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Progress and future challenges in gene vectors, gene therapy systems and gene expressions

¹Ravindra and ²Dr. Prafulla Prakash Adkar Patil

¹Research Scholar, Sunrise University, Alwar, Rajasthan, India

²Professor, Sunrise University, Alwar, Rajasthan, India

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Corresponding Author: Ravindra

Abstract

Despite these advancements, gene therapy faces several challenges. Immune responses to viral vectors, the potential for insertional mutagenesis, and difficulties in delivering the therapy to specific tissues are major hurdles that need to be addressed. Moreover, the high cost of gene therapy and ethical concerns surrounding genetic modifications continue to spark debate. Clinical outcomes have been promising, with successful treatments reported for conditions like haemophilia, some cancers, and spinal muscular atrophy are examples. Nevertheless, in order to have a complete understanding of the effectiveness and safety of these therapies. As research continues, overcoming the existing challenges vital to the expansion of gene therapy's potential uses in clinical settings.

Keywords: Clinic, gene expression, gene therapy, cancer treatment

Introduction

The process of gene therapy entails altering the genetic code on the cells of the body. The genetic code, which includes instructions for your biological systems and instructions for getting taller, determines the majority of your physical characteristics. Disrupted gene expression may lead to disease. Generative medicine aims to cure disease or enhance the immune system by fixing defective genes or introducing new ones. Many disorders may be amenable to better therapies using gene therapy, including cancer, CF, cardiovascular disease, diabetes, hemophilia, and AIDS. Research on the best times and ways to use gene remedy is ongoing. Right now, the only way to get gene therapy in the US is via a clinical study. It has been an aim of medicine to make site-specific modifications to the human genome ever since the gene was recognised as the basic unit of heredity. Consequently, gene therapy refers to the capacity to modify an individual's genetic composition via the restoration of normal gene function or the introduction of targeted mutations for therapeutic purposes treatment 4. Progress in genetics and bioengineering has made this treatment feasible

by allowing the use of vectors to deliver extrachromosomal fiber to specific cells. In India, gene therapy research is receiving crucial financial support from a number of organisations that provide financial support, including the Indian Council of Medical Research (ICMR), the Departments of Science and Technology (DST) and Biotechnology (DBT), and elsewhere. In terms of gene therapy, India is almost caught up to other Asian countries. Among achieving better vectors is the main focus of the technique usually viruses, nanostructured materials, or plasmids 5. The ability of viruses to invade cells and insert genetic material makes them a frequent subject of scientific inquiry. Major concerns, however, center on genetic alterations and increased immunological responses, especially in cells that make the germ lines. Positive outcomes from somatic cell-based *in vivo* research were obtained from clinical trials that used established procedures. Gene therapy refers to the process of deleting an abnormal gene that causes contamination and replacing it with a normal gene. Transposon insertion into stem cells is one of the operation's most critical obstacles. For large-scale

production and distribution, A "vector" is used to liberate the gene from a molecular carrier. This vector has to be very precise, immune system-free, able to effectively release a gene or genes of a certain size, and purified in big enough numbers and concentrations for use in scientific programs. When implanted into the body, the vector shouldn't cause any kind of allergic reaction or irritation; instead, it should improve regular functioning, fix impairments, or prevent harmful actions.

Literature Review

Rajguru, R *et al.* (2020) ^[1]. Gene therapy has been approved by regulators for a number of diseases and disorders, and it has recently shown promising outcomes. This makes gene therapy a cutting-edge therapeutic intervention. Rapid advancements in The resurgence of gene therapy has been marked by the use of both viral and non-viral vectors, as well as accelerated preclinical and clinical testing procedures. With a focus on recent developments in the use of vectors derived from the herpes simplex virus (HSV) and adeno-associated virus (AAV), this special issue aims to address the evolution of gene therapy by offering reviews of viral and non-viral vectors. Thus, the purpose of this review is to provide a brief introduction to gene therapy in general, with an emphasis on the field's emerging medicines for monogenic rare diseases affecting the central nervous system and hematological, which are now undergoing clinical studies and are on the cusp of regulatory approval.

Gonçalves, Giulliana *et al.* (2017) ^[2]. The ability to insert DNA at specific sites has been an aim of medical study since the discovery of the gene as the basic unit of heredity. In summary, gene therapy is the ability to modify a person's genetic composition in order to treat a condition by either fixing broken or mutant genes or by making specific changes to specific areas. The main objective of this approach is vector optimisation, which mostly employs viruses, plasmids, or nanostructured RNA. The superiority of viruses in penetrating cells and introducing their genetic material makes them the subjects of increasingly frequent investigation. On the other hand, genome editing and immunological response augmentation, particularly in germ line cells, are major causes for alarm. U.S., European, Australian, and Chinese *in vivo* investigations of somatic cell therapies have shown promising outcomes when following established methods. The study delves into recent developments in biotechnology, including stem cells for patients with liver disease, immunotherapy using chimeric antigen receptor T cells, and genome editing with CRISPR/Cas9.

Patil, Santosh *et al.* (2018) ^[5]. Many people are excited about gene therapy because it might one day heal almost all ailments. However, there is also criticism around the idea of changing human imperfection. Regardless, gene therapy offers a new kind of medical treatment that most of us would never have imagined feasible. Using a variety of

keywords, SCOPUS, EMBASE, and the National Library of Medicine Databases were used to do a thorough literature search. Reviewing the history, present status, and prospective future of gene therapy was conducted by reviewing all pertinent papers and ranking them according to their significance. Nothing short of a medical miracle is gene therapy, with its potential to eradicate and prevent AIDS, cancers, genetic illnesses, and its possible treatment for heart ailments.

Bueren, Juan *et al.* (2023) ^[3]. A number of hereditary and acquired disorders now have a viable treatment option because to innovative recombinant DNA techniques and viral vectors that have occurred since the 1960s. With the maturation We are now seeing a new era in the history of medicine, thanks to various *in vivo* and *ex vivo* gene therapy. The widespread acceptance of gene therapy in recent years is evidence of this pharmaceutical goods for commercialization. As an added bonus, a plethora of others are now being considered after thorough examination in human clinical studies. In this overview, we will go over some of the major steps taken to create gene therapy medicines, which have helped cure many uncommon disorders. The translation of these new treatments into clinical practice is likewise encountering significant hurdles, despite advancements in the gene therapy area. This introductory review focuses on this special edition of Human Gene Therapy commemorating the 30th ESSGCT Congress. It discusses both the successes and failures of gene therapy in treating uncommon disorders.

Veeman, Dhinakaran *et al.* (2022) ^[4]. Important strides have been achieved in the fields of technology, agriculture, and medicine thanks to genetic engineering. Different methods, based on viral and nonviral vector designs, been developed to manipulate cells' genetic fabric. The objective of gene therapy is to correct genetic abnormalities by transferring genes using viral and nonviral vectors. Gene transfer motors have come a long way in that they are now safer, less harmful, nonimmunogenic, and able to express transgenes for longer periods of time. Strict and ongoing regulation of gene expression is the objective of gene therapy is to in clinical settings. In this study, we will focus on a variety of gene expressions and their potential biological uses utilizing viral vectors. Thanks to advancements in viral vector engineering and better gene regulatory systems, the technique that uses genes to provide a desired therapy has shown to be successful.

Adenoviral Vectors

In their capsids, adenoviruses store their genomes, which are linear assemblies of double-stranded DNA measuring 36 kilobases (kb). In (Fig. 1), we can see the various methods of treating AAV gene disorders classified. When adenovirus and coxsackievirus receptors on cell surfaces bind to the fiber protein,

Table 1: The main groups of viral vectors used in gene therapy

Type	Vector	Immunogenic potential	Tropism	Limitation	Major advantage	Ref
Integrating	Retrovirus	Low	Dividing cells only	Potential oncogenicity	Long-term gene expression in dividing cells	24
	Lentivirus	Low	Dividing cells only	Potential oncogenicity	Long-term gene expression in dividing cells	25
Episomal	AAV*	Low	Dividing cells only	Small packaging capacity	Non-inflammatory and non-pathogenic	26
Herpes simplex	virus	High	High neurons	Transient expression	Large packaging capacity	27
	Adenovirus	High	Dividing cells only	Viral capsid could induce immune response	Efficient transduction of most cells	28

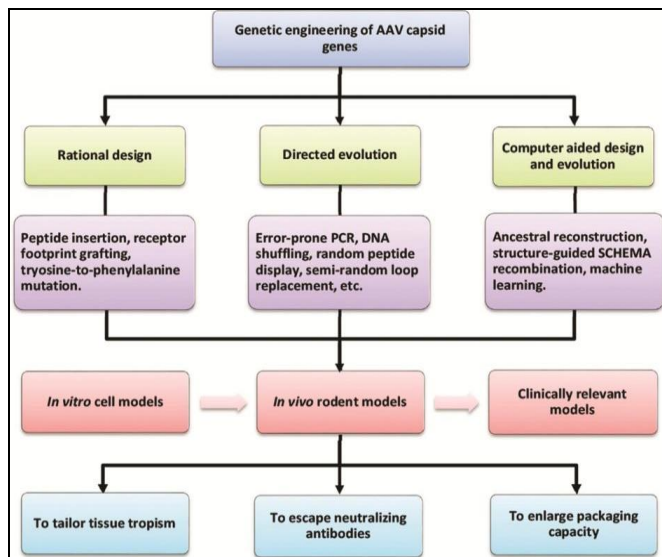


Fig 1: AAV capsid gene technique

Infection with a virus happens. Once the penton binds to the v3 and v5 integrins on the cellular surface, the virus is able to enter the cell by endocytosis. In the process of viral DNA is released into the nucleus during virion breakdown and continues to exist as an episome; the adenovirus hexon capsid protein is retained in the nuclear membrane. In Figure 2, we can see the AAV cancer therapy strategy in action.

Retroviral and lentiviral vectors

RNA virus vectors that have the potential to incorporate themselves into the host genome, are a kind of RNA virus that is developed from retroviruses and lentiviruses. This makes them unique among viruses. For gene therapy applications, these vectors are interesting because of their propensity to combine with host DNA. Upon incorporation of the vector into the host cell, the transgenic hobby may be replicated during DNA replication, allowing for long-term expression of transgenes (for up to two years), a crucial component for long-term medicinal uses. Nonetheless, instances of transgenic expression being gradually downregulated have been documented.

Type 1 herpes simplex vectors

Roughly 80% of the world's population contracts the type 1 herpes simplex virus at some point in their lives.

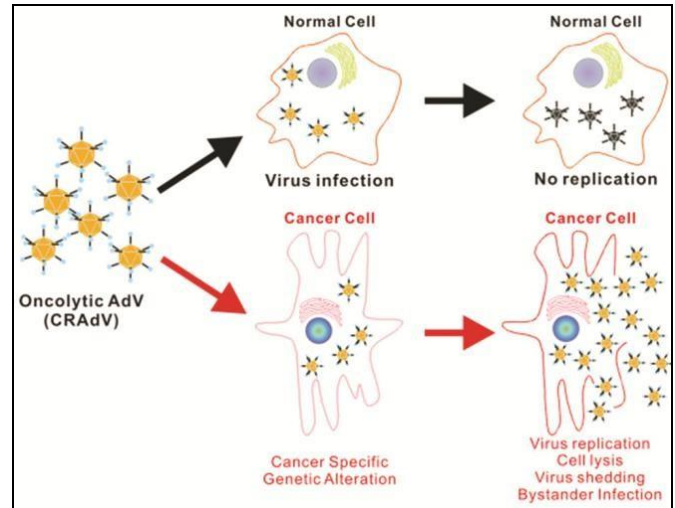


Fig 2: Treatment of cancer by AAV method

Population. After entering the CNS ganglia by retrograde axonal transport, epidemic viruses enter latency as episomes. When activated, they re-enter the epithelium through anterograde delivery from the ganglia and replicate. Because it causes worry system delay, HSV infection may be permanent. The herpes simplex virus (HSV) is a DNA molecule with two strands and separate series of statements that can be accompanied by terminal repetitions and linked by internal repetitions of the roughly 80 genes found in the viral genome, 50% are deemed superfluous.

Gene expression systems that can be regulated for gene therapy

The region, duration, and quantity of gene expression are three of the many factors that decide the efficacy of gene therapy. In order to control the gene's expression at certain times, regulatory methods were created for use in both laboratory and living organism settings. For the present being, the most widely used and adaptable instrument is the tetracycline law tool. Table 2 displays the specificity of control of transgene expression with respect to time.

The capacity to regulate gene expression was first postulated for steroid-responsive promoters, promoters that responded to heat shock, metal ions, interferons, or double-stranded RNA, and other physiological and environmental changes. A lac operator-IPTG-based machine and an FKBP12-rapamycin-related protein/FK106 binding protein have recently become the subjects of *in vitro* and *in vivo* investigations. Right now, these structures aren't good candidates for therapeutic gene therapy because the prevalence of abnormalities in them. On the other hand, it seems that the steroid hormone receptor regulating device may be similarly studied and may have gene therapeutic applications potential.

The mammalian proteome is dominated by the vast majority of transcription factors, including steroid hormone receptors. The epithelium and plasma membranes are consistently crossed by endogenous steroid receptor ligands. Translocation of ligand-receptor complexes from the

cytoplasm to the nucleus allows ligands to regulate gene expression. However, there are a few downsides to techniques that regulate steroid hormone receptors. Aim gene inducers and repressors may influence inside cells, the expression of genes. Alterations to the body's physiology

that manifest as ligands may, on the one hand, also influence the expression of the right genes for the right music. An examination of steroid hormone receptor issues regulation have prompted some businesses to provide solutions.

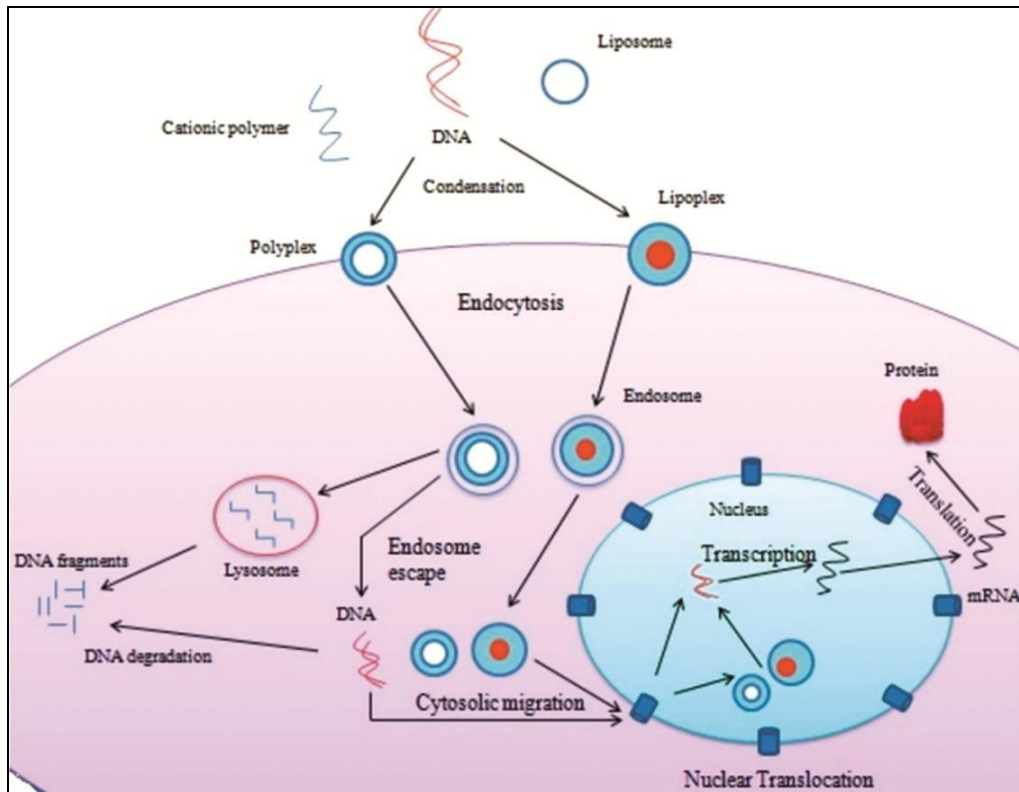


Fig 3: Gene Delivery through Nonviral Vector-Mediated

Table 2: Temporal specificity: regulation of transgene expression

Regulating stimulus	Effector molecules	Benefits	Limitations	Ref
Extrinsic stimuli	Tetracycline response element or nuclear hormone receptor elements in a chimeric transcription factor	obtainable in a wide range of viral vectors	It's challenging to get the right dose of regulator medications and apply them at the right time.	43
Dimerizer drugs	Heterodimeric transcription factor	Improved versions have less promoter leakiness.	Concerns about HIV-based systems' safety	44
Intrinsic stimuli	cytokine-inducible gene promoter elements	Remitting-relapsing illnesses are covered.	Experimentation data is scarce.	45
Hypoxia	Hypoxia-inducible transcription factor	Disease activity influences the level and duration of gene expression.	Concerns about HIV-based systems' safety	46

Gene therapy in the treatment of Gaucher disease

Gaucher disease (GD), the most common autosomal recessive lysosomal storage disorder, is defined by a decrease in the α -glucosidase (glucocerebrosidase). The accumulation of glucocerebrosides in macrophages is produced due to abnormalities in the acid glucosidase gene, which causes an inadequate synthesis of the glucocerebrosidase enzyme. A subset of macrophages called "Gaucher cells," which could be carrying glucocerebroside, are the hallmark of the disease. You may find big, wrinkled cells called Gaucher cells in the bone marrow and spleen. They store glycolipids. When treating GD, enzyme opportunity remedy (ERT) is the standard of care. Unfortunately, the recombinant enzyme is very expensive, and patients cannot afford to have it infused into their veins twice a week only to alleviate stress. Another kind of treatment is substrate reduction therapy (SRT), which entails using ceramide glucosyltransferase inhibitors to decrease glucosylceramide production. But it seems like SRT is more dangerous and pointless than ERT.

Future challenges

Gene therapeutic options have been advanced thanks to the identification of specific metabolic disorders that underlie neurological ailments. Additionally, Novel, highly targeted vehicles will be developed for the treatment of neurological disorders as basic mobile biology, ex vivo mobile refurbishment, and transplantation procedures improve. There has to be an effort to fix the technical issues with viral vector systems. Important areas of research for the future include transduction efficiency, infection prevention, and long-term controllable expression. There is growing hope that gene therapy may one day be able to cure neurological disorders and anomalies, thanks to creating lentiviral vector systems with excellent transduction efficiencies in brain cells.

Future scope

When considering recent advances and future possibilities, gene therapy has surpassed all other technological fields in terms of intrigue. In the coming decade, new areas of medicine will be made possible by technologies that were unthinkable even a decade ago, such as specific human genome change, immune system management, and unprecedented levels of control over the distribution of nucleic acids. At the same time, fresh academic disciplines including synthetic biology, reprogramming cells, and practical genomics based on high-throughput sequencing have emerged in response to this first sight of a vast array of technological possibilities, promising to further transform the biomedical research landscape. In the not-too-distant future, gene treatment may provide a viable therapeutic option for a range of illnesses. Roughly four thousand medical conditions have their roots in inherited genetic defects. If any of these genetic abnormalities can be corrected by gene editing or replacement, those who suffer from them may enjoy longer, healthier lives without symptoms and medical costs. To be successful, a viral-vector gene therapy must be able to evade the many different types of human immune system defenses. Transgenic products, viral capsids, and viral vector DNA may all be mistaken for foreign invaders by the immune

system, which would then have several opportunities to eliminate the gene therapy. The efficiency of gene therapy might be limited if the patient develops an immunity against viral capsids. As most viral-vector gene treatments now utilize vectors derived from innocuous viruses that are common in humans, many patients (up to 60%) may already have protection from previous exposure. Despite the fact that the impact's clinical result differs depending on the vector serotype, many medical trial organizers exclude participants with antibodies to the problematic vector from their studies. The broader population of patients may also end up ineligible for treatment as a result of this. Received immunity to viral vectors is one of the long-term difficulties with viral-vector gene therapy. Future 2D gene treatments will be inaccessible to patients treated with gene therapy today if the same viral vector is used in all cases.

Conclusion

In a single study that used PCR and DNA sequencing, respectively, 5.5% (10 out of 188) of the water samples for drinking and 22.2% (10 out of 45) of the water samples from rivers were found to contain ad DNA. The same researchers also found Ad DNA in 11–22 percent of the treated pool water samples that met all established standards. There are a variety of ways to disable ads, even though they are quite stable. Free chlorine in water supplies successfully deactivates ads, including Ad5. One comprehensive investigation using Ad8, which may cause epidemic keratoconjunctivitis, indicated that surfaces in the environment might be disinfected by washing with a combination of ethanol and quaternary ammonium compounds plus 1,900 parts per million of chlorine. After India earned its independence in 1947, there was a surge in support for reviving traditional medical practices. In order to provide health care to the country's population, the systems were officially recognized and integrated into the National Health Care Network. In order to address the country's primary healthcare needs, the Indian government has implemented many measures to elevate genetic engineering to the position of a leading healthcare system. One highly targeted strategy, gene targeting, involves creating novel genetic alterations by use of vector sequences that are homologous. "Gene addition" is the opposite of this process; it happens when a vector becomes an additional chromosomal episome or integrates at nonhomologous chromosomal sites, and it usually contains an expression cassette genomic locations.

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