International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 4 Issue 3, April 2020 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Gene Therapy- Challenges & Success

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ABSTRACT

Gene therapy is a contemporary therapeutic intervention with recent positive results and regulatory approvals either completed or expected in the next several years for various conditions. The evolving view is that gene therapy will ultimately offer hope across a range of otherwise debilitating or difficult to-treat conditions. The renaissance in gene therapy has seen major development of both non-viral and viral vectors and accelerated preclinical studies and clinical trials. It is therefore timely to address the progress in gene therapy through a special issue presenting reviews on non-viral and viral vectors including relevant updates on applications on herpes simplex virus (HSV) and adeno-associated virus (AAV) vectors. Thus, the purpose of this review is to summarize the general concepts of gene therapy with a specific focus on monogenic rare disease in hematology and central nervous system disorders where burgeoning therapies are currently entering clinical investigations and approaching regulatory approval.

KEYWORDS: *Gene, viral vector, non viral vector, operon, cistron, muton, expression*

How to cite this paper: Ms. Snehal D. Jadhav | Mr. Jeevan R. Rajguru | Ms. Hina U. Momin | Dr. Mrunal K. Shirsat "Gene Therapy- Challenges & Success" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-4 | Issue-3, April 2020, pp.870-874, URL: www.ijtsrd.com/papers/ijtsrd30612.pdf

IJTSRD30612

INTRODUCTION

Gene therapy (also called human gene transfer) is a medical field which focuses on the utilization of the therapeutic delivery of nucleic acid into a patient's cells as a drug to treat disease.[1][2] The first attempt at modifying human DNA was performed in 1980 by Martin Cline, but the first successful nuclear gene transfer in humans, approved by the National Institutes of Health, was performed in May 1989.[3] The first therapeutic use of gene transfer as well as the first direct insertion of human DNA into the nuclear genome was performed by French Anderson in a trial starting in September 1990. It is thought to be able to cure many genetic disorders or treat them over time.

Discovery:

Between 1989 and December 2018, over 2,900 clinical trials were conducted, with more than half of them in phase I.[4] As of 2017, Spark Therapeutics' Luxturna (RPE65 mutation-induced blindness) and Novartis' Kymriah (Chimeric antigen receptor T cell therapy) are the FDA's first approved gene therapies to enter the market. Since that time, drugs such as Novartis' Zolgensma and Alnylam's Patisiran have also received FDA approval, in addition to other companies' gene therapy drugs. Most of these approaches utilize adeno-associated viruses (AAVs) and lentiviruses for performing gene insertions, in vivo and ex vivo, respectively. ASO / siRNA approaches such as those conducted by Alnylam and Ionis Pharmaceuticals require non-viral delivery systems, and utilize alternative mechanisms for trafficking to liver cells by way of GalNAc transporters. The introduction of CRISPR gene editing has opened new doors for its application and utilization in gene therapy.[5] Solutions to medical hurdles, such as the eradication of latent human immunodeficiency virus (HIV) reservoirs and correction of the mutation that causes sickle cell disease, may soon become a tangible reality.[6][7][8]

Not all medical procedures that introduce alterations to a patient's genetic makeup can be considered gene therapy. Bone marrow transplantation and organ transplants in general have been found to introduce foreign DNA into patients.[9] Gene therapy is defined by the precision of the procedure and the intention of direct therapeutic effect.

Definition & History:

Gene therapy is a promising treatment for several inherited or acquired hematologic disorders. Gene therapy involves the introduction of a functional gene to replace a mutated gene or a therapeutic gene to provide a missing or defective protein to the organism. In some cases, the patient's blood cells are removed and special, targeted cells such as hematopoietic stem cells (HSCs) are selected for engineering. The therapeutic genes are introduced into a vector and delivered into the targeted cells. These targeted, gene-modified cells are reinfused back into the patient. Because this method modifies cells outside the patient's body, it is called ex vivo gene therapy [22]. By contrast in vivo gene

therapy describes the therapeutic gene-containing vectors being directly injected into the patient [23]. In the in vivo case, the gene is expressed, producing a therapeutic protein for treatment. Theoretically, if the gene-modified cells are long-lived and able to expand inside the body, a single gene therapy can be sufficient to provide a lifelong therapeutic effect. Current gene therapy technologies have reached the point that many types of single-gene hematologic deficiency diseases can be permanently corrected, for example, X-linked severe combined immunodeficiency (X-SCID) and adenosine deaminase deficiency severe combined immunodeficiency (ADA-SCID).

Modern Concept of Gene –

Genes are unit of inheritance. A gene is a segment of DNA that provides instructions for synthesis of a specific protein or a particular type of RNA. It may be defined as a segment of DNA which is responsible for inheritance and expression of a particular character. Seymour Benzer (19995) introduced the term, cristron, muton and recon.

Cistron: (Unit of function) It is responsible for expression of a trait. It is a segment of DNA having information for synthesis of a particular protein or RNA. It can be several hundred bp (base pairs) long.

Muton: (Unit of mutation). It consists of a few nucleotides, (one to few bp long). It is segment of DNA that can undergo mutation.

Recon: (Unit of recombination). It is segment of DNA that particular on recombination through crossing over during meiosis. It consists of few to many base pair.

Gene Expression and Gene Regulation:

We know that in a living cell a gene expresses itself and as a result, either a structural protein or an active protein e.g. enzyme or RNA is produced. All these are required for different metabolic activities. However the gene expression can be controlled or regulated at various levels such as transcriptional or post transcriptional or translational level. Here we are going to discuss the transcriptional regulation of gene expression. Usually, small extracellular or intracellular metabolites trigger either initiation or inhibition of gene expression. The clusters of genes with related functions are called operons. They usually transcribe single mRNA molecules. In E. coli some 260 genes are grouped in 75 different operons.

Structure of the Operon:

Each operon is a unit of gene expression and regulation which includes the structural genes and their control elements (promoters and operators). The structural genes code for protein, t RNA and t RNA required by the cell. Promotes are signal sequences in DNA that start RNA synthesis. These are the sites where the RNA polymerase are bound during transcription. The operators are present between the promoters and structural genes. The repressor protein binds to the operator region of the operon. Regulatory genes are responsible for formation of repressors which interact with operators.

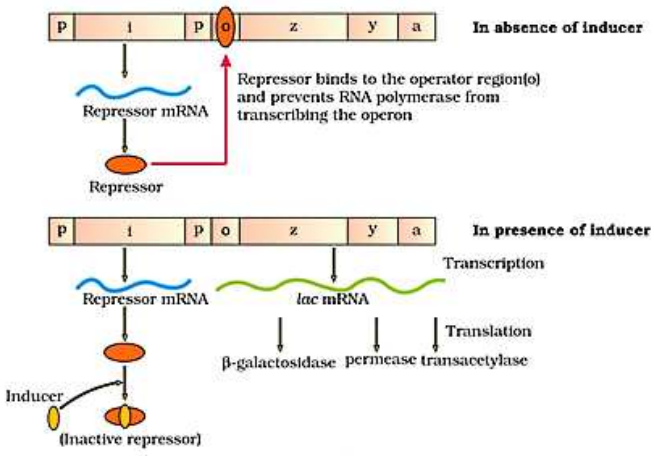

Fig: The Lac Operon

The Lac (Lactose) Operon:

The metabolism of lctose in cells requires three enzymes – permease, β – galactosidase and transacetylase. The enzyme permease is needed for entry of lactose in the cell, β – galactosidase brings about hydrolysis of lactose into glucose and galactose, while transacetylase transfer acetyl group from acetyl Co 'A' to β – galactoside. The lac operon has promoter sites (p), regulatory site (i) and operator site (o). Besides this it has three structural gens namely z, y and a. The 'z' gene codes for β – galactosidase, 'y' gene codes for permease and 'a' gene for transacetylase.

Francois Jacob and J. Monod proposed the classical model of lac operon which can explain properly gene expression and regulation in E. coli. In lac operon a polycistronic structural gene is regulated by common promoter and regulatory genes. When the cell is using its normal energy source glucose, the 'i' gene transcribes a repressor m RNA, after translation of which, a repressor protein is produced. It binds the operator region of the operon and prevents the RNA polymerase from transcribing the operon. As a result β – galactosidase is not produced. In absence of glucose, if lactose is available as energy source for the bacteria then following events occur in the cell. The lactose enters the cell as a result of the activity of permease enzyme. It acts as inducer and interacts with the repressors to inactive it. As the repressor is inactivated, the RNA polymerase can bind itself to the operator site and transcribe the operon to produce lac m RNA which enables formation of all the required three enzymes needed for lactose metabolism. This regulation of lac operon by the repressor is an example of negative control of transcription initiation.[21]

Objective:

Gene therapy is used to correct defective genes in order to cure a disease or help your body better fight disease. Researchers are investigating several ways to do this, including:

1. Replacing mutated genes:

Some cells become diseased because certain genes work incorrectly or no longer work at all. Replacing the defective genes may help treat certain diseases. For instance, a gene called p53 normally prevents tumor growth. Several types of cancer have been linked to problems with the p53 gene. If doctors could replace the defective p53 gene that might trigger the cancer cells to die.

2. Fixing mutated genes:
Mutated genes that cause disease could be turned off so that they no longer promote disease, or healthy genes that help prevent disease could be turned on so that they could inhibit the disease.[24]

Risks:
Gene therapy has some potential risks. A gene can't easily be inserted directly into your cells. Rather, it usually has to be delivered using a carrier, called a vector. The most common gene therapy vectors are viruses because they can recognize certain cells and carry genetic material into the cells' genes. Researchers remove the original disease-causing genes from the viruses, replacing them with the genes needed to stop disease.[16]

This technique presents the following risks:
1. Unwanted immune system reaction:
Your body's immune system may see the newly introduced viruses as intruders and attack them. This may cause inflammation and, in severe cases, organ failure.

2. Targeting the wrong cells:
Because viruses can affect more than one type of cells, it's possible that the altered viruses may infect additional cells not just the targeted cells containing mutated genes. If this happens, healthy cells may be damaged, causing other illness or diseases, such as cancer.

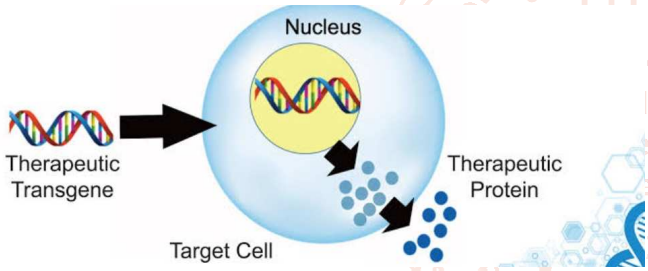

Fig: Targeting Cell

3. Infection caused by the virus:
It's possible that once introduced into the body, the viruses may recover their original ability to cause disease.[25]

Viruses aren't the only vectors that can be used to carry altered genes into your body's cells. Other vectors being studied in clinical trials include:
1. Stem cells:
Stem cells are the cells from which all other cells in your body are created. For gene therapy, stem cells can be trained in a lab to become cells that can help fight disease.

2. Liposomes:
These fatty particles have the ability to carry the new, therapeutic genes to the target cells and pass the genes into your cells' DNA.

Several significant barriers stand in the way of gene therapy becoming a reliable form of treatment, including:
1. Finding a reliable way to get genetic material into cells
2. Targeting the correct cells
3. Reducing the risk of side effects

Gene therapy continues to be a very important and active area of research aimed at developing new, effective treatments for a variety of diseases.[17]

Vectors in gene therapy:
The delivery of DNA into cells can be accomplished by multiple methods. The two major classes are recombinant viruses (sometimes called biological nanoparticles or viral vectors) and naked DNA or DNA complexes (non-viral methods).

Viral vector:
In order to replicate, viruses introduce their genetic material into the host cell, tricking the host's cellular machinery into using it as blueprints for viral proteins. Retroviruses go a stage further by having their genetic material copied into the genome of the host cell. Scientists exploit this by substituting a virus's genetic material with therapeutic DNA. (The term 'DNA' may be an oversimplification, as some viruses contain RNA, and gene therapy could take this form as well.) A number of viruses have been used for human gene therapy, including retroviruses, adenoviruses, herpes simplex, vaccinia, and adeno-associated virus.[4] Like the genetic material (DNA or RNA) in viruses, therapeutic DNA can be designed to simply serve as a temporary blueprint that is degraded naturally or (at least theoretically) to enter the host's genome, becoming a permanent part of the host's DNA in infected cells.

Non-viral:
Non-viral methods present certain advantages over viral methods, such as large scale production and low host immunogenicity. However, non-viral methods initially produced lower levels of transfection and gene expression, and thus lower therapeutic efficacy. Newer technologies offer promise of solving these problems, with the advent of increased cell-specific targeting and subcellular trafficking control. Methods for non-viral gene therapy include the injection of naked DNA, electroporation, the gene gun, sonoporation, magnetofection, the use of oligonucleotides, lipoplexes, dendrimers, and inorganic nanoparticles. More recent approaches, such as those performed by companies such as Ligandal, offer the possibility of creating cell-specific targeting technologies for a variety of gene therapy modalities, including RNA, DNA and gene editing tools such as CRISPR. Other companies, such as Arbutus Biopharma and Arcturus Therapeutics, offer non-viral, non-cell-targeted approaches that mainly exhibit liver trophism. In more recent years, startups such as Sixfold Bio, GenEdit, and Spotlight Therapeutics have begun to solve the non-viral gene delivery problem. Non-viral techniques offer the possibility of repeat dosing and greater tailorability of genetic payloads, which in the future will be more likely to take over viral-based delivery systems. Companies such as Editas Medicine, Intellia Therapeutics, CRISPR Therapeutics, Casebia, Cellectis, Precision Biosciences, bluebird bio, and Sangamo have developed non-viral gene editing techniques, however frequently still use viruses for delivering gene insertion material following genomic cleavage by guided nucleases. These companies focus on gene editing, and still face major delivery hurdles. Moderna Therapeutics and CureVac focus on delivery of mRNA payloads, which are necessarily non-viral delivery problems. Alnylam, Dicerna Pharmaceuticals, and Ionis Pharmaceuticals focus on delivery of siRNA (antisense oligonucleotides) for gene suppression, which also necessitate non-viral delivery systems. In academic contexts, a number of laboratories are working on delivery of PEGylated particles, which form serum protein coronas and chiefly exhibit LDL receptor mediated uptake in cells in vivo.[10]

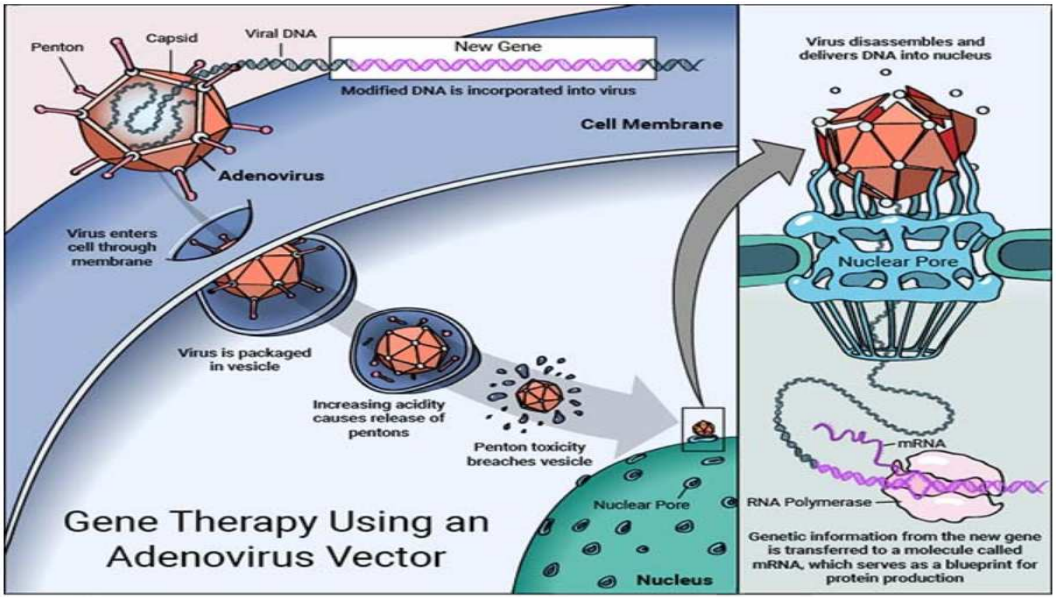

Fig: Gene Therapy

Challenges of This Type of Therapy

Finding the best delivery system for transporting normal CFTR genes is only one challenge that scientists must solve to develop an effective treatment for cystic fibrosis. Scientists must also:

1. Determine the life span of the affected lung cells
2. Identify the "parent cells" that produce CFTR cells.
3. Find out how long treatment should last and how often it needs to be repeated.

The first CF gene therapy experiments have involved lung cells because these cells are readily accessible and because lung damage is the most common, life-threatening problem in CF patients. However, scientists hope that the technologies being developed for lung cells will be adapted to treat other organs affected by cystic fibrosis.

Conclusion:

Though gene therapy can offer the opportunity to significantly improve symptomology or slow progression of non-cancerous blood and/or neurologic disorders, it currently remains in the research arena. As such, those being treated do so with uncertain expectation of the outcome. It will be a number of years before a fully informed position is available, but clinicians should be thinking about who might benefit most from gene therapy based on extrapolation of early clinical trial data, potential impact on health care costs and patient quality of life.

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