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REVIEW ARTICLE

Developing non-viral or viral vectors for efficient and targeted delivery of genetic material, such as DNA or RNA, for gene therapy applications

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ABSTRACT

Gene therapy has emerged as a promising approach for treating a wide range of genetic and acquired diseases by introducing or modifying genetic material within cells. To achieve successful gene therapy, efficient and precise delivery of genetic material, such as DNA or RNA, to target cells is essential. This abstract explores the development of both non-viral and viral vectors for the delivery of genetic material in gene therapy applications. Non-viral vectors, including lipid nanoparticles (LNPs), polymer-based carriers, and cell-penetrating peptides, have gained significant attention due to their safety profile and ease of production. These vectors are designed to protect genetic material from degradation, facilitate cellular uptake, and release the cargo at the desired location. Recent advancements in nanotechnology have enabled the design of customizable non-viral vectors with enhanced delivery efficiency and reduced off-target effects. Viral vectors, on the other hand, harness the natural infectivity of viruses to transport genetic material into target cells. Retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses (AAVs) are commonly used viral vectors in gene therapy. Viral vectors offer high transduction efficiency but may trigger immune responses or pose risks of insertional mutagenesis. Efforts in vector engineering have led to the development of safer viral vectors with improved targeting capabilities and reduced immunogenicity. AAVs, in particular, have gained prominence due to their ability to achieve long-lasting gene expression with minimal adverse effects. Targeted delivery strategies aim to enhance vector specificity, ensuring that genetic material reaches the intended cell type or tissue. These strategies include modifying vector surface proteins, employing tissue-specific promoters, or utilizing ligand-receptor interactions. In conclusion, the successful application of gene therapy relies on the development of efficient and targeted delivery systems for genetic material. Non-viral and viral vectors offer distinct advantages and continue to evolve to meet the demands of gene therapy applications. Advances in vector design, safety, and targeting strategies hold promise for the continued progress of gene therapy as a transformative medical intervention.

KEY WORDS: DNA, Gene, Non-viral, RNA, Targeted delivery, Viral

OVERVIEW OF GENE THERAPY

Gene therapy was first proposed in the 1960s and early 1970s. Tobacco mosaic virus was the first virus to undergo genetic modification. In this same year, Rogers and Pfuderer hypothesized that DNA could be utilized to replace defective DNA in individuals suffering from genetic illnesses. In 1972, Friedman and Roblin evaluated

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the needs and dangers and recommended a moratorium. In 1975, the scientific literature reported on a failed early attempt at gene therapy.[1] In the 1990s, a patient with severe combined immunodeficiency (SCID) caused by adenosine deaminase deficiency took part in the first clinical trial of gene therapy.[2] In the year 2000, researchers used gamma retroviral vectors to introduce IL2RG cDNA into hematopoietic stem cells. They did this as part of gene therapy trials for X-linked SCID, which is caused by mutations in the IL2RG gene.[3] However, unfavorable leukemia events caused by insertional mutagenesis were described as a result of the discovery of T and natural killer cells that expressed IL2RG.[4] In addition, the first case of death linked to gene therapy was documented in 1999 in a patient with ornithine transcarbamylase deficiency who passed away from systemic inflammatory response syndrome as a result of the systemic administration of adenoviral vectors, which strongly activated the innate immune system.[5] Clinical gene therapy research stalled as a result of these unfavorable outcomes, although work on safer and more effective transgenic vectors has persisted. Gene therapy has recently attracted new attention, and clinical trials are being carried out globally. Due to its accessibility and appeal as a tissue for gene therapy, clinical trials of gene therapy have also been carried out in the dermatological sector for a number of skin illnesses. Determining the current status of this technique's utilization in the dermatological area is difficult, nevertheless, due to the wide range of disorders that have been investigated. As a result, this review concentrates on specific gene-transfer methods and details how each method is currently being used in dermatology.[1]

In the past few years, gene therapy has drawn a lot of attention as a potential cure for a variety of deadly illnesses. To control gene expression with the least amount of off-target harm, nucleic acids, their synthetic analogs (also known as nucleic acid analogues [NAA]), or genome editing proteins (particularly nucleases) are introduced into the cells in gene therapy.[6] Gene therapy thereby addresses the issue at its root. The transport of NAA and genome editing proteins to the target site, despite numerous advancements in the field of gene therapy, remains a challenge for the widespread usability of applications based on gene therapy. To get past biological barriers and deliver the payload to the action site, the delivery must be aided by synthetic, biocompatible nanocarriers.[7] In additon, various recent study about gene therapy are mentioned below.

Lin *et al*., explored the potential of F-DPC/pIL-12/iPDL-1 gene therapy against ovarian cancer. The cancer was induced to the mice by intraperitoneal injection of 5×10^6 ID8 cells. The results of the study demonstrated that the F-DPC/pIL-12/iPDL-1 gene therapy treated group exhibited 1.2-fold and 1.3-fold decrease in tumor volume as compared to vehicle control and GS treated groups, respectively. In addition, of F-DPC/pIL-12/iPDL-1 gene therapy-treated groups exhibited antitumor activity by decreasing the level of IL-6, INF-gamma, and TNF-alpha at the targeted site.[8]

Mei *et al*., explored the potential HA-TPP/A nanoparticle against gastrointestinal cancers. The GIT cancer was induced to the BALB/c nude mice by subcutaneous injection of 5 × 106 MIA PaCa-2 cells. The result showed that HA-TPP/A Nps gastrointestinal cancers treated group exhibited 16.66-fold and 44.2-fold decrease the volume as compare to control group and placebo, respectively. Similarly, HA-TPP/A Nps gastrointestinal cancers treated group exhibited 6-fold and 4-fold decrease the tumor weight as compared to placebo and control group, respectively.[9]

Lan *et al*., the DW closure potential of topical formulation of matrix mettaloproteinase-9 (MMP-9) siRNA-loaded hydrogel was checked in SD diabetic rats. The results showed that MMP-9 siRNA-loaded hydrogel-treated groups showed decrease in wound area of DW by 1.61-fold, 1.24-fold, and 1.23-fold as compared to diabetic control, placebo hydrogel, and MMP-9 siRNA solution-treated groups, respectively. Furthermore, MMP-9 siRNA-loaded hydrogel-treated group demonstrated 2.6-fold, 3.4-fold, and 3.8-fold faster collagen deposition at wound site as compared to MMP-9 siRNA solution, placebo hydrogel, and DC treated groups, respectively.[10]

Lan *et al*., the treatment of DW in SD diabetic rats with G-EDA siRNA-based therapy exhibited 1.5-fold and 1.6-fold faster wound closure in comparison to G-EDA and DC groups, respectively. In addition, G-EDA siRNA-treated groups exhibited 1.5-fold and 1.3-fold decrease in MMP-9 mRNA levels by in comparison to G-EDA and DC groups, respectively.[11]

Diseases treated with gene therapy drugs

Gene therapy has demonstrated significant success in addressing a variety of genetic disorders. Notably, SCID has been effectively treated by introducing functional IL2RG genes, allowing patients to develop functional immune systems and protect against severe infections.[12] Hemophilia, a hereditary bleeding disorder, has shown promise with gene therapy reducing the need for frequent clotting factor infusions, offering better control over bleeding episodes.[13] Leukodystrophy, which affects the nervous system's myelin sheath, is being explored as a candidate for gene therapy, aiming to correct underlying genetic mutations.[14] Inherited retinal diseases, such as Leber congenital amaurosis and retinitis pigmentosa, have been targeted successfully with gene therapy, as seen with Luxturna. Spinal Muscular Atrophy has witnessed remarkable progress with gene therapy, halting disease progression, and restoring motor function in some young children using Zolgensma.[15] Beta-thalassemia and sickle cell anemia are also under investigation for gene therapy, offering potential cures, while cystic fibrosis research is

advancing toward correcting CFTR gene mutations. These developments mark promising strides in treating genetic disorders through gene therapy.[16]

Various tools have been created to carry a therapeutic gene to the specific place where it's needed. These tools, known as gene-transfer vehicles, can be classified into two main groups: viral and non-viral vehicles, as shown in [Figure 1 and detailed in Table 1].

NON-VIRAL VECTORS

By adding new genetic material into a cell, it is possible to modify nearly any part of the cell's genetic instructions, including all the sequences in the genome and all the different types of RNA in the cell, both coding and non-coding, for the purpose of treatment. In the past two decades, scientists have been exploring how gene-based therapies can be used to treat or prevent a wide variety of diseases.[17] However, various technical barriers have limited clinical trial success. The creation of safe and effective delivery vectors is a major technical challenge for gene-based treatment. For systemic delivery, clinical studies use both non-viral and viral vectors.[18] In actuality, modified viruses such as retroviruses, lentiviruses, adenoviruses, and adeno-associated virus (AAVs) have been used in about 70% of gene therapy clinical trials to date. Despite considerable advances in gene therapy, viral vectors have a number of limitations, including the ability to induce cancer, immunogenicity, wide tropism, low DNA packing capacity, and difficulty in generating them.[7] Many of these restrictions, particularly in terms of safety, may be solved by non-viral gene therapy. For example, unlike certain viral systems, patients do not often have pre-existing immunity, and synthetic "vehicles" have lower immunogenicity than viral vectors.[19] Non-viral vectors are frequently easier to develop than viral vectors and can carry bigger genetic payloads. A variety of artificial vectors

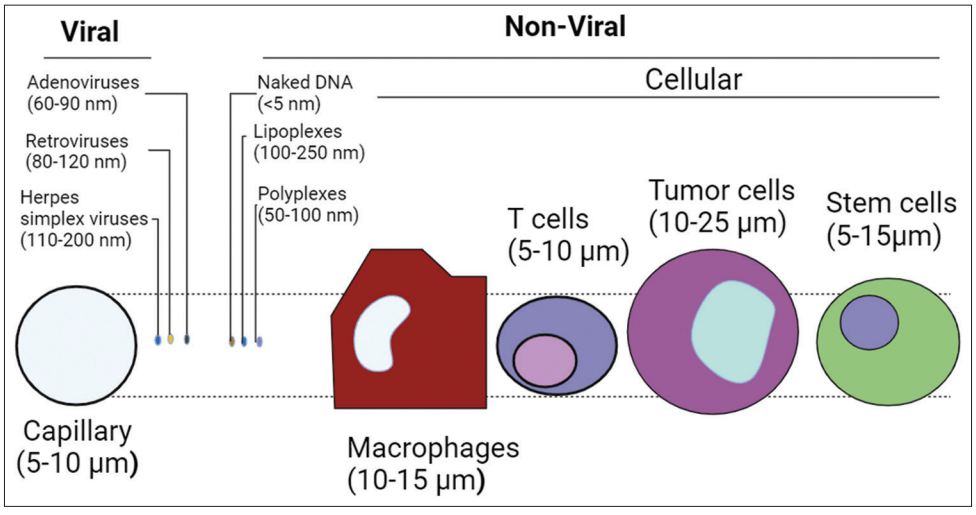

Figure 1: In this diagram showing different vehicles used to transfer genes into the body, and their sizes compared to a small blood vessel called a capillary. These gene transfer vehicles vary in size, as shown on the right side of the figure. Because they are different sizes, we need different strategies and methods to make sure these gene vehicles go where we want them to in the body to have the intended therapeutic effect. Naked DNA, which means just using DNA without any special carriers, is not the best choice for precise targeting

Table 1: Characteristics of gene therapy vehicles

Vehicle	Packaging capacity	Ease of production	Transfection efficiency	Genomic integration	Transgene expression	Immunogenic
Adenovirus	30 kb	+/-	++	No	Transient	Yes
Retrovirus	8 kb	+/-	+	Yes	Stable	No
Herpes simplex virus	30-50 kb	+/-	+	No	Transient	Yes
Lipoplex	Unlimited	++	+/-	Infrequently	Usually transient	No
Polyplex	Unlimited	++	+/-	Infrequently	Usually transient	No
Cellular vehicle	Unlimited	+	-	Yes/No	Usually transient	Only after heterologous transplantation

have been developed to transport therapeutic nucleic acids to their sites of action.[20]

Lipid-based vectors

One of the most often utilized non-viral gene carriers is a lipid-based vector. The ability of liposomes made of the phospholipid phosphatidylserine to entrap and transfer SV40 DNA to monkey kidney cells was first demonstrated in 1980.[21] Therapeutics based on small molecules and macromolecules have both been delivered using liposome formulations. Liposomes are hydrophilic polar head groups and hydrophobic tails on a spherical delivery device. Water-soluble substances can be effectively encapsulated in liposomes' hydrophilic core and water-insoluble substances in their lipid membrane.[22] N-[1-(2, 3-dioleyloxy) propyl] the first cationic lipid used to create liposomes was -N, N, N-trimethylammonium chloride (DOTMA). In comparison to calcium phosphate and diethylaminoethyl-dextran, it demonstrated 5 to 100-fold higher transfection efficiency and about 100% trapping of plasmid DNA.[23] Lipoplexes are DOTMA-based liposomes that bind electrostatically with nucleic acids to form compounds with them. Along with lipid chemistry optimization to create stable, ideal-sized liposomes, several attempts have been made to understand the entrapment and release of nucleic acids from the lipid bilayers.[24] However, liposomes attach non-specifically to serum proteins due to their cationic nature and are harmful.[25] Helper lipids like 1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine,[26] cholesterol,[27] phosphatidylcholines,[28] and polyethylene glycol (PEG) ylating lipids[29] have become popular as a result. These lipids have the ability to decrease the surface charge of cationic lipids, provide stability to LNPs, and decrease reticuloendothelial system.[30]

Polymer-based vectors

A different class of non-viral DNA vectors, cationic polymers, are appealing in part due to their tremendous chemical variety and their capacity for functionalization. Poly (l-lysine) (PLL) and polyethylenimine (PEI) are two early examples of polymeric DNA vectors. PLL, a homopolypeptide of the fundamental amino acid lysine, has been known to condense DNA at least since the 1960s.[31] Back in the late 1980s, scientists thought about using PLL attached to a special glycoprotein called asialoorosomucoid for delivering genes to the liver without using viruses.[32] However, PLL by itself does not work very well for carrying genes into cells. This is because PLL has a hard time doing its job without some help, like lysosomal disruption agents such as chloroquine. The reason for this is that the amine groups in PLL tend to be positively charged at the pH level found in our bodies, and they do not have a strong ability to deal with the acidic environment in the endosomes where they need to go.[20] In addition, unaltered PLL exhibits quite noticeable *in vitro* cytotoxicity. There have been many reported modified PLL variants with improved gene delivery capabilities. PLL is one example, which is coated in the hydrophilic polymer PEG to reduce non-specific interactions with blood components and lengthen circulation time.[33]

Nanoparticles

Nanocarriers are materials with a diameter of 1–100 nm that can transport a wide range of pharmaceuticals and/or imaging agents. Due to their large surface-to-volume ratios, it is possible to target molecules with high ligand densities on the surface.[34] Polymer conjugates, polymeric nanoparticles, lipid-based carriers such as liposomes and micelles, dendrimers, carbon nanotubes, and gold nanoparticles, which include nanoshells and nanocages, are all part of the group of nanocarriers.[35] Nanocarriers have various uses, like delivering medicines precisely to specific areas, aiding in medical imaging, using heat to treat tumors, increasing the effectiveness of radiation therapy, studying cell death (apoptosis), and mapping sentinel lymph nodes.[36] Every area of medicine has seen a constant increase in interest in the topic of nanoparticles. Nanoparticles are generally believed to be beneficial in improving therapeutic index and providing improved healthcare services along with higher patient satisfaction, particularly due to lower side effects.[37] Nanocarriers play a significant role in the improvement of medicinal formulations. For presently used medications, they could be able to increase bioavailability while decreasing dosage and frequency requirements.[38] The advancement of nanocarriers allows for several benefits, including delivering drugs precisely to a particular spot, controlling the release of drugs at that spot, easily passing through protective barriers in the body, providing combination therapies with multiple drugs for diseases, and improving the stability and capacity of carrying drugs.

Nanomaterials and special compounds with metal atoms are used to make tiny particles for delivering medications. One example is the formulation of paclitaxel as nanoparticles carried by serum albumin, known as nanometer-sized albumin-bound paclitaxel or Abraxane. This approach offers a new way to treat metastatic breast cancer.[39]

Furthermore, evaluation of Abraxane is crucial in phase I, pharmacokinetics, and phase II trials for a number of diseases, including advanced non-hematologic malignancies and non-small-cell lung cancer. Gene-based cancer therapy uses new TPGS-b-(PCL-ran-PGA) nanoparticles with PEI vectors.[40]

Investigations into the expressions of TRAIL and endostatin, which are suitable candidates for *in vivo* gene delivery to cancer cells, are conducted using plasmids transferred to HeLa cells through nanoparticles together with RT-PCR and western blot analyses.[41]

VIRAL VECTORS

Recombinant viruses with the ability to insert DNA or RNA into the host cell serve as the basis for viral vectors, which are used to transport genes to cells. Adenovirus, AAV, retrovirus, and herpes simplex virus are the most often employed viruses in gene therapy. Despite widespread knowledge of the use of viral vectors due to their high levels of transfection effectiveness, drawbacks associated with specific viruses necessitate using them sparingly to prevent adverse reactions or immune responses.[42]

Scientists transport specific genes to the brain by altering viral carriers, mainly using AAVs in clinical trials for central nervous system diseases. However, there is an exception in lentiviral vectors, which can carry larger DNA segments but have been used in only one clinical trial for this purpose until now.[43] Based on their capsid characteristics, AAVs can be categorized into serotypes. In the brain, viral vector particles can move anterogradely (through the transduction of cells in a region that receives projections from the injection site) or retrogradely (through the transduction of cells in a region that sends projections to the injection site). In preclinical tests, the transduced species and the serotype determine how this transfer occurs.[44]

Retroviruses

Among the several gene-transfer vectors created, retroviral vectors are the most effective. Retrovirus integration into dividing cells has many benefits for gene-based cancer therapy.

Retroviral vectors are also thought to be suitable vectors for ongoing insertion of foreign DNA into the target cells due to their high *ex vivo* transduction effectiveness. Retroviral vectors are subject to a variety of potential limitations, though.[45]

Adenovirus

Utilizing more flexible vectors became necessary because to the limitations of recombinant retroviral vectors. The ability of a type of double-stranded DNA viruses known as adenoviruses to efficiently transduce a wide range of cells, regardless of the cell's mitotic phase, places them in need of additional research.[46] The recombinant viruses' cellular toxicity and cell death are greatly reduced by their intact genes. Due to an immunological reaction to viral antigens, the length of gene expression is therefore constrained, and the virus may not be able to be supplied to recipients who have already received treatment.[47] Increased knowledge in the field of virology led to the introduction of several suitable and relevant viruses with unique properties for gene therapy. The majority of the viral genome must be changed with DNA encoding a gene that may be therapeutic for the AAV to be able to reproduce. As a result, there is a lower likelihood that this virus may cause an immunological reaction.[48] In addition, it can infect both dividing and no dividing cells, just like hematopoietic cells, and is used in cystic fibrosis gene therapy. However, there are issues with this vector as well, which are mostly brought on by the removal of the viral genome.[49]

AAV

In the past, a preparation for simian adenovirus type 15 had an unintended contaminant called AAV, which was discovered back in the 1960s. Since then, Glybera (alipogene tiparvovec) became the first officially approved gene therapy medicine, and AAV has been developed into a viral carrier widely used in clinical treatments.[50] AAV belongs to the Parvoviridae family and falls under the depend parvovirus category. There are at least 12 naturally occurring types, and each of them has a preference for specific tissues in the body. Gene therapy using AAV vectors takes advantage of these preferences to target specific locations. The prevalence of AAV antibodies in the human population varies, with numbers ranging from 15% to over 90% depending on factors such as the AAV type and the group being studied. For instance, in a study of a Chinese population, 96.6% had antibodies against AAV2, 82% had antibodies against AAV8, and 40.2% had antibodies against AAV5.[51]

Infections with AAV usually do not show any symptoms, and they can stick around in the body for a long time. Some other viruses, such as adenoviruses (like AdV5), papillomaviruses (such as human papillomavirus type 16, HPV-16), and members of the herpes virus family (like HSV-1 and human cytomegalovirus, HCMV), as well as other viruses such as baculovirus and human Boca virus 1, can help AAV replicate. Interestingly, AAV replication can also be triggered when cells infected with AAV are exposed to physical or chemical agents that can cause cancer. This means that AAV does not solely rely on other viruses but can also use changes in the cellular environment to replicate.[52] Without the aid of helper proteins, AAV inserts its genome into the host cell, where most copies are quickly removed but others remain for a long time. Long-term persistence is thought to most frequently manifest as an episomal, circular shape. On confections with a helper virus, latently persistent AAV reactivates, resulting in the creation of progeny virus. Section 1.2 below provides more information about AAV biology. Since AAV2 has been the subject of the most research of any AAV serotype, it is the main focus of this article.[53]

Herpes simplex viruses (HSV)

The herpes simplex virus is a large, double-stranded DNA virus that has drawn attention for its unique capacity to cause latent infection in the brain. Therefore, it is used to introduce altered genes into neurons, which may then help

with gene-based brain cancer treatments.[45] As previously indicated, a number of different herpes viruses, including HCMV, HSV-2, VZV, or HHV-6, can serve as helpers for AAV. In contrast to HCMV and HHV-6, which are members of the subfamily Betaherpesvirinae, HSV-2 and VZV are members of the subfamily Alphaherpesvirinae, like HSV-1.[54] It might seem reasonable to think that herpes viruses within the same subfamily could provide similar support to AAV because many herpesviruses in a subfamily share genetic and structural features. Early studies involving HSV-1 and HSV-2 revealed that when AAV preparations were created in cells infected with either of these helper viruses, the results were equally successful. This suggests that the support functions of these two herpesviruses are quite alike. Moreover, when cells were coinfected with HCMV, there were signs of successful AAV replication as well.[55]

The specific helper factors for HCMV have not received extensive research attention and, consequently, remain unidentified. In contrast, the HHV-6 Rep protein, which shares similarities with the AAV Rep68/78 proteins, has been associated with HHV-6 helper functions and appears to enhance AAV replication by potentially mimicking the molecular actions of AAV Rep proteins.[56]

Interesting, it has been suggested that the coevolution of these two viruses resulted in a spontaneous genetic information transfer that led to the acquisition of the HHV-6 Rep homolog from AAV.

Limitation of viral vector and non-viral vector

Viral and non-viral vectors, integral to gene therapy, each present distinctive limitations that warrant consideration in therapeutic applications. Viral vectors, despite their efficiency in delivering genetic material, are associated with immunogenicity concerns, potentially triggering immune responses that compromise their efficacy on repeated administration.[57] Their limited cargo capacity poses challenges for accommodating larger genetic constructs, while the integration risks inherent in some viral vectors may lead to unintended genetic alterations. Scalability issues in production and the presence of pre-existing immunity further add complexity to their widespread use.[58] Conversely, non-viral vectors, though safer and more straightforward to produce, often exhibit lower transduction efficiency and transient gene expression. Challenges in achieving precise delivery to target cells within intricate tissues persist, and certain non-viral vectors may induce cytotoxic effects at higher concentrations. In addition, their *in vivo* stability may be compromised, impacting their ability to reach target cells intact. Acknowledging and addressing these limitations are imperative for optimizing the choice and design of gene therapy vectors, driving advancements in the field toward safer and more effective interventions.[59]

TARGETED DELIVERY STRATEGIES

By increasing the specificity and effectiveness of gene transfer, targeted gene therapy aims to enhance therapeutic results while minimizing negative side effects. Targeted gene therapy's fundamental principles center on understanding the disease's underlying molecular pathways and distinguishing between healthy and sick cells. For instance, the cancer cells must preferentially express a chemical that should not be present on healthy tissue to target tumor tissues.[60] Such a molecule, also known as a target molecule, should ideally be able to interact with a particular antibody or ligand and should be immune to changes or mutations both within and across individuals.[61]

A gene delivery vehicle must be created to trigger gene expression only in the cells of interest after an appropriate target molecule has been found. Transductional or transcriptional targeting is shown in [Figure 2]. Transductional targeting alters the delivery vehicle's normal interaction to ensure that the gene is exclusively delivered to the targeted cells. A thorough understanding of vehicle biology and tissue-specific receptors is necessary for this. In transcriptional targeting, the gene is put under the control of cell-specific promoters and/or enhancers to produce tissue-specific expression. The fact that only a few number of particular promoters with appropriate activity have so far been discovered hinders the latter strategy.[62]

Ligand-based targeting

A promising biomedical approach that could raise the therapeutic index of pharmaceuticals and spare healthy organs is the targeted delivery of bioactive molecules to disease locations. In addition to being accessible to drugs in the bloodstream, newly created blood vessels appear to be a particularly alluring target for *in vivo* selective localization techniques due to the importance of angiogenesis in both tumor and non-tumor diseases.

Strategies for ligand-based vascular targeting depend on finding reliable vascular indicators of disease and isolating high-affinity ligands with the right pharmacokinetic characteristics. In most instances, these ligands will also need to be altered in a way that yields therapeutic benefits *in vivo* (for instance, by coupling to a poisonous moiety). Vascular-targeting ligands may enable new biomedical imaging techniques in addition to therapeutic methods, enabling the visualization of active angiogenesis sites.[63] One way to develop therapies, as shown in Figure 3, is by delivering a bioactive substance (such as a drug, cytokine, coagulation factor, photosensitizer, or radioactive material) precisely to the site of a disease. This is achieved using a binding molecule, often a human antibody, that specifically attaches to a marker associated with the disease, such as a protein on the cell membrane or within the cells. There are two main types of therapeutic approaches: one focuses on

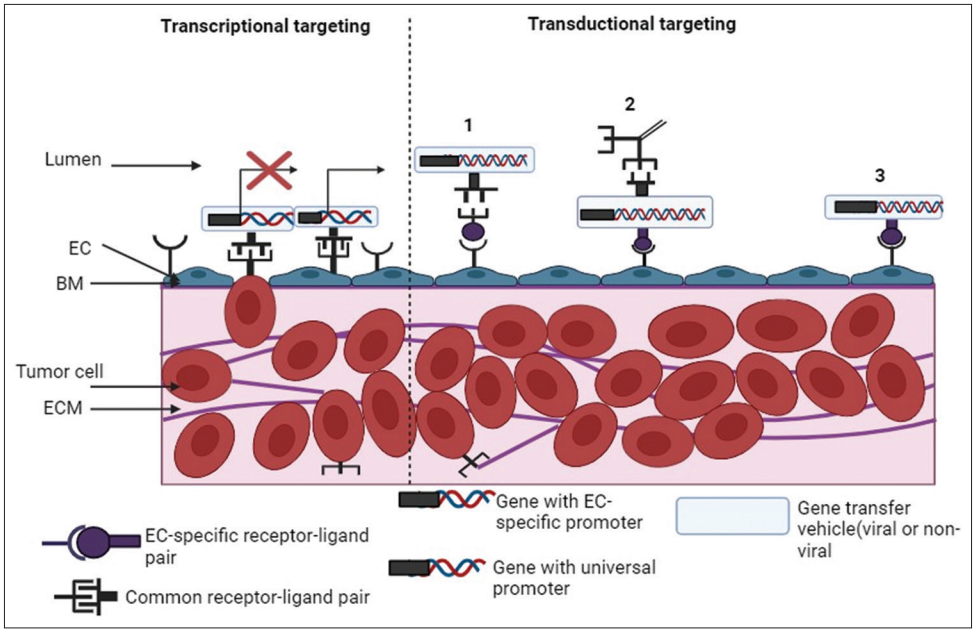

Figure 2: EC-specific transcription can be achieved using targeting techniques. By putting the gene under the control of cell-specific promoters and/or enhancers, transcriptional targeting (left) allows for tissue-specific expression of the gene. Transductional targeting (right) modifies the delivery vehicle's normal interaction to ensure that the gene is only delivered to the targeted cells. This can be accomplished in a number of ways, including the use of adaptor molecules that can inhibit the natural interaction between the vehicle's ligand and a cellular receptor while facilitating the interaction with a particular receptor on the target cell (1), the use of an antibody to inhibit the natural interaction between the vehicle's ligand and a cellular receptor (2), the expression and/or conjugation of a new ligand that is specific for the target cell (3), and the use of adaptor molecules (1)

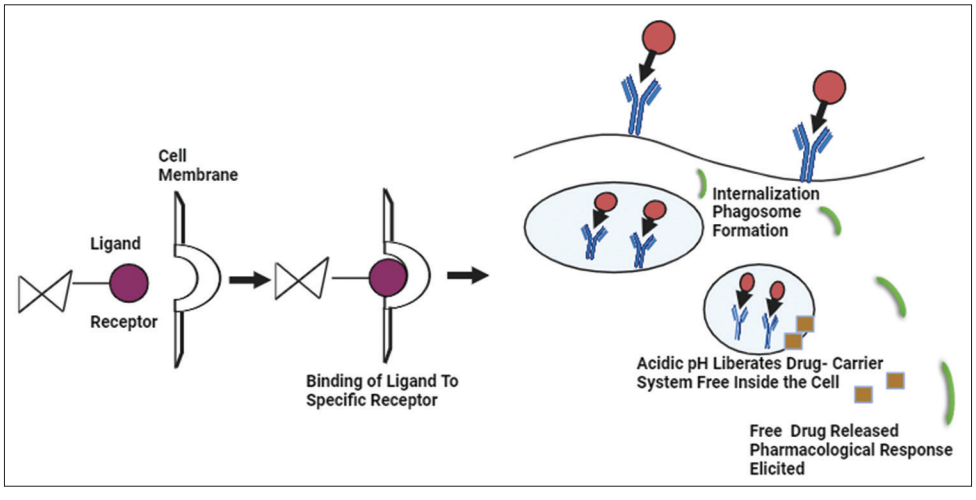

Figure 3: Site-specific delivery of a carrier system can be achieved through, conjugation of a carrier to a ligand

stopping the biological function of a specific target (like an enzyme or receptor), while the other aims to deliver therapeutic agents directly to the affected organ or tissue. In this review, we define targeting as the intentional delivery of a molecular agent to a location where the disease is present. This way, healthy tissues are spared, and the medicine accumulates in the diseased area, increasing its concentration where it is needed. This usually makes the treatment more effective with fewer side effects. Another advantage of targeted techniques is their ability to work well for both therapy and diagnosis.[64] The delivery of both a therapeutically effective molecule and an imaging agent that enables the diagnosis of the disease (an imaging effector function such as a radionuclide, fluorophore, or contrast agent) can theoretically be accomplished using the same binding molecule. This synergy also enables imaging investigations in humans and quantitative biodistribution studies in animals, which are crucial tools for characterizing disease indicators (such as tumor-associated antigens) and their related ligands (such as monoclonal antibodies).[65]

Tissue-specific promoters

A promotor is a broad term for a region that participates in the binding of the RNA polymerase necessary for the start of mRNA transcription. Each usually has a promotor that is situated 5' from the transcription start location. Although generally poorly understood, the structure-function link of the promotor area and the development of Tumor-Specific Promoters (TSPs) are both quite empirical. However, a transcription factor that is provided under tissue-specific regulation activates a promotor.[66] Therefore, a specific tissue type must express a particular factor that recognizes the promotor in order for a promotor to be activated in that tissue type. Key of regulating AD genes, like EIA, with TSPs is a method for CRAD production.[67] The genetic revolution has produced an abundance of candidates. Many TSPs have been studied for their potential in cancer gene therapy. However, some of them have proven to be not effective enough, not specific for targeting cancer cells, or lacking in both aspects. As a result, recent research has focused on thoroughly evaluating and selecting the most suitable promoter candidates. Controlling several genes, refining the promotor, and combining deletion and promotor may increase the specificity of first generation CRAD (containing single deletion or promotor for E1 regulation), as shown in below Table 2.[68]

Physical targeting strategies

Physical targeting refers to methods where greater cell concentration is achieved with the aid of suitable tools or techniques. It makes sense that delivering treatments, including cell carriers, closer to the intended target site will instantly aid in removing mechanical and physiological obstacles that lower the attained effective dose. Therefore, applying it directly to the area where the therapeutic activity is required is the easiest way to boost the local concentration of cells. This idea's seeming simplicity, nevertheless, can be deceiving.[69] Most organs cannot be directly accessed without open surgery or the use of guided tools like catheters. Even though physical targeting may be an option, there are still other aspects to take into account. For instance, even when cells are injected through catheter, heartbeat poses a significant challenge for cell retention. Therefore, even when direct cell insertion is possible, additional considerations may still restrict therapeutic efficacy.[70] Even though there are challenges in the process, delivering treatments or therapeutic carriers directly to a specific area in the body can be an effective targeting strategy. This means introducing cells directly into the tissue, bloodstream, or body cavities such as the peritoneum and pleura using tools like catheters. Positive results have been observed using these physical targeting methods to place cell carriers directly into different models involving the brain, heart, and wounds.[71] The mapping of diseased regions made possible by recent technical developments in some circumstances enables guided instruments to deliver therapeutic cells directly to the site of the disease, such as a myocardial infarction.

Cell introduction is made possible for a variety of target organs, including the heart, trachea, lung, and brain, using instrument-guided devices.[72]

Advances in genetic material delivery

The study of genetics has made significant advances in recent years, particularly in the delivery of genetic elements to target cells and animals. The capacity to transfer genetic material such as DNA, RNA, and other therapeutic agents to particular cells rapidly and accurately has transformed several disciplines, including medicine, biotechnology, and agriculture. This advancement in genetic material delivery has created new opportunities for gene therapy, genetic engineering, and personalized medicine, with the ability to treat genetic illnesses, develop innovative medicines, and better understand biological processes.[73]

DNA delivery

DNA, the blueprint of life, holds the key to an organism's genetic makeup. Advances in DNA delivery methods have ushered in a new era of possibilities. Viral vectors, synthetic vehicles derived from modified viruses, have emerged as powerful tools. These vectors efficiently ferry therapeutic genes into target cells, addressing genetic deficiencies. AAVs and lentiviruses are prime examples, demonstrating remarkable success in clinical trials for gene therapy applications. This approach offers promise in treating a diverse range of genetic disorders by providing functional copies of defective genes.[74,75]

RNA delivery

RNA, a versatile molecule with diverse functions, has garnered significant attention in genetic material delivery. Messenger RNA (mRNA) has taken the spotlight with the rapid development of mRNA-based vaccines against infectious diseases. Engineered nanoparticles, including lipid-based carriers, play a pivotal role in encapsulating and protecting fragile RNA molecules during delivery. This innovation has been showcased in the swift creation of COVID-19 vaccines, highlighting the potency of RNA-based therapeutic strategies.[76]

GENE THERAPY APPLICATIONS

Gene therapy stands as a revolutionary medical frontier, holding immense potential to revolutionize the treatment

Table 2: Natural promotor used in experimental cancer gene therapy

Promotor	Target	*In vivo* application	Reporter/ effector gene	vector	Therapeutic effect	
					In vitro	In animal model
Tissue-specific						
Tyrosinase TRP	Melanocytes	i.t.	LacZ	nv, re	-	-
		i.t.,	tk	nv	+	+
		transduced cells	tk	re	-	+
		i.v.	Luc, PNP	nv	+	-
			IL-2	re	-	-
PSA	Prostate Cancer	-	Luc	nv	-	-
		-	LacZ	nv	+	-
		i.t.	antisense tk, PNP	ad	-	+
Albumin	Liver	-	VZV-tk	re	+	-
		-	hAAt	re	-	-
		i.v.	F Vlll	ad	-	-
MCK	Muscle	Transduced cells	LacZ	re	-	-
		i.m. and other tissues		ad	-	-
		i.m. (producer)	Luc, LacZ	re	-	-
			LacZ			
MBP	Oligodendrocytes	-	LacZ, tk	re	+	-
	glial cells	Injection into brain	LacZ	ad	-	-
			GFP	AAv	-	-
GFAP	Glial cells	-	LacZ, tk	re	+	-
		Injection into brain	LacZ	HSV-1	-	-
		-	tk	nv	+	-
		i.v.	Fasal	ad	+	+
NSE	Neurons	i.v.	FasaL	ad	+	+
Tumor endothelium-directed						
KDR		-	LacZ, TNF	re	-	-
E-selectin		-	LacZ, TNF	re	-	-
		-	Luc	ad	-	
Endoglin		-	Luc	nv	-	-

landscape for a myriad of genetic disorders. By strategically introducing functional genes or modifying existing ones within the cells of afflicted patients, gene therapy aims to correct the underlying genetic anomalies responsible for previously untreatable conditions.[77]

The technique's promise lies in its ability to address the root cause of disorders rather than merely alleviating symptoms. Genetic mutations, which underlie many devastating diseases, can lead to dysfunctional or absent proteins critical for cellular function. Gene therapy aims to rectify this by delivering therapeutic genes into specific target cells. The employment of various delivery methods, such as viral vectors derived from genetically modified viruses, facilitates the transport of the corrective genetic material into the cells.[78]

Once within the cellular domain, these introduced genes act as genetic engineers, orchestrating the production of vital proteins that were either lacking or defective due to the underlying genetic defect. Notably, gene therapy's triumphs have manifested in treating conditions such as SCID and hemophilia, where the introduction of functional genes has enabled the production of immune components or clotting factors, respectively.[79]

Despite these successes, gene therapy confronts hurdles that warrant diligent research and innovation. Long-term effectiveness, ensuring the persistence of corrected genes within target cells, remains a challenge. Minimizing immune responses against the introduced genetic material and refining delivery methods to ensure precise targeting are critical objectives.[80]

Nonetheless, gene therapy's journey continues with undeterred momentum, offering the promise of alleviating the suffering of those grappling with a spectrum of genetic disorders. As scientific understanding deepens and technological capabilities evolve, the prospect of reshaping the realm of modern medicine looms larger. The path forward holds potential breakthroughs, making the once-unthinkable concept of treating and potentially

Table 3: Recent clinical trial for the treatment of Parkinson's disease

Clinical trial No.	Intervention/ *treatment*	Clinical trial status	No of patients	Start/estimated completion date	Title	Reference
NCT00427726	-	Observational	12	Mar-2003 May-2014	Follow-up of Breast Cancer and Multiple Myeloma Patients Previously Enrolled in NIH Gene Therapy Studies	[87]
NCT04286815	Lentiviral Vector Gene Therapy	Not Applicable	10	May-2020 May-2025	Gene Therapy for X Linked Severe Combined Immunodeficiency	[88]
NCT04728841	Genetic: Injection of GS001	Not Applicable	12	Mar-2021 July-2028	Gene Therapy for Chinese Hemophilia A (HA)	[89]
NCT03894852	Diagnostic Test: PCR and cytogenetics	Observational	139	June-2019 April-2020	SRSF2 Gene Mutation in Patients With t-MDS/AML	[90]
NCT03432520	Genetic: SPK-8011	Observational	40	Aug-2018 Dec-2032	Long-Term Safety and Efficacy of Spark-Sponsored Gene Therapies in Males With Hemophilia A	[91]
NCT03039751	Drug: AdvVEGF-D Drug: Control Rx	Phase 2	180	Oct-2019 Dex-2023	Adenovirus Vascular Endothelial Growth Factor D (AdvVEGF-D) Therapy for Treatment of Refractory Angina Pectoris (ReGenHeart)	[92]
NCT03311503	Biological: autologous CD34+ cell transduced with G2SCID vector	Phase 1 Phase 2	10	Jan-2018 Oct-2025	Phase I/II Trial of Lentiviral Gene Transfer for SCID-X1 With Low Dose Targeted Busulfan Conditioning	[93]
NCT04093362	• Drug: TAS-120 • Drug: Cisplatin/ Gemcitabine	Phase 3	216	Mar -2020 Dec-2023	Futibatinib Versus Gemcitabine-Cisplatin Chemotherapy as First-Line Treatment of Patients With Advanced Cholangiocarcinoma Harboring FGFR2 Gene Rearrangements	[94]
NCT04274231	Genetic: different effection	Not Applicable	100	Feb-2020 Dec-2022	Cancer-associated Gene Mutations in CML Treatment With TKIs by NGS	[95]
NCT04273243	Genetic: AT-GTX-501	Observational	10	Jan-2020 Oct-2024	Long-Term Follow Up of CLN6 Batten Disease Subjects Following Gene Transfer	[96]

curing inherited genetic conditions an increasingly tangible reality.[81]

Different methods of gene therapy

Gene therapy encompasses a wide range of techniques designed to address both genetic and acquired diseases, with the CRISPR-Cas9 gene editing method standing out as a prominent and revolutionary approach. CRISPR-Cas9 enables precise modifications to genes by targeting specific DNA sequences for additions, deletions, or corrections. This breakthrough technique holds immense promise for correcting genetic anomalies, especially in monogenic disorders. In addition to CRISPR-Cas9, RNA interference offers a potent means of gene regulation by silencing specific genes using small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs). RNA interference is particularly promising for diseases rooted in genetic misregulation.[82]

Gene therapy can be categorized into *ex vivo* and *in vivo* approaches. *Ex vivo* therapy involves extracting a patient's cells, modifying them outside the body, and then reintroducing the modified cells. This method has demonstrated success in treating various disorders. In contrast, *in vivo* gene therapy aims to modify genes directly within the patient's body, often utilizing viral and non-viral vectors to deliver therapeutic genetic material. The choice between these strategies depends on the specific disease and the intended therapeutic outcome.[83]

For monogenic disorders, where a single gene mutation is responsible for the disease, gene therapy holds significant promise as it offers the potential for a cure. Disorders such as SCID and cystic fibrosis have seen positive results in clinical trials for gene therapies targeting these specific genetic mutations. However, complex diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders present unique challenges due to their multifactorial nature. Research is ongoing to develop gene therapy approaches that can modulate gene expression, target specific signaling pathways, and bolster the body's defenses against these intricate diseases, with the potential benefits being substantial.[84] Table 3 provides a glimpse into ongoing endeavors in the treatment of Parkinson's disease.

Epigenome editing introduces a distinctive strategy for gene therapy by focusing on the regulation of gene expression through epigenetic modifications. Epigenetic marks, including DNA methylation and histone modifications, play a critical role in gene activity. Epigenome editing techniques seek to modify these marks to regulate gene expression, particularly in conditions such as cancer and developmental disorders where epigenetic regulation is pivotal.[85]

Finally, antisense oligonucleotide therapy utilizes short genetic sequences to modulate gene expression by binding to specific RNA molecules. This approach is particularly effective in conditions where targeting RNA is more beneficial than modifying DNA, as seen in diseases such as muscular dystrophy and SMA. The diverse array of gene therapy methods underscores the field's versatility and adaptability, enabling tailored approaches for different disease types and genetic abnormalities.[86]

FUTURE DIRECTIONS AND CHALLENGES

Looking ahead, the field of genetic material delivery stands on the brink of transformative advancements, driven by innovative technologies and ambitious objectives. A key focus lies in refining precision and efficacy to unlock the vast potential of genetic manipulation across diverse applications.[97] The trajectory toward achieving these goals is not without hurdles, demanding thoughtful strategies and collaborative efforts across disciplines. Future directions emphasize the need for enhanced targeting and delivery strategies, aiming for greater accuracy and specificity in reaching specific cell types within intricate tissues.[98] This pursuit relies on fine-tuning techniques and leveraging advances in nanoparticles, biomaterials, and cellular engineering. Concurrently, the persistent challenge of ensuring safety amidst technological progress remains paramount, necessitating a delicate balance between therapeutic efficacy and immunogenicity. Regulatory considerations are evolving alongside these innovations, requiring collaboration among scientists, clinicians, regulatory bodies, and ethicists to establish clear guidelines and frameworks that uphold both innovation and patient safety.[99] Meanwhile, the scalability and manufacturing challenges associated with producing therapeutic genetic materials on a larger scale underscore the need for robust processes ensuring consistent quality, purity, and stability. Addressing these challenges will be pivotal in advancing genetic material delivery methods and making novel therapies accessible on a broader scale.[100]

CONCLUSION

The realm of genetic material delivery stands at the cusp of transformative change, driven by advancements that hold immense promise for various fields, from medicine to biotechnology. The rapid evolution of DNA and RNA delivery methods has paved the way for groundbreaking applications, ranging from gene therapy to personalized medicine. Viral vectors, engineered nanoparticles, and innovative techniques have propelled the field forward, enabling the correction of genetic anomalies at their root causes.

Gene therapy, a trailblazing medical approach, seeks to correct genetic disorders by introducing functional genes into affected cells. This strategy has yielded successes in treating conditions such as SCID and hemophilia, offering renewed hope for patients with once-incurable diseases. However, the journey forward is not without its challenges.

The future of genetic material delivery envisions enhanced precision through targeted delivery strategies, where therapies are tailored to specific cell types within complex tissues. Balancing efficacy with safety remains crucial, as researchers strive to mitigate immune responses and off-target effects. Regulatory considerations and manufacturing scalability are also pivotal, ensuring that innovative therapies meet stringent standards while being accessible on a broader scale.

As the field progresses, collaborative efforts among scientists, clinicians, regulators, and ethicists will be paramount in navigating the complexities ahead. By overcoming challenges and leveraging technological advancements, the potential to revolutionize modern healthcare and scientific progress through genetic material delivery remains unprecedented. This journey promises to reshape the boundaries of medicine, ushering in a new era of personalized treatments and transformative solutions for genetic disorders.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

Not applicable.

COMPETING INTERESTS

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