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A REVIEW ON OVERVIEW OF GENE AND RNA BASED THERAPIES

Tandel Muskan Vijaykumar[1], Prajapati Hardikkumar Bharatbhai[2], Dr. Anuradha P. Prajapati[3], Dr. Sachin Narkhede[4], Dr. Shailesh Luhar[5]
[1,2,3,4,5]*Smt. B. N. B. Swaminarayan Pharmacy College Salvav*

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ABSTRACT
Therapeutics based on genes and RNA are revolutionary in medicine because they address the underlying causes of disorders. Enabling precise treatments for genetic problems, enhancing the body's defences, and addressing a variety of ailments ranging from infectious diseases to inherited disorders, these therapies use genes and RNA molecules to correct, replace, or control defective genes.
KEYWORDS: *Gene Therapy, RNA based Therapies,Genetic Disorders, mRNA based Vaccines.*

INTRODUCTION [1, 2, 3]

Medicine is revolutionised by gene and RNA-based medicines, which target the underlying causes of disease. Adenosine deaminase deficiency and severe combined immunodeficiency have been successfully treated using gene therapy, which replaces damaged genes. Research on applications for complicated diseases such as Alzheimer's and polygenic malignancies is still ongoing. RNA treatment, which uses RNA molecules to manipulate biological pathways, becomes more popular. Effective COVID-19 vaccine development is powered by a subset called messenger RNA (mRNA) treatment. The FDA has authorised RNA treatments, which show promise in treating a variety of ailments. These therapies have been proven effective in clinical trials. When combined, gene and RNA therapies offer highly accurate, state-of-the-art medical treatments that are redefining the healthcare landscape with focused precision.

Gene [4]

Genes are orderly sequences of nucleotides on chromosomes that encode features inherited from parents. Genes are the basic building blocks of heredity.Gene therapy shows promise for addressing genetic problems at their core and provides hope for the treatment or elimination of inherited conditions.

Gene Therapy[5-7]

Gene therapy typically involves the insertion of a functioning gene into cells to correct a cellular dysfunction or to provide a new cellular function. It is defined as the administration of genetic material to modify, manipulate gene expression or alter the properties of living cells for therapeutic purposes. The primary goal is to introduce therapeutic genetic material into a patient's cells to correct a genetic defect, replace a faulty gene, or enhance the body's ability to fight against specific diseases.

Objectives

Gene therapies can work by several mechanisms:

- Replacing a disease-causing gene with a healthy copy of the gene
- Inactivating a disease-causing gene that is not functioning properly
- Introducing a new or modified gene into the body to help treat a disease

Types Of Gene Therapy: [4]

Somatic Gene Therapy	Germline Gene Therapy
Therapeutic genes transferred into the somatic cell	Therapeutic genes transferred into the germ cell
E.g. Introduction of genes into bone marrow cells, blood cells, skin cells etc.	E.g. Introduction of genes into eggs and sperms.
Will not be inherited later generations.	It is heritable and passed on to later generation.

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Mechanism of Gene Therapy: [8, 9]

1) **Ex Vivo Gene Therapy:** The ex vivo gene therapy can be applied to only selected tissues (e.g., bone marrow) whose cells can be cultured in the laboratory. The technique of ex vivo gene therapy involves the following steps:
   - Isolate cells with genetic defect from a patient.
   - Grow the cells in culture.
   - Introduce the therapeutic gene to correct gene defect.
   - Select the genetically corrected cells (stable trans-formants) and grow.
   - Transplant the modified cells to the patient.

2) **In Vivo Gene Therapy:** The direct delivery of the therapeutic gene (DNA) into the target cells of a particular tissue of a patient constitutes in vivo gene therapy. These include liver, muscle, skin, spleen, lung, brain and blood cells. The success of in vivo gene therapy mostly depends on the following parameters:
   - The efficiency of the uptake of the therapeutic gene by the target cells.
   - Intracellular degradation of the gene and its uptake by nucleus.
   - The expression capability of the gene.

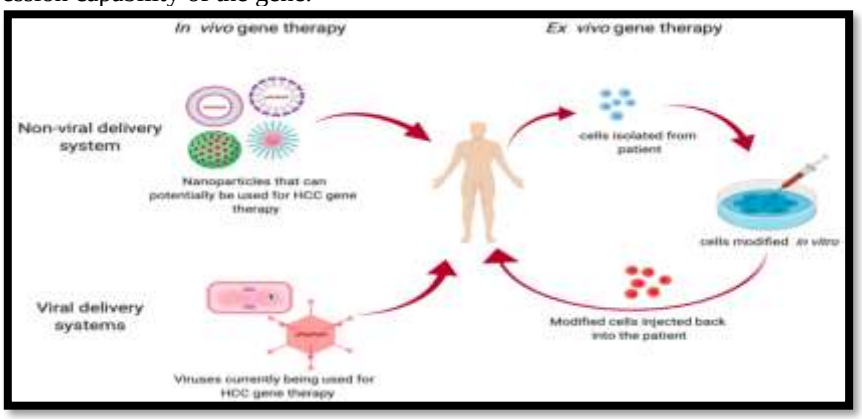

Figure 1: In Vivo and Ex Vivo Gene Therapy

Vectors in Gene Therapy [5, 10, 11]

Facilitating the transfer of genetic information into a cell are vehicles simply called as
Vectors. Vectors can be divided into viral and non-viral delivery systems. Viral vectors have played a central role in gene therapy because of their superior gene delivery capacity compared to non-viral vectors. Moreover, the virus-based transgenic expression, depending on the needs, for both short-term and long-term duration can be achieved.

Viral Vector

a. **Adenovirus Vectors**: Since the advent of gene transfer in mammalian cells, adenoviruses (Ad) vectors have been commonly used as viral delivery vehicles. They are non-enveloped viruses possessing a double-stranded DNA (dsDNA) genome.
b. **Adeno-Associated Virus Vectors:** The small non-enveloped single-stranded DNA (ssDNA) adeno-associated virus (AAV) can only accommodate 4 Kb of foreign DNA, although, the packaging capacity has been improved by constructing fragmented, overlapping, or trans-splicing Dual AAV vectors.
c. **Herpes Simplex Virus Vectors:** A class of double-stranded DNA viruses that infect a particular cell type, neurons. Herpes simplex virus type 1 is a common human pathogen that causes cold sores. It is a human neurotropic virus, which is mostly used for gene transfer in nervous system. Antibodies to HSV-1 are common in humans.
d. **Retrovirus and Lentivirus Vectors:** The enveloped single-stranded RNA (ssRNA) retroviruses (RVs) possess a packaging capacity of 8 kb of foreign sequences. The special feature of RVs comprises their reverse transcriptase activity, which allows the production of dsDNA copies of the RNA genome for integration into the host genome.

Non-Viral Vector

a. **Naked plasmid DNA:** A series of approaches for naked plasmid DNA based gene delivery strategies have been reported in recent years like, naked plasmid DNA transfer method where in a cytotoxic T-lymphocyte antigen 4- immunoglobulin (CTLA4-Ig) gene was delivered using a naked plasmid DNA.
b. **Cationic lipids:** Cationic liposomes are an important class of compounds suitable for carrying negatively charged DNA. There are at present several commercial transfection reagents that are based on cationic lipids like DOTMA(Lipofectin), DOTAP, DOSPA, DOSPER, DDAB, DODAC, Neophectin (PCL-2), DMRIE, DC-Chol, DOGS (Transfectam).

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c. **Polymeric gene carriers:** Synthetic polycationic polymers have gained wide attention as non-viral vectors for gene delivery. Polyplexes form these polymers spontaneously as a result of electrostatic interaction between phosphate groups of DNA and oppositely charged groups of polycationic polymer.

Gene Therapy Approaches[12]

1. **Gene Addition:** Vectors, which are often viruses, are used to deliver the working gene to the cell's nucleus, where the DNA is stored.Sometimes the therapy is designed for the new gene to insert itself into the main DNA storage while other times it will stay next to the main DNA storage, like an extra set of instructions.
2. **Gene Silencing:** It is where the delivered genetic material prevents or inhibits the activity of a gene that is already present in a cell. Gene silencing often decreases the amount of a specific protein being made.
3. **Gene Editing:** Genetic material is sent to directly edit or change pieces of DNA already located within a cell to correct the protein being made by that DNA. Gene editing uses technology that is highly precise to make these types of changes.

Evolving Technologies and New Gene Therapy Platforms [13, 14]

Genome Editing: Gene therapy is defined as the addition of new genes to human cells.

1) **Zinc finger nucleases (ZFNs):** Zinc finger nucleases (ZFNs) are specialised nuclease domains with a specific DNA-binding domain that act as molecular scissors in gene editing. Zinc finger domains that have been engineered to precisely recognise and bind particular DNA sequences work in tandem with the nuclease to cause double-strand breaks. This activates cellular repair pathways to precisely alter the genomic code.
2) **Transcription activator-like effector nucleases (TALENs):** Transcription Activator-Like Effector Nucleases, or TALENs, are molecular tools used in gene editing that use modular proteins from bacteria that are harmful to plants to precisely recognise DNA and cause specific genetic alterations.
3) **Meganucleases:** Enzymes called meganucleases, often referred to as homing endonucleases, are specialised DNA sequence-specific enzymes that are present in microbes such as bacteria and archaea. They are useful for precise genome editing in applications like genetic engineering and gene therapy because of their lengthy recognition sites.
4) **CRISPR/Cas nucleases:** Genetic manipulation is revolutionised by the revolutionary genome editing technologies known as CRISPR/Cas nucleases. CRISPR and Cas proteins, which work as a bacterial immune system and are led by RNA, allow for precise DNA editing, providing previously unheard-of accuracy in research and possible medicinal uses.

APPLICATION OF GENE THERAPY: [11]

1) **Cystic fibrosis (CF):** It is a progressive lung disease causing respiratory issues. Directly delivering the CFTR gene to lung cells is promising, yet challenges arise from the lung's barriers. Advances in gene transfer, tissue engineering, and animal models drive ongoing CF research for improved treatments in overcoming these obstacles.
2) **Cancer Treatment:** Viral vectors are used in gene therapy for cancer patients to change their genes, or immune cells are edited to better target tumours, lessen adverse effects, and boost immune response.
3) **Severe combined immunodeficiency (SCID)**: Particularly the IL2RG gene mutation variant, may be possible through gene therapy. This strategy has demonstrated efficacy in restoring immunological function by introducing a functioning gene copy by viral vectors, providing promise for long-term treatment.
4) **Muscular dystrophy:** It is characterised by muscle degradation. Viral vectors are used in emerging gene therapy to deliver a functioning dystrophin gene, which may decrease or stop muscle deterioration and provide hope for better treatments.
5) **Alzheimer's disease:** Potential approaches involve modifying genes to enhance neuroprotection, reduce beta-amyloid accumulation, or boost cognitive resilience. While in early stages, these strategies aim to address the underlying causes of Alzheimer's, offering hope for more effective treatments.
6) **Parkinson's disease:** It introduces genes that increase dopamine production. This strategy, which uses viral vectors for targeted delivery, shows promise in symptom relief, but early-stage development still faces obstacles with long-term efficacy and targeting.

Progress and Current Trends in Gene Therapy: [15-17]

CAR T cells Therapy

Utilising the power of genes and RNA molecules, gene and RNA therapies represent ground-breaking developments in medicine. With the use of methods like CRISPR-Cas9 and viral vectors, which allow for precise DNA editing, gene therapy treats hereditary problems. RNA therapies work by altering RNA molecules, such as mRNA, which is present in COVID-19 vaccines and other mRNA-based vaccinations.

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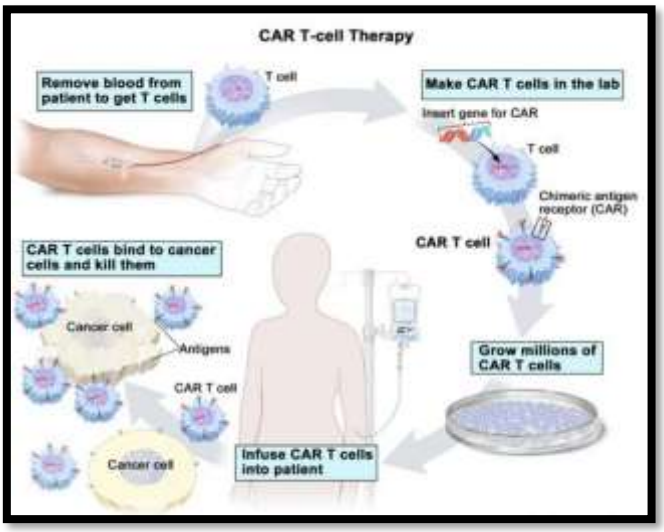

Figure 2: CAR T-Cell Therapy

RNA BASED THERAPEUTICS[3, 18, 19]

The discovery of the genetic code in 1868 provided the impetus for RNA therapies, a next-generation method of treating disease. These medications control gene expression by utilising Watson-Crick base-pairing, offering economical, effective substitutes for conventional treatments.Types of RNA Based therapeutics:

1) **Messenger RNAs (mRNAs):** By using messenger RNA to direct cells to produce proteins, mRNA therapies transform medical intervention.
2) **Antisense oligonucleotides (ASOs):** These brief synthetic sequences have a specific binding affinity for complementary RNA, so providing tailored regulation of gene expression at the pre-mRNA or mRNA level. This therapeutic approach can be used to a variety of medical conditions, including neurological illnesses, muscular dystrophies, malignancies, and genetic abnormalities.
3) **Small interfering RNAs (siRNAs):** In RNA interference (RNAi), small interfering RNAs (siRNAs) play a critical role in controlling gene expression. Double-stranded RNA molecules known as siRNAs specifically target genes in RNA therapies, directing the RNA-induced silencing complex to inhibit or degrade messenger RNAs.
4) **MicroRNAs (miRNAs):** This are small, conserved RNA molecules that bind to target mRNAs to control the expression of certain genes. They were first identified in 1993. Compared to their synthesised RNA counterparts, miRNAs have reduced immunogenic risks due to their powerful targeting capabilities, which allow them to downregulate a wide range of genes.
5) **Aptamers:** Aptamers are short single-stranded RNA, DNA, or RNA-DNA hybrids that fold into precise structures, much like chemical antibodies. This allows them to bind selectively to a variety of targets that are found by the SELEX method.Aptamers offer diverse therapeutic mechanisms:
   (1) Target-specific aptamers can deliver therapeutic agents to specific cells or tissues.
   (2) They can act as agonists, functionally activating their target molecules.
   (3) Serving as antagonists, aptamers block interactions in disease-associated pathways.

Recent Advancement in RNA based Therapies [20-22]

mRNA Vaccine: mRNA vaccines, which function as flexible cell instruction manuals, are a novel method to disease prevention. The COVID-19 pandemic demonstrated their unparalleled efficacy and rapidity of development, as evidenced by the FDA's approval of the Pfizer-BioNTech and Moderna vaccines. RNA therapeutics goes beyond treating infectious diseases and includes cancer vaccines, illustrating the ever-changing field of preventive approaches for a range of illnesses.

CircRNAs Therapies: Unlike mRNAs, circular RNAs are unique single-stranded molecules with closed loops that have certain characteristics. Because circRNAs are safer, less immunogenic, and more stable than viral vectors and DNA treatment, they have the potential to be used in nucleic acid therapy. CircRNAs can function as both non-coding RNAs that regulate biological processes and protein-producing mRNAs. It also highlights the possibility for precision medicine and tailored treatments.

Applications of RNA Therapies [23-26]

1. **Cancer Therapy with siRNA:** Small interfering RNA (siRNA) can selectively suppress the expression of disease-causing genes, holding great promise in the treatment of human diseases, including malignant cancers. Identification of genetic drivers is crucial for advancing cancer therapeutics. There were 19.3 million new cancer cases and 10.0 million deaths worldwide in 2020. It is estimated that cancer incidence cases will rise to 28.4 million in 2040.

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2. **mRNA Vaccines with siRNA:** Combining siRNA with mRNA vaccines offers a novel approach to vaccine development. COVID-19 vaccines are an example of mRNA vaccines, which use genetic instructions to trigger immunological responses. By precisely modulating immune responses, integrating siRNA has the potential to improve vaccination efficacy through its capacity to specifically block gene expression.
3. **Antisense Oligonucleotides (ASOs) for Genetic Disorders:** Antisense oligonucleotides (ASOs) are powerful agents against genetic illnesses. ASOs, which are engineered to engage with particular RNA molecules, regulate gene expression at the fundamental level, providing a means of treatment for ailments such as muscular dystrophy, spinal muscular atrophy, and uncommon genetic disorders.
4. **RNA Therapeutics for Neurogenerative Diseases:** RNA therapies, which use different RNA molecules to target disease-causing factors directly or to modify gene expression, offer a promising strategy to treat neurodegenerative illnesses. MicroRNAs, which are small RNA molecules, have the ability to control gene expression associated with neurodegenerative diseases. RNA therapies have the ability to provide individualised treatments as research advances, providing optimism for better results in this difficult subject.
5. **RNA Therapeutics in Cardiovascular Diseases:** Cardiovascular disease (CVD) is the leading cause of death and disability in developed countries despite advances in risk stratification strategies and treatment RNA therapeutics show promise in revolutionizing cardiovascular disease treatment. MicroRNA (miRNA) therapies target aberrant miRNA expression linked to heart conditions, while messenger RNA (mRNA) therapies aim to enhance protective protein expression.

CONCLUSION

Treating diseases with genetic foundations, such as muscular dystrophy and cancer, may be possible with the use of RNA-based therapeutics and gene therapy. By correcting genetic flaws, these cutting-edge methods hope to cure ailments that were once thought to be incurable and offer long-term comfort.

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