory and structural parts, is responsible for the production of a particular polypeptide/protein. All genes on all chromosomes together, a genome, constitute the information a person inherits from his or her parents' information that sets limits on body structure, function and behavior.[1,2] In humans, a copy of the entire genome - more than 3 billion base pairs - is contained in all cells that have a nucleus.[3]

A permanent change in DNA that has pathological consequence is known as mutation, which may be spontaneous or induced and classified as genomic mutation, chromosomal mutation and sub-microscopic gene (point) mutation that involves vast majority of hereditary diseases. Accordingly, genetic disorders are categorized as single gene disorders, chromosomal disorders, somatic cell gene disorders, mitochondrial gene mutations and multifactorial genetic disorders.(Table-I) Can medical treatment leading to permanent genetic cure or at least long term therapeutic benefit or remission possible for these disorders?

The answer is probably 'yes' as the initial thought of 'Gene Therapy' have been transformed recently into reality because of intense medical research efforts. In the mid-1980s, the focus of gene therapy was entirely on treating diseases caused by such single gene defects as hemophilia, Duchenne's muscular dystrophy and sickle cell anemia. In the late 1980s and early 1990s, the concept of gene therapy was expanded into a number of acquired diseases. When human testing of first-generation vectors began in 1990, scientists learned that the vactors didn't transfer genes efficiently and that they were not sufficiently weakened. Expression and use of the therapeutic genes did not last very long. In 1995, a public debate led to the consensus that gene therapy has value although many unanswered questions require continued basic research. As the field has matured over the last decades, it has drawn the attention of biopharmaceutical industry, which has begun to sort out its own role in gene therapy. This is critical because ultimately this industry will bring gene theraphies to large patient populations. The remarkable progress has been achieved in the field of gene therapy over the last 15 years or so.[4] Gene therapy is the application of various technologies including recombinant DNA technology for the introduction of a relevant functional gene, i.e. exogenous DNA or genetic material, into a cell to achieve therapeutic effects for heriditary and acquired human genetic disorders.[5,6] In the present review article an attempt has been made to give an updated account of the present status of gene therapy for primary immunodeficiency diseases in human

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A. Single gene defects

Table-I: Some genetic disorders or diseases Single gene defects[1,2]

Autosomal dominant	Autosomal recessive	X- linked recessive
Familial hypercholesterolemia	Sickle cell anemia	Frailge X syndrome
Adult polycystic kidney disease	Cystic fibrosis	Duchenne muscular ystrophy
Huntingtondisease	Tay-Sachs disease	Hemophilia A
Neurofibromatosis	Phenylketoneureia	Hemophilia B
	β-Thalassemia	

B. Multifactoral disorders

a) Diabetes mellitus b) Hypertension c) Manic depression, etc are the example of multifactoral disorders that are not inherited in simple mendelian pattern, but are strongly influenced by the genetic constituent of the individual.

C. Chromosome disorders

Specific chromosomal abnormalities occur as – 1. Numerical or alteration in number: a) Polyploidy b), Aneuploidy - Down's syndrome, Turner's syndrome; 2. Structural: deletion, translocation, isochromosome, ring chromosome; 3. Combined.

D. Somatic cell gene defects

These mutations are not inherited except by the cells in the lineage of the mutated cell. Somatic cell gene mutations are important in tumurogenesis.

E. Mitochondrial mutations

Identification of mitochondrion has been a tremendous achievement of accomplished scientists who tried to understand the essence of life. We now know that the mitochondrion is the intracellular powerhouse that generates ATP, the chemical energy source, though a chain of reactions called oxidative phosphorylation, Now, the mitochondrion genes are known to play a major role in many important health problems of the 21st centry including Alzhimer's disease, diabetes mellitus, senescence, autism, carcinogenesis, encephalopathies.[7-10]

Ideal criteria, approaches and types of gene therapy

Minimal requirements of gene therapy of a genetic disorder are the following .[6,11-13]

- Identification of the affected locus or at least of the biochemical basis of the disorder.
- A complementary DNA (cDNA) clone of the gene itself (particularly if it is not large), or a functional version of the gene from which noncritical components have been removed to reduce its size.
- A substantial disease burden and a favorable risk-benefit ratio in comparison with alternative therapy.
- Sufficient knowledge of the molecular basis of the disease to be confident that the gene transfer is likely to ameliorate or correct the biochemical pathology and to prevent or reverse critical phenotypic abnormalities. Whereas loss of functional mutations will require replacement with a functional gene, some dominant alleles, such as dominant negative alleles, will require inactivation of the mutant gene or its product.
- Appropriate regulatory components for the transferred gene, tight regulation of the level of gene expression is relatively important in some disease and critical in others. In thalassemia, for example, over-expression of the transferred gene would cause a new imbalance of globin chains, whereas low levels of expression would be ineffective. In contrast, in some enzymopathies, abnormally high levels of expression may have no adverse effect.
- An appropriate target cell with ideally, a long half-life or good replicative potential in vivo.
- Adequate data from cultured cell and animal studies to indicate that the vector, gene construct, and target cell are both efficacious and safe.
- Protocol review and approval by an institutional Review Board and in most countries an oversight governmental agency.

Types of gene therapy

To deliver genes to specific tissues and correct genetic disorders two general approaches and strategies are used, i.e. Ex-vivo approach: The target cells are removed from the body and a normal gene is then introduced in vitro by one of the gene delivery methods; In vivo approach (Direct gene transfer): The therapetrtic gene is introduced into the affected tissues without removing cells from the body.[6,11-13] Type of gene therapy are– Somatic cell therapy: The

insertion of a therapeutic gene into somatic cells which include fibroblasts, myoblasts, epithelial cells, endothelial cells, nervous cells, glial cells, etc; Germline therapy: It is the introduction of a foreign gene into cells, i.e. sperm, ovum or fertilized egg, resulting in their expression in both somatic as well as germ cells, which is not advocated in humans. There are two classes of vehicles for gene transfer i.e. viral and non-viral vectors.

Gene therapy for primary immunodeficiency diseases

Since the demonstration that genes could be successfully transferred into humans in 1990, gene therapy has made much progress. It has become established as an alternative strategy to bone marrow transplantation (BMT) in carefully selected individuals.

One of the primary immunodeficiency diseases (PIDs), adenosine deaminase (ADA) deficiency form of severe combined immunodeficiency (SCID), meets the criteria for successful gene therapy. ADA deficiency is the first human disease in which somatic gene therapy was attempted. The gene for ADA has been identified on chromosome 20 and the cDNA cloned. To transfer the ADA gene to patient cells, a modified retrovirus vector called SAX was prepared. Treatment involved repeated apheresis to collect blood T cells, culture of these cells with anti-CD3 and IL-2 to induce T-Cell expansion, gene transfer by SAX and reinfusion of cells into the patient as explained in Figure-1.[14,15] Treated patients showed transient immunological improvement with an increase in ADA activity in T cells, as well as short-lived clinical benefit. Subsequently, stem cells were transferred and reinfused to provide renewable source of 'normal' cel1s.

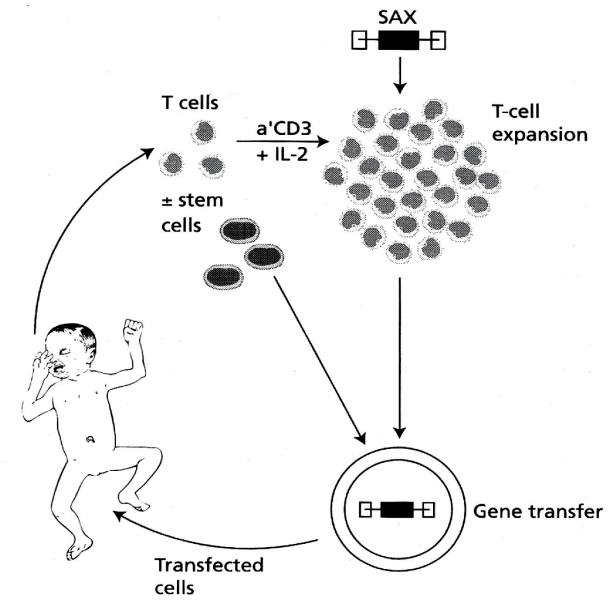

Figure-1: *Gene therapy for adenosine deaminase (ADA) deficiency*

Building on promising results in SCID due to ADA deficiency, gene therapy has recently been extended to X-linked SCID associated with mutations in the common γ-chain cytokine receptor using a retroviral vector (Figure-2).[16]

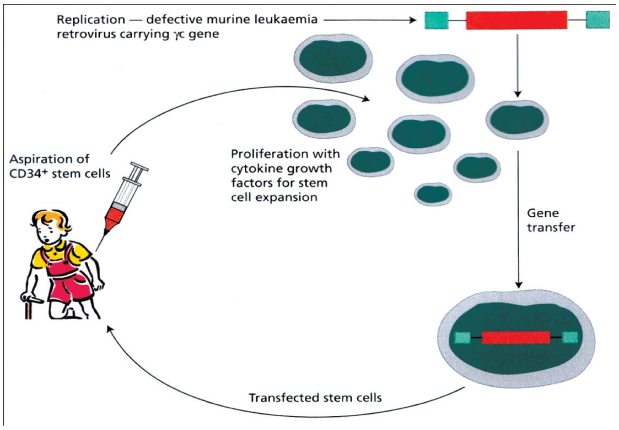

Figure-2: *Gene therapy for severe combined immunodeficiency*

Several children with this form of SCID are currently in remission with durable restitution to date of T and B cell fuction.[15,16] Recipients, however, face the risk of vector-induced inflammation (as seen with adenoviral vectors), overwhelming viral infection by the vector and the possibility, with retroviral vectors, of insertional mutagnesis, i.e. induction of new mutations in the host cell genome by the retrovirus. Delight at the apparent success of gene therapy has recently been dampened by the development, of T-cell leukemia several years after engraftment due to insertional mutagnesis, affecting the LMO2 oncogene, a possibility that had been feared. New vectors may help to outcome this potential limitations in the near future.[15,16]

Recently, it has been reported that 60 patients affected by SCID due to IL2R gene (IL2RG) deficiency (SCID-X1) or ADA-SCID have received hematopoientic stem cell therapy in the past 15 years using gammaretroviral vectors, resulting in immune reconstitution and clinical benefit in the majority of them. However, the occurrence of insertional oncogenesis in the SCID-X1 trials has led to the development of new clinical trials based on integrating vectors with improved safety design as well as investigation on new technologies for highly efficient gene targeting and site-specific gene editing.[17]

Wiskott –aldrich syndrome & chronic granulomatous disease

Gene therapy has become an attractive alternative therapeutic strategy to allogeneic transplant for primary immunodeficiencies (PIDs) owing to known genetic defects. Clinical trials using gammaretroviral vectors have demonstrated the proof of principle of gene therapy for Wiskott-Aldrich Syndrome (WAS) and Chronic Granulomatous Disease (CGD), but have also highlighted limitations of the technology. New strategies based on vectors that can achieve more robust correction with less risk of insertional mutagenesis are being developed. The status of gene therapy of WAS and CGD have encouraged the emerging application of similar strategies to a broader range of PIDs, such as IPEX syndrome.[18,19]

Gene therapy of SCIDs has been proven to be effective to provide sustained correction of the T cell immunodeficiency's. This has been achieved for 2 forms of SCID, i.e. SCID-X1 and ADA deficiency as mentioned earlier. Occurrence of gene toxicity generated by integration of first generation retroviral vectors, as observed in the SCID-X1 trials has led to replace these vectors by self inactivated retroviruses that my provide equivalent efficacy with a better safety profile. Results of ongoing clinical studies in SCID as well as in other PIDs such as WAS, will be thus very informative.[19,20]

PIDs have played a major role in the development of gene therapy for monogenic diseases of the bone marrow. The last decade has seen convincing evidence of long-term disease correction as a result of ex-vivo viral vector-mediated gene transfer into autologous haematopoietic stem cells. The success of these early studies has been balanced by the development of vector-related insertion mutagenic events. More recently the use of alternative vector designs with self-inactivating designs, which have an improved safety profile.[19]

The ongoing development of safer vector platforms and gene-correction technologies together with improvements in cell-transduction techniques and optimized conditioning regimes is likely to make gene therapy amenable for a greater number of PlDs. lf long-term efficacy and safety are shown, gene therapy will become a standard treatment option for specific form of PIDs. We eagerly wait until the results of ongoing clinical studies, the experiences and perspectives of gene therapy for SCID-X1 and ADS-SCID, WAS, CGD & IPEX in comparison to allogenic transplantation are know. Recent developments in gene therapy for other human diseases, such as cancers and autoimmunity are considerable which are our next target to review in the near future.

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