Review Article

Genetic medicines and genome editing - possible cure for monogenic and polygenic hereditary disorders

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Abstract

Modern therapeutics and drugs use DNA/RNA transfer techniques for the modification of gene expression levels, correction and compensation of mutant genes. Molecular approaches like genetic medicines and genome editing technology can lead to comprehensive and wide-range of medical and scientific acceptance. Due to the feasibility of these techniques, these can help in the treatment of monogenic disorders, neurodegenerative diseases, multifactorial inheritance disorders or polygenic hereditary disorders, including liver, bone, nervous and skeletal syndromes. Not only this, circulating enzyme, hormonal, and coagulation factors deficiencies can also be investigated through genome editing technology. However, successful application of these strategies on clinical human trials can only be implemented by overcoming regulatory, economic and socio-political concerns. The review would focus on the practice of genetic medicines and gene therapy for the treatment of heredity disorders.

Keywords: DNA transfer; Genetic medicines; Genome Editing; Human trials; Monogenic disorders

Introduction

Human genome sequencing and genotypic-phenotypic studies are now focusing more on the treatment of genetic diseases other than identification strategies [1]. During pre-genetic medicine era, metabolic manipulation and protein augmentation have been considered as extraordinarily effective for the treatment of various monogenic disorders including phenylketonuria, sickle cell anemia, thalassemia and endocrine disorders respectively [1]. Many of the innate metabolic disorders have been cured with gene therapy and manipulation of genome codes for the defective enzymes and proteins in the metabolic trails [28].

Metabolic manipulation

Metabolic manipulation includes a detailed investigation of metabolic pathways and then handling them for clinical diagnosis and management. It basically uses nutritional or small molecule remedy to treat the disorder. Diet adjustment (just as phenylalanine constraint for phenylketonuria treatment) is one of the most fundamental types of metabolic management [2]. For a number of disorders, effective treatment is built upon combining diet management with medical prescription (for example, a blend of cholesterol-lowering diet and statin inhibitors also known as HMG-CoA reductase inhibitors is used for familial hypercholesterolemia).

Metabolic remedies can also be used to treat the difficulties of hereditary conditions, like thalassemia's treatment with the iron-complex mediator desferrioxamine. It basically averts the failure of an organ that else can be affected by the excess iron from the recurrent red blood cell's transfusions that are necessary for the primary phenotype's treatment [1, 3].

Protein augmentation

Protein augmentation therapy focuses on the purification of the proteins from mammalian cell or tissue and then this purified form is given to the patient. It would supplement a severely depleted or missing factor, thereby overcomes the deficiency and restores the function. Protein augmentation strategy can be used for the effective treatment of genetic disorders like coagulation disorders, cystic fibrosis, immunoglobulin deficiencies, Alpha-1 antitrypsin deficiency (A1AD), endocrine ailments and LSDs (lysosomal storage diseases) [4]. However, for the treatment of hereditary disorders involving protein deficient functionality in the extracellular matrix, this strategy can be considered as one of the most appropriate strategies. However, some potential challenges including maintenance of venous entree to oversee the protein, contamination, supply deficiencies of the therapeutic mediator; budget; condition of regular, repetitive administrations; and the probable hypersensitive, inflammatory and resistant comebacks to the permeated proteins cannot be neglected [1].

According to Online Mendelian Inheritance in Man (OMIM) reports, out of 25,000 genes (approximately), 18,000 genes have been identified in human genome that are involved in genetic disorders [1]. Thus, there is a need to design strategies to cure monogenic and polygenic disorders. Identification of the causative gene is the first step but a definitive treatment for genetic treatment must be focused. This treatment can be a direct or indirect action to the site of the defect itself fairly than the effect of mutant gene product [5].

Monogenic disorders that are occurring due to single gene mutations can be prevented or treated with genetic medicine. However, complex phenotype accompanying hundreds of genes having multiple genetic variations cannot be corrected with genetic medicines. These disorders can be treated by following the procedure of compensation or modification of defective organ. For example, regeneration of cardiac myocytes for the treatment of failed myocardium by stem cell therapy [1].

Genetic medicines

Genetic medicine is an innovative term which combines areas such as gene therapy, personalized medication, and the rapidly developing new medical area known as predictive medicine. In clinical testing, main consortiums of genetic medicines include somatic stem cells (SSCs), gene transfer, gene therapy, genome editing, RNA modification, and embryonic stem cells (ESCs) [1, 6]. We would focus on all the above-mentioned strategies, their application to treat the genetic disorders and the biological challenges to make the genetic medication an authentic reality.

Stem cell therapies

Immature and unspecialized cells with sustained self-renewal ability and depending upon their origin and source are called stem cells [7]. Two prime groups of stem cells include; embryonic stem cells (ESCs) and somatic stem cells (SSCs).

Embryonic stem cells (ESCs) are pluripotent cells and at the blastocyst period, they are usually a derivative of inner cell mass of embryos [8, 9]. Conversely, somatic stem cells are obtained from several organs at fetal and post-natal stages and they can minimally individualize into the cell types set up in the tissue [10]. Somatic stem cells are usually characterized on the base of parent organ from which they are obtained, for example, hematopoietic stem cells. Transplantation of hematopoietic stem cells (HSCT) has become one of the successful treatments for many patients with distinct congenital or acquired conditions of the hematopoietic

structure or with chemo, radio or immuno-sensitive malignancies [11]. Hematopoietic stem cell transplantations (HSCTs) can be a possible cure of lymphomas, leukemias, immune-deficiency disorders, hereditary metabolic deficiencies, hemoglobinopathies, myelodysplastic and myeloproliferative conditions. During bone marrow transplantation, the defective bone marrow cells from the patient's body are interchanged with the cells that are taken from a physically fit and analogous donor. To carry out this, surviving and abnormal leukocytes of the patient are destroyed by using chemotherapy and radiation techniques. Subsequently, bone marrow sample from the donor holding healthy stem cells is hosted into the bloodstream of the patient.

After transplantation, newly introduced stem cells will navigate into the bone marrow of patient and start making fresh, healthy leukocytes replacing the irregular and defective cells. However, umbilical cord blood stem cells (UCBSCs) and peripheral blood stem cells (PBSCs) can also be used for the treatment as they are easier to collect and less prone to infection respectively [12].

Additionally, somatic stem cells have been identified in many other organs including brain, heart, gut, liver, pancreas, skeletal muscles, skin/hairs and they have the ability to get differentiated into several extractions from the parent organ from which the somatic stem cells are obtained. However, this is an open debate whether somatic stem cells can differentiate into crosswise lineages or not [1].

For a disorder caused by single gene mutation, gene transfer strategy is considered as one of the most recognizable therapeutic options. According to a report, entries on 2597 gene therapy clinical trials undertaken in 38 countries have been finalized, which are further continuing or have been approved worldwide [29].

Gene transfer strategies

Two basic strategies of gene therapy for the treatment of genetic disorder are:

1. *Ex vivo* gene transfer

In this technique, patient's blood or bone marrow cells are taken out and then developed in the laboratory. Thus, cells are altered external to the body and then transplanted back. The first step is the exposure of cells to the desired gene-carrying virus. After entering the cells, the virus incorporates the chosen gene into the DNA sequence of the targeted cell. The laboratory grown-up cells are then reimplanted to the patient body by an intravenous injection. As the cells are treated outside the body so referred as *ex vivo* gene therapy. However, *ex vivo* approach has a major limitation that it is applicable for the conditions in which the significant population of cells can be detached from the affected individual and then interchanged after genetic modification [13, 14].

2. *In vivo* gene transfer

It is the best approach for direct gene transfer and thus can be used for the treatment of many hereditary disorders. In this technique, genes are altered and transformed into cells within the body. For monogenic disorders, human *in vivo* studies follow the procedure in which recombinant vector holding therapeutic DNA is either precisely administered to the organ of interest or into blood vessels feeding that organ [1].

Viruses –vehicles for gene therapy

Having the talent in entering the cells, viruses are considered as ideal candidates for gene delivery [30]. To treat hereditary disorders, gene-transfer vectors and a prototypic multigenic expression control systems are mostly considered.

1. Non-Viral vectors

These include naked plasmids and liposomes [1].

2. Viral vectors

These include; Retrovirus and Lentivirus as integrating viral vectors. While, first-generation adenovirus vector and Adeno-associated virus are non-integrating vectors [15]. Mechanism of adenovirus-

associated vector gene delivery has been shown in (Figure 1)

However, two major problems exist which need to overcome before they can safely be used:

1. The Self-replicating ability of the virus must be obstructed, besides the viral mRNA's production that not only codes
- 3.

for proteins thus maintaining the infection but also helping the virus to outflow the cell.

2. Since capsid is essential for cell entry, thus the therapeutic gene should be injected into the viral genome in such a way that it will not constrain the development of a normal capsid [16].

Adenovirus 36kb genome (approx.)

Adeno-associated virus 4.7kb genome

Retrovirus 7-10kb genome

Lentivirus 9-10kb genome

Liposome + plasmid unlimited size of genome

Expression Cassette

Figure 1. Types of viruses and mechanism of respective gene transfer [1].

Genome editing

Based on processed and programmable nucleases, genome editing technologies involve the role of different type of

nucleases including transcription activator-like effector nucleases (TALEN), mega nucleases, zinc finger nucleases (ZFNs), and clustered regularly interspaced short

palindromic repeat (CRISPR) associated nuclease Cas9 as an opening strategy for the achievement of therapeutic genome excision in diseased and defective cells and tissues thus following the elimination/improvement of lethal alterations or addition of defensive transformations [1].

The basic principle of precise genome editing or transgenic technology is the addition of DNA double-stranded breaks (DSBs) at particular genomic loci by programmable nucleases. To facilitate genome editing, these DNA double-stranded breaks then allow successive recruitment of endogenous repair apparatus either for non-homologous end-joining (NHEJ) or homology-directed repair (HDR) to the double-stranded break site [17].

Therapeutic genome modifications and its types

A number of approaches used for genome editing based therapies include improvement or inactivation of lethal mutations, an introduction of protective and defensive mutations, therapeutic transgenes addition, and viral DNA disruption [1]. Table (1) shows the examples of genome editing's applications for the treatment of different disorders

A. Gene disruption

In this strategy, NHEJ locus targeting is used for protein's pathogenic function silencing. Indels formation on the gene of interest frequently results in frameshift transmutations that cause the generation of premature stop codons and consequently a non-functional protein product, or nonsense mediated degeneration of transcripts thus suppressing gene function.

B. NHEJ based gene correction

NHEJ gene correction involves direction of two DSBs equally towards the edges of a pathogenic development or insert. It would cause a deletion of the dominant sequences to mediate therapy. This dealing requires multiplexed targeting of mutation that is responsible for the cause of particular disease [17].

C. HDR based gene correction

It is mostly used for correction of lethal and deleterious mutations hence, in this strategy, in the presence of an exogenously delivered counteractive HDR template, a double-stranded break is induced in the vicinity of mutation site. This HDR repair mechanism at the break site with the exogenous template then applies correction at the mutation site. Accordingly, the gene function is restored [17, 33].

D. Gene addition

This approach introduces a therapeutic transgene into the native or a non-native locus in the genome. At the desired locus, a DSB is induced while an HDR template comprising homology to the break site, a promoter, a transgene and a polyA sequence is introduced to the nucleus. This HDR repair system improves the function of gene at the targeted locus without exact functional mechanism over the gene expression [1].

RNA-modification therapy

RNA-based therapeutics are also under experimental research and exploration for diseases ranging from heredity syndromes to HIV infection up to numerous cancer types. This incipient strategy includes therapeutic ribozymes, aptamers, and small interfering RNAs (siRNAs) thus demonstrating the unmatched adaptability of RNA [18].

The main target of RNA-modification therapy is mRNA. It targets either by suppressing mRNA levels or by applying correction or addition in the functioning of mRNA. Five major approaches for the modification of gene expression at the pre-mRNA or mRNA levels include Antisense oligonucleotide-mediated cleavage of mRNA, RNAi cellular pathways by dsRNA, Spliceosome-mediated trans-splicing, Spliceosome-mediated segmental trans-splicing and Corrective trans-splicing ribozyme. By knocking down the target mRNA, all the mentioned strategies result in the reconciliation of the targeted repression of defective genes [17].

As the surplus amount of ribonucleases are present in serum and in the cells, therefore RNA is unstable *in vivo*. Consequently, certain chemical alterations can be applied to improve preferred characteristics without dropping the activity. However, chemical modifications carried out on small interfering RNAs (siRNAs), aptamers,

ribozymes, antisense (AS) oligonucleotides (ONs), and miRNAs may develop the pharmacokinetic, pharmacodynamics properties and thus reducing immunogenicity [1].

To treat monogenic disorders, four basic RNA modification strategies can be applied. These are as under:

Table 1. Showing the examples of genome editing's applications for the treatment of different disorders [17].

Type of disease	Nuclease Platform Employed	Therapeutic Approach	References
Hemophilia B	ZFN (Zinc-finger nucleases)	Insertion of correct gene sequence via HDR (homology-directed repair) mechanism.	[20]
HIV (human immunodeficiency virus)	ZFN and CRISPR (Clustered regularly interspaced short palindromic repeats)	Inactivation of C-C chemokine receptor type 5 (CCR5) by NHEJ (Non-homologous end joining).	[21]
DMD (Duchenne muscular dystrophy)	CRISPR and TALEN (Transcription activator-like effector nucleases)	Removal of stop codon via NHEJ (Non-homologous end joining)., and gene correction through HDR (homology-directed repair).	[22]
HBV (Hepatitis B virus)	CRISPR and TALEN	Viral DNA weakening through NHEJ mechanism	[23]
SCID (Severe combined immunodeficiency)	ZFN	Insertion of correct gene sequence via HDR strategy.	[24]
Cataract	CRISPR	HDR-mediated genome editing of mutant mice	[25]
Cystic fibrosis	CRISPR	Correction of CFTR allele in intestinal stem cell organoid by HDR.	[26]
HT-1 (Hereditary tyrosinemia)	CRISPR	Improvement of mutation in mouse liver through HDR.	[27]

Antisense oligonucleotides (ASO)

In this strategy, single-stranded DNA (ssDNA) sequences (approx. 18-30bp) cause the degradation of mRNA and this targeting is because of sequence resemblance. As a result, the expression level of the gene is knocked down at targeted region. Thus, ASO strategy can be applied to decrease the levels of mutant

proteins subsequently modifying the phenotype. However, mechanism of ASO strategy, its cellular uptake, binding affinity and target specificity are not clearly understood yet [1].

RNAi

This natural modification approach includes intracellular processing of dsRNA with 2-3 nucleotide 3' overhangs resulting

in the formation of RISC (RNA induced silencing complex) that further facilitates translational blockage of targeted mRNA [1].

Trans-splicing

This technique is unique in a sense that it not only results in the reduction of the expression level of target genes but for correction of phenotype at pre-mRNA level, thus modifying overall genetic makeup. This strategy has not been assessed in human clinical trials but it can successfully correct animal models of hemophilia A, X-linked severe combined immunodeficiency (SCID) with hyper IgM and CF (cystic fibrosis) [1, 34].

Ribozymes

Within the target fragment, RNA molecules having an enzymatic action to identify definite RNA sequences and then catalyze site-specific phosphodiester bond cleavage are called Ribozymes [35]. In hereditary disorders like loss of function based dominant disorders, they can be used for replacing mutant sequences or reducing the levels of mutant mRNA. Most commonly used type of ribozymes in RNA modification therapeutics are hammerhead ribozymes. However, their applicability in clinical trials faces hurdles like efficiency level in delivery and stability [1].

Embryonic stem cell therapy

About 25 years ago, first embryonic stem cells were isolated and cultured from a mouse. Later on, human and other mammalian stem cells have been derived and used for various therapeutic strategies. This principle of embryonic stem cell therapy (ESCT) for hereditary disorder starts with culturing technique. By taking patient's skin fibroblasts, culturing them and by applying gene transfer modes such as retrovirus or lentivirus, compensation for the abnormal gene can be applied. This would result in the incorporation of a correct normally functioning gene with correct regulatory sequences in the fibroblast cells [19].

Using SCNT technology, the nucleus from modified cell would be removed and then

introduced into enucleated oocyte obtained from a single donor cell. This modified egg (containing genetically corrected genome) is stimulated and triggered to grow into a blastocyst stage *in vitro* and modified autologous iPSCs (Induced pluripotent stem cells) would result from the inner cell mass (ICM, pluriblast, embryoblast). Depending on the patient's complexity and genotype-phenotype correlations, the stem cells are then administered to differentiate into a specific cell type consequently repairing the disorder [19].

Besides the fact that approaches to genomic therapeutic editing are improving the ability to make accurate changes in eukaryotic genomes, different ethical and policy issues related to them must be addressed. For instance, genome editing technologies consent larger volumes of sequence records to be acquired from individuals, thus secrecy and confidentiality becomes of greater apprehension. Also, informed approval, privacy and data rights and allotment, technology instructions and issues of admittance are necessary [32].

Challenges for the development of genetic medicines and future prospects

Genetic medicines face regulatory concerns as the focus here is to modify or alter the genomic data or genetic expression. One of the main scenarios about hereditary disorders is that they are regarded as untreatable thus causing severe disabilities and frequently premature deaths [31]. Therefore, clinical agents, researchers, biotech companies and pharmaceutical manufacturers frequently express loss about these additional directing sprints. One other main hurdle is the finances. The economics of drug development and therapeutic strategies often faces financial or resource unavailability that's why pharmaceuticals and biotechnology industries divert their attention. Socio-political concerns to use human ESCs are one of the most common national debate in many countries including Pakistan.

Although, genetic research has a revolutionary aspects in an intense fashion in the last decade still various ethical realms in the society exists. Privacy issues, unethical motivations, centralization of health evidence and automation of access are the main concerns of general public. Because of all these ethical and societal issues, no genetic medicine has been approved for use in the treatment and management of any genetic human disorder or chromosomal anomaly. Besides all the hurdles, correction of mutation and a normally functioning protein's production under an endogenous signal is one of the main tasks for the scientist of the current era. Drug development and application of genomic therapeutic would take time not only to get approval from the government but to change the mind of society towards the treatment. To develop this technology by experimental analysis not only on animal models but on humans every regulatory, socio-political and economic matter must also be overwhelmed before genetic medicines can get a place in the real world and a logical practicality.

Authors' contributions

Conceived and designed the idea: R Bibi, Review the paper: R Bibi & T Hussain, Wrote the paper: R Bibi, Z Tariq & T Hussain.

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