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Gene Therapy: Principles, Non-Viral Vectors, and Therapeutic Applications – A Mini-Review

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Abstract

Gene therapy has emerged as one of the most promising innovative therapeutic approaches for the treatment of genetic, inflammatory, and malignant diseases. By introducing, modifying, or regulating genetic material within target cells, gene therapy aims to correct defective genes, restore cellular functions, or modulate pathological biological processes. Over the past decades, significant progress in molecular biology, genomics, and biotechnology has enabled major advances in gene transfer strategies, vector design, and clinical applications. While viral vectors have demonstrated high transfection efficiency, their safety concerns, immunogenicity, and manufacturing constraints have driven increasing interest toward non-viral delivery systems.

This mini-review provides an overview of gene therapy fundamentals, focusing on delivery strategies, mechanisms of action, and current therapeutic applications. The main approaches of gene therapy, including gene addition, inhibition of gene expression, and genome editing, are discussed alongside their respective molecular tools. Particular attention is devoted to non-viral vectors, such as lipid nanoparticles, cationic polymers, and inorganic nanoparticles, highlighting their advantages, limitations, and recent technological developments. Routes of administration, biological barriers to gene delivery, and key challenges related to targeting, efficacy, and safety are also examined. Finally, major clinical applications of gene therapy in genetic disorders, inflammatory diseases, and cancer are reviewed, with emphasis on current challenges and future perspectives.

Overall, this review underscores the growing potential of non-viral gene therapy systems as safer and more versatile alternatives to viral vectors, while emphasizing the need for continued research to overcome delivery barriers, improve targeting specificity, and ensure long-term therapeutic efficacy.

Keywords: *Gene therapy; Non-viral vectors; Lipid nanoparticles; Nucleic acids; Genome editing; Targeted delivery; Molecular medicine.*

1. INTRODUCTION

Gene therapy has emerged as a transformative therapeutic approach following major advances in molecular biology and genetics. The discovery of the human genome and the identification of genetic mutations responsible for inherited and acquired diseases have fundamentally changed the understanding of disease mechanisms. Rather than addressing only symptoms, gene therapy aims to correct or compensate for the underlying genetic cause of pathology by introducing functional genetic material into target cells [1,2].

The first clinical trials of gene therapy began in the late 1980s, motivated by the possibility of correcting genetic "errors" at their source. Although early attempts faced technical and safety challenges, they provided proof of concept and laid the foundation for current strategies. Over time, improvements in gene delivery systems, molecular tools, and regulatory frameworks have contributed to the progressive maturation of this field [3–5].

Gene therapy is defined as the therapeutic transfer of nucleic acids—DNA or RNA—into cells in order to restore, modify, or regulate gene expression. The delivered genetic sequence, often referred to as a therapeutic gene, is placed under the control of regulatory elements that

ensure its expression and biological activity. This approach can be achieved using viral or non-viral vectors and can be applied through different therapeutic strategies depending on the disease context [11,26,31].

Despite its significant therapeutic promise, gene therapy remains associated with scientific, technical, and ethical challenges. These include ensuring efficient and targeted gene delivery, minimizing toxicity and immune responses, achieving sustained gene expression, and addressing ethical concerns related to germline modification. Nevertheless, continuous progress has positioned gene therapy as one of the most innovative and promising modalities in modern medicine. The general principle of gene therapy is summarized in Figure 1.

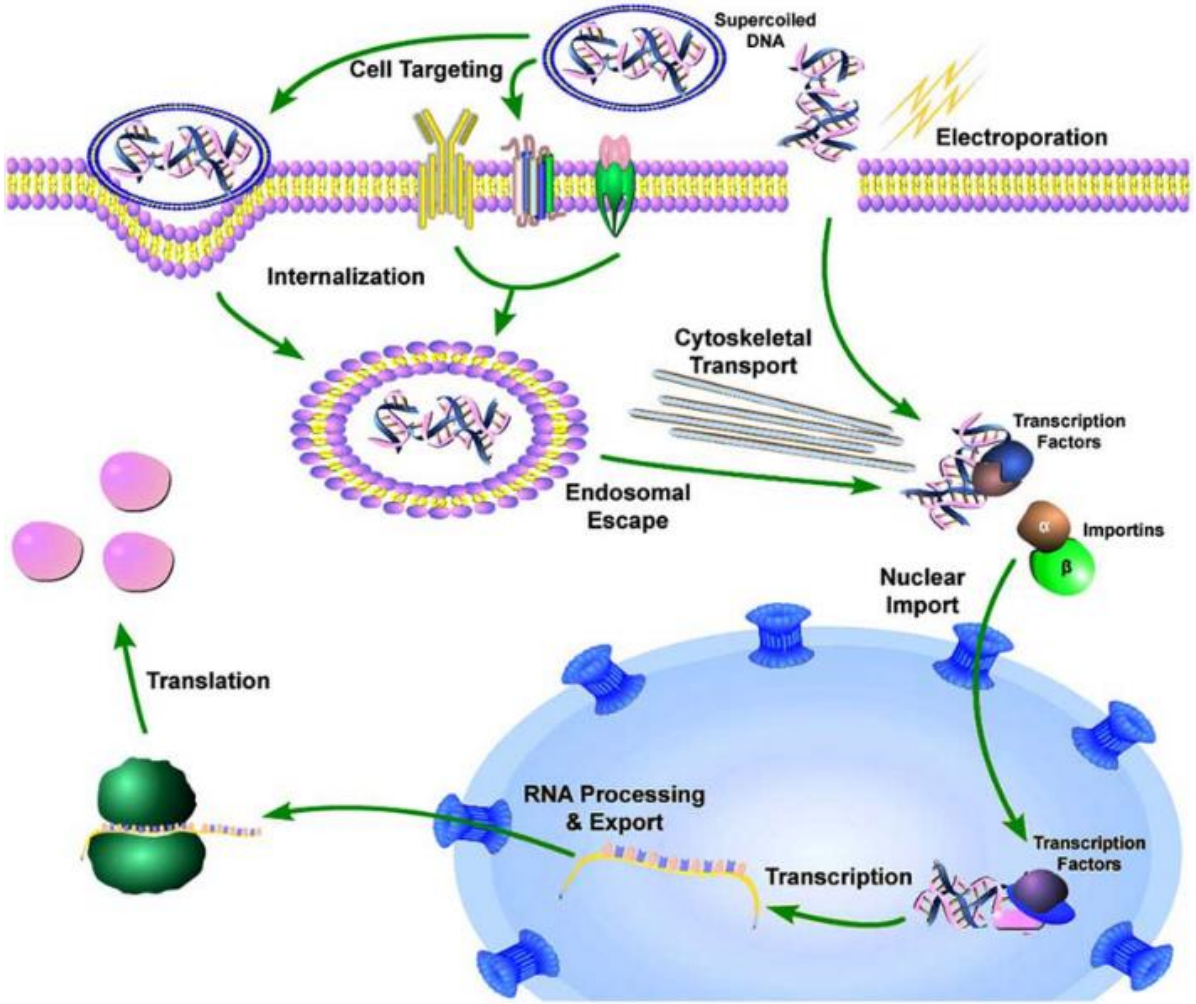

Figure 1. *General principle of gene therapy*

Gene therapy involves the delivery of therapeutic nucleic acids (DNA or RNA) into target cells using appropriate delivery systems. Once internalized, the genetic material reaches the nucleus or cytoplasm, where it enables protein expression, gene silencing, or genome modification, ultimately leading to a therapeutic effect.

2. General Overview Of Gene Therapy

2.1. Fundamental Concepts of Gene Therapy

Gene therapy is based on the manipulation of genetic information to treat or prevent disease. At the molecular level, genes are defined as specific DNA sequences located at precise loci on chromosomes within the cell nucleus.

Each gene contains the information necessary for the synthesis of functional proteins or regulatory RNA molecules that govern cellular structure, metabolism, and signaling pathways.

Under physiological conditions, genetic information is expressed through a tightly regulated process involving transcription of DNA into messenger RNA (mRNA), followed by translation of mRNA into proteins. Any alteration in the DNA sequence, known as a mutation, may disrupt this process by modifying the amino acid sequence of the protein, altering its quantity, or completely abolishing its function. Such genetic alterations are responsible for a wide range of inherited and acquired disorders, commonly referred to as genetic diseases.

The principle of gene therapy relies on introducing a functional genetic sequence into target cells in order to compensate for or correct the defective gene. The therapeutic nucleic acid may consist of a healthy copy of a gene, a regulatory sequence capable of modulating gene expression, or RNA molecules designed to inhibit or silence pathogenic genes. Once delivered into the cell, the therapeutic gene acts as a gene drug, producing the desired biological effect through cellular transcription and translation mechanisms.

Depending on the therapeutic objective, gene therapy may aim to restore protein expression, suppress abnormal gene activity, induce apoptosis in diseased cells, or stimulate an immune response. The success of these approaches depends on efficient delivery of the genetic material, its stability within the cellular environment, and appropriate control of its expression to ensure safety and efficacy.

2.2. Types of Gene Therapy

Gene therapy can be classified into two main categories according to the type of cells targeted: somatic gene therapy and germline gene therapy [9,10].

2.2.1. Somatic Gene Therapy

Somatic gene therapy involves the introduction of genetic modifications into somatic (non-reproductive) cells of an individual. In this approach, the therapeutic genetic alteration affects only the treated patient and is not transmitted to future generations. As a result, the offspring of treated individuals retain the same genetic risk of disease as the general population [8].

This form of gene therapy is currently the only approach authorized for clinical applications in humans due to its limited ethical implications. Somatic gene therapy is particularly suitable for the treatment of diseases affecting accessible tissues such as blood cells, muscle tissue, skin, and certain organs. It represents the dominant strategy in current clinical trials and approved gene-based therapies [8].

2.2.2. Germline Gene Therapy

Germline gene therapy consists of modifying the genetic material of germ cells or early-stage embryos, thereby introducing heritable genetic changes. These modifications are transmitted to all cells of the developing organism and to subsequent generations [9,10].

Although this approach offers theoretical potential for permanently eliminating genetic diseases from family lineages, it raises significant ethical, legal, and societal concerns. Consequently, germline gene therapy is currently prohibited in humans. However, it is widely used in experimental research, particularly in animal models, to study gene function and generate transgenic organisms for biomedical research and the production of biologically relevant substances [9,10].

2.3. Modes of Gene Therapy Implementation

Gene therapy may also be categorized according to the mode of gene delivery: ex vivo and in vivo approaches [11,12].

2.3.1. Ex Vivo Gene Therapy

Ex vivo gene therapy involves the isolation of cells or tissues from a patient or donor, followed by genetic modification in a controlled laboratory environment. Once the desired genetic modification is achieved and verified, the modified cells are reintroduced into the patient via local or systemic administration [11].

This strategy offers several advantages, including reduced exposure to vectors, enhanced control over gene expression, and minimized off-target effects. Depending on the source of the cells, ex vivo gene therapy may be autologous (patient-derived cells) or allogeneic (donor-derived cells). This approach is widely used in hematological disorders and oncology, where target cells are easily accessible and expandable [11].

2.3.2. In Vivo Gene Therapy

In contrast, in vivo gene therapy involves the direct administration of therapeutic genetic material into the patient's body. The nucleic acid sequence is delivered locally or systemically using viral or non-viral vectors that facilitate cellular uptake, protect the genetic cargo, and enable its functional expression. [11, 26,31]

This strategy is particularly relevant when target cells cannot be easily harvested or manipulated ex vivo. However, it requires highly efficient and specific delivery systems to avoid unintended gene expression in non-target tissues and to ensure safety [11]. The two main modes of gene therapy implementation are illustrated in Figure 2.

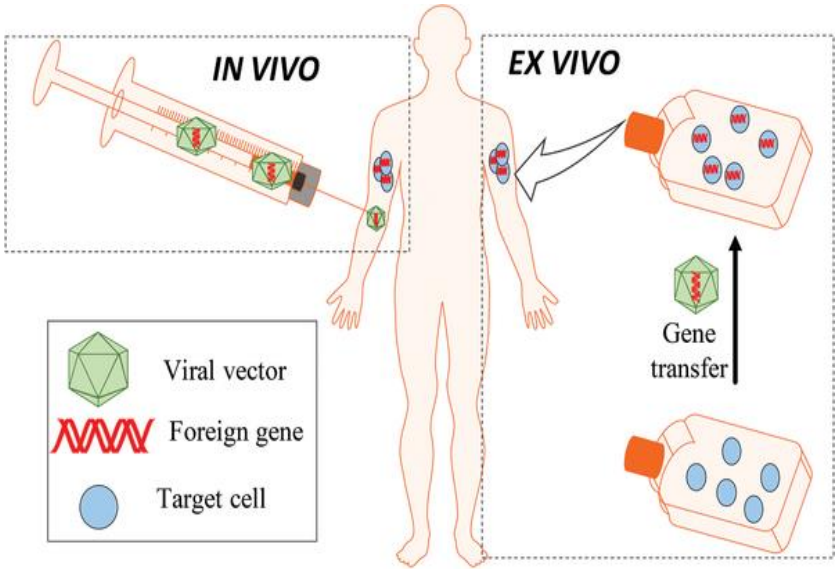

Figure 2. *Ex vivo and in vivo gene therapy approaches*

In ex vivo gene therapy, patient-derived cells are genetically modified outside the body and subsequently reintroduced. In contrast, in vivo gene therapy consists of the direct administration of therapeutic nucleic acids into the patient using viral or non-viral vectors.

3. Strategies Of Gene Therapy

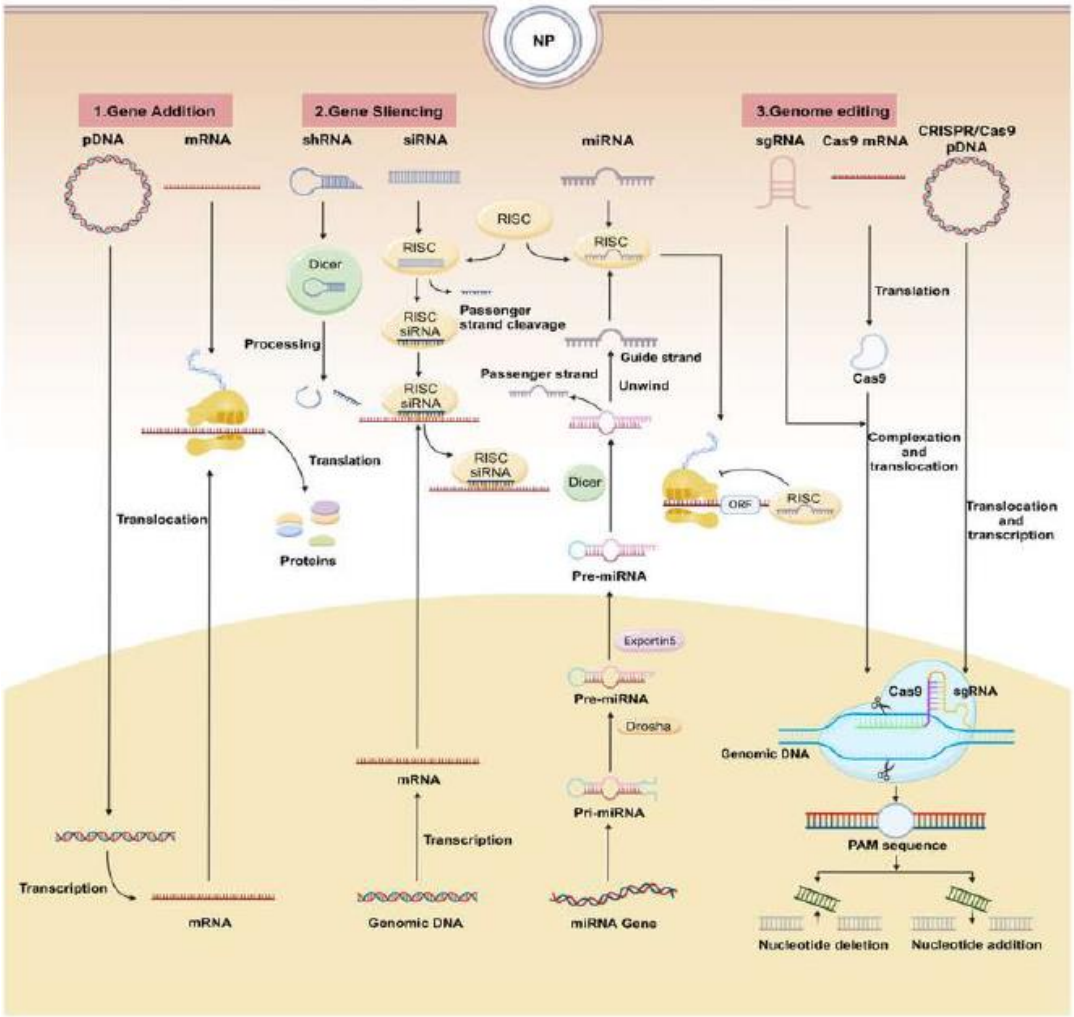

Figure 3. *Major gene therapy strategies.*

Gene therapy strategies include gene addition to restore deficient protein expression, targeted inhibition of gene expression using antisense or RNA interference approaches, and genome editing techniques enabling precise correction of genetic mutations.

Gene therapy encompasses several distinct therapeutic strategies that differ according to their molecular mechanisms and therapeutic objectives. These strategies are designed to restore normal cellular function, suppress pathological gene expression, or modify cellular behavior in a targeted manner. The main strategies of gene therapy are summarized in Figure 3.

3.1. Gene Addition

Gene addition, also referred to as gene supplementation or gene compensation, is one of the earliest and most widely used strategies in gene therapy. It consists of introducing a functional copy of a gene into target cells to compensate for a defective or absent endogenous gene. This approach allows the restoration of normal protein expression and cellular function without directly modifying the mutated gene.

Gene addition has been extensively applied in the treatment of monogenic diseases and certain cancers. In oncology, this strategy may involve the introduction of so-called suicide genes, which encode enzymes capable of converting non-toxic prodrugs into cytotoxic compounds within tumor cells. Classic examples include the herpes simplex virus thymidine kinase (HSV-tk) in combination with ganciclovir and the cytosine deaminase/5-fluorocytosine system. These strategies selectively induce apoptosis in malignant cells while sparing healthy tissues.

In addition to cancer therapy, gene addition plays a crucial role in immunotherapy. DNA- and RNA-based vaccines rely on the delivery of genetic sequences encoding antigenic proteins. Following cellular uptake, these sequences are transcribed and translated, leading to antigen presentation by antigen-presenting cells and subsequent activation of both humoral and cellular immune responses. This strategy has gained particular prominence with the development of nucleic acid vaccines.

3.2. Targeted Inhibition of Gene Expression

In cases where disease results from the overexpression or inappropriate expression of a gene, therapeutic strategies aim to inhibit or silence gene expression rather than add a functional gene. This approach relies on synthetic nucleic acid molecules designed to interact specifically with target RNA sequences.

Among these tools, antisense oligonucleotides (ASOs) are short single-stranded DNA or RNA molecules complementary to target mRNA sequences. Upon hybridization, ASOs induce mRNA degradation via RNase H activity or block translation. Their therapeutic efficacy is enhanced by chemical modifications that improve nuclease resistance, binding affinity, and cellular uptake.

RNA interference (RNAi)-based strategies, including small interfering RNAs (siRNAs) and microRNAs (miRNAs), constitute another powerful approach. These molecules trigger sequence-specific mRNA degradation through intracellular silencing complexes, effectively suppressing pathological gene expression. Targeted inhibition of gene expression has shown particular promise in oncology, where oncogene silencing can limit tumor progression.

3.3. Genome Editing

Genome editing represents a more recent and highly precise gene therapy strategy aimed at directly correcting genetic mutations at the DNA level. Rather than adding or silencing genes, genome editing enables targeted modification of specific genomic sequences.

One widely explored approach involves exon skipping, which uses antisense oligonucleotides to modulate pre-mRNA splicing. By masking specific splice sites, mutated exons are excluded from the mature mRNA, leading to the production of a shorter but partially functional protein. This strategy has been successfully applied in diseases such as Duchenne muscular dystrophy [12, 46,47].

Advanced genome editing tools, including meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR-Cas9 system, allow site-specific DNA cleavage and subsequent repair. These technologies offer unprecedented precision and efficiency but remain associated with challenges related to off-target effects, delivery, and ethical considerations.

4. Nucleic Acids Used In Gene Therapy

The different types of nucleic acids used in gene therapy are presented in Figure 4.

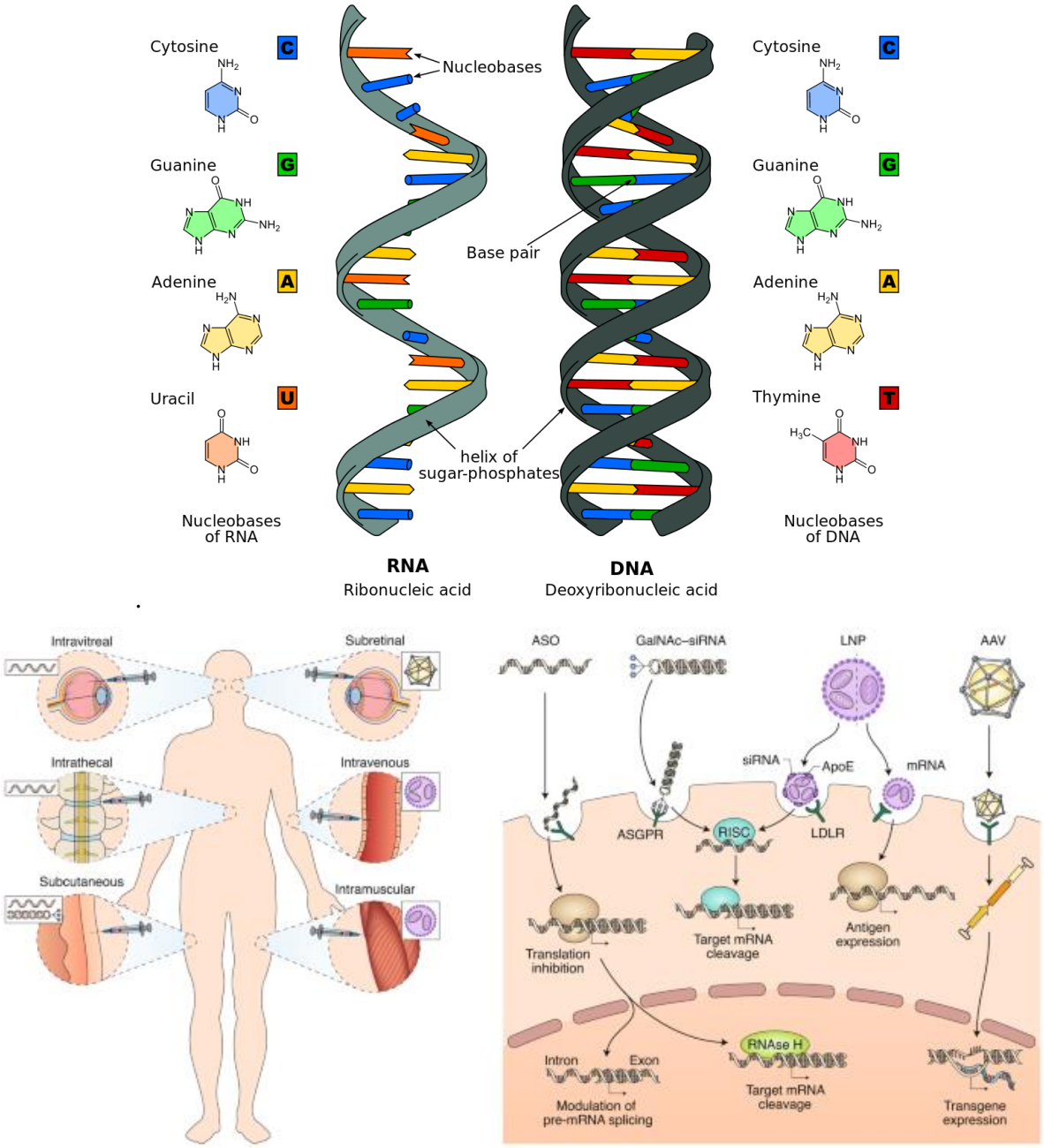

Figure 4. *Nucleic acids used in gene therapy*

Therapeutic nucleic acids include plasmid DNA, messenger RNA, antisense oligonucleotides, and regulatory RNAs such as siRNA and miRNA, each fulfilling specific therapeutic functions in gene replacement or gene regulation.

Nucleic acids constitute the fundamental therapeutic agents in gene therapy. Depending on the therapeutic strategy, either DNA or RNA molecules may be employed to restore, regulate, or suppress gene expression. Each type of nucleic acid presents distinct structural, functional, and biological properties that influence its therapeutic application.

4.1. DNA: Structure and Function

Deoxyribonucleic acid (DNA) serves as the primary carrier of genetic information in living organisms. It contains the complete genetic blueprint required for the synthesis of proteins and the regulation of cellular functions.

Structurally, DNA is composed of repeating nucleotide units, each consisting of a deoxyribose sugar, a phosphate group, and one of four nitrogenous bases: adenine, thymine, cytosine, or guanine.

DNA adopts a characteristic double-helical structure formed by two complementary antiparallel strands stabilized through hydrogen bonding between base pairs (adenine–thymine and cytosine–guanine). This configuration

ensures the faithful replication of genetic information during cell division and the long-term stability of the genome.

In gene therapy, DNA is commonly delivered in the form of plasmids or recombinant vectors containing an expression cassette. This cassette typically includes the therapeutic gene, a promoter sequence to control transcription, and regulatory elements required for efficient gene expression in eukaryotic cells. Once inside the nucleus, the DNA may remain episomal or, less frequently, integrate into the host genome, leading to sustained transgene expression.

4.2. RNA: Structure, Function, and Therapeutic Roles

Ribonucleic acid (RNA) plays a central role in gene expression by acting as an intermediary between DNA and proteins. Unlike DNA, RNA is generally single-stranded and contains ribose as its sugar component, along with uracil instead of thymine. Despite its single-stranded nature, RNA can adopt complex three-dimensional structures essential for its diverse biological functions. Several types of RNA are exploited in gene therapy:

4.2.1. Messenger RNA (mRNA)

Messenger RNA (mRNA) is transcribed from DNA and serves as the template for protein synthesis in the cytoplasm. In gene therapy, synthetic mRNA molecules encoding therapeutic proteins are delivered directly to cells, bypassing the need for nuclear entry. This approach offers rapid protein expression and eliminates the risk of genomic integration, making mRNA-based therapies particularly attractive for vaccination and transient protein replacement.

4.2.2. Transfer RNA and Ribosomal RNA

Transfer RNA (tRNA) and ribosomal RNA (rRNA) play essential roles in protein synthesis by transporting amino acids and forming the ribosomal machinery, respectively. Although they are not directly used as therapeutic agents, their biological functions are crucial for the translation of therapeutic mRNA delivered in gene-based treatments.

4.2.3. Regulatory RNAs: miRNA and siRNA

MicroRNAs (miRNAs) and small interfering RNAs (siRNAs) are short RNA molecules involved in post-transcriptional regulation of gene expression. miRNAs bind partially complementary sequences on target mRNAs, leading to translational repression, while siRNAs induce specific degradation of target mRNAs via the RNA-induced silencing complex (RISC).

These regulatory RNAs are valuable tools for targeted gene silencing in diseases characterized by aberrant gene expression, such as cancer and viral infections.

4.2.4. Antisense Oligonucleotides and Other Non-Coding RNAs

Antisense oligonucleotides (ASOs) are synthetic nucleic acid sequences designed to hybridize with specific RNA targets, thereby modulating splicing or inhibiting translation. Other non-coding RNAs, including small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs), participate in RNA processing and maturation and contribute indirectly to therapeutic gene expression.

5. Non-Viral Vectors In Gene Therapy

5.1. Classification and General Principles of Non-Viral Vectors

Non-viral vectors have emerged as a promising alternative to viral systems in gene therapy due to their improved safety profile and lower immunogenicity. While viral vectors exhibit high transfection efficiency, their potential to induce immune responses, insertional mutagenesis, and long-term toxicity has driven extensive research into non-viral delivery strategies [11, 26,31].

The primary role of non-viral vectors is to protect therapeutic nucleic acids from degradation, facilitate their cellular uptake, and promote their release within target cells. To achieve these objectives, non-viral vectors must form stable complexes with nucleic acids, remain biocompatible and biodegradable, and efficiently overcome both extracellular and intracellular biological barriers. These barriers include enzymatic degradation, limited membrane permeability, endosomal entrapment, and nuclear import [11, 26,31].

Non-viral gene delivery systems are generally classified into three main categories: chemical, physical, and biological non-viral systems. Among these, chemical and physical systems represent the most extensively studied approaches in gene therapy research. The main classes of non-viral vectors are illustrated in Figure 5.

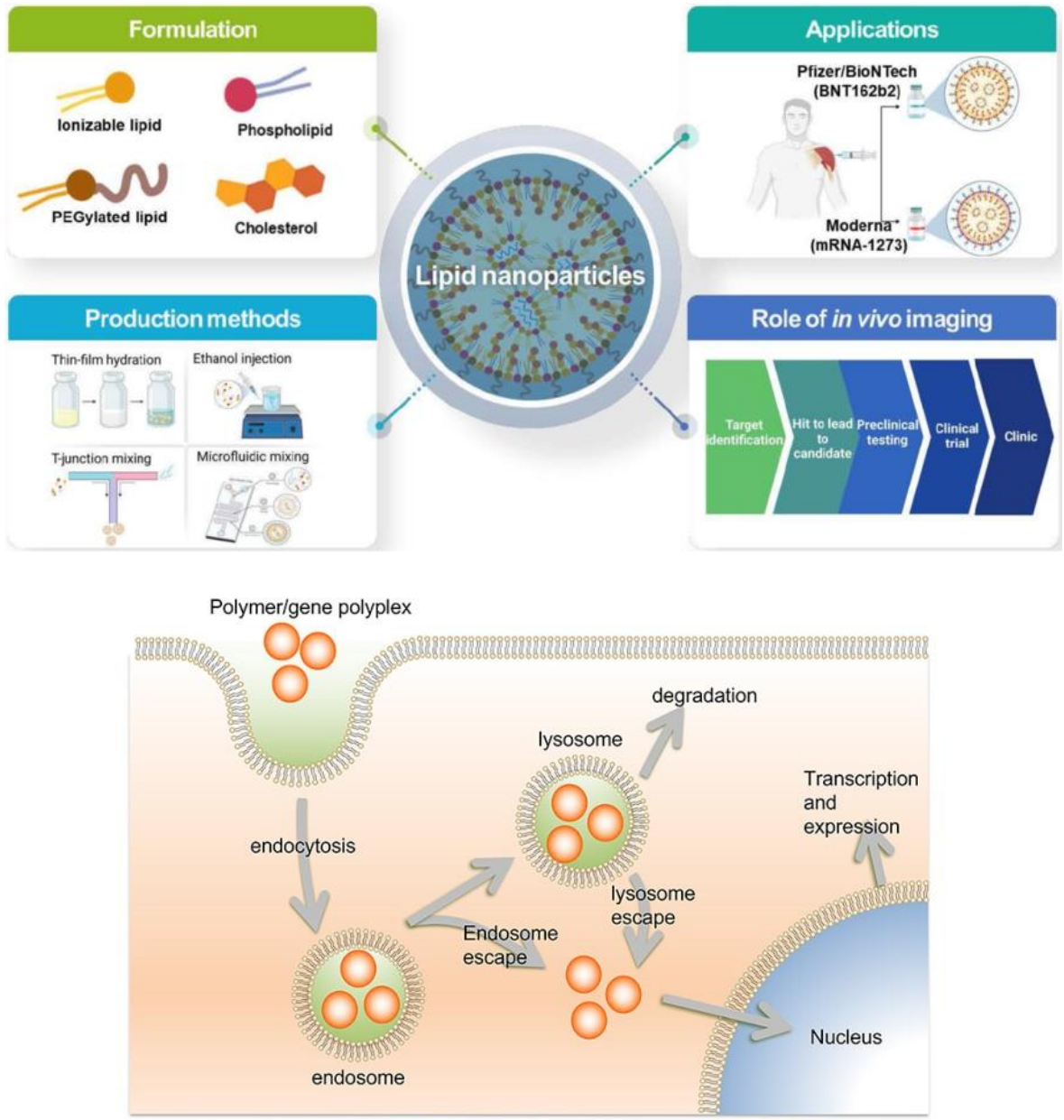

Figure 5. *Non-viral vectors used for gene delivery*

Non-viral gene delivery systems include lipid-based vectors, polymeric carriers, and physical delivery methods. These systems protect nucleic acids from degradation and facilitate cellular uptake while offering improved safety compared to viral vectors.

5.2. Chemical Non-Viral Delivery Systems

Chemical delivery systems rely on electrostatic or hydrophobic interactions between nucleic acids and synthetic or natural carriers. These systems aim to neutralize the negative charge of nucleic acids while preserving their biological activity [6,7].

5.2.1. Lipid-Based Vectors

Cationic lipids are widely used non-viral carriers that interact electrostatically with negatively charged nucleic acids to form lipoplexes. These complexes protect the genetic material from nuclease degradation and promote cellular uptake through membrane fusion or endocytosis. Lipid-based vectors exhibit favorable biocompatibility and have been successfully employed in both in vitro and in vivo gene delivery [11,12].

Lipid nanoparticles (LNPs) represent an advanced class of lipid-based systems composed of ionizable lipids, helper lipids, cholesterol, and polyethylene glycol (PEG)-conjugated lipids.

The ionizable lipids enable efficient nucleic acid encapsulation and endosomal escape, while PEGylation enhances circulation time and stability. LNPs have demonstrated remarkable clinical success, particularly in mRNA-based vaccines, highlighting their translational potential [17–20].

5.2.2. Polymeric Vectors

Cationic polymers constitute another major class of chemical non-viral vectors. These polymers form polyplexes with nucleic acids through electrostatic interactions, offering enhanced stability compared to lipoplexes. Commonly investigated polymers include polyethylenimine (PEI), chitosan, poly(lactic-co-glycolic acid) (PLGA), dendrimers, and polymethacrylates [21,22,40–42].

Polyethylenimine is considered a benchmark polymer due to its high transfection efficiency and ability to promote endosomal escape via the "proton sponge" effect. Natural polymers such as chitosan offer reduced toxicity and improved biocompatibility, making them attractive for mucosal and oral gene delivery. Biodegradable polyesters like PLGA enable sustained release of genetic material, while dendrimers provide high nucleic acid binding capacity through their branched architecture [21, 22,40–42].

Despite their advantages, polymeric vectors may exhibit cytotoxicity depending on their molecular weight, charge density, and structure, necessitating careful optimization for clinical use.

5.3. Physical Non-Viral Delivery Systems

Physical methods of gene delivery rely on external forces to temporarily disrupt cellular membranes, allowing naked or complexed nucleic acids to enter target cells. These approaches are generally simple to implement and avoid the use of potentially toxic carriers [6,7].

5.3.1. Electroporation

Electroporation involves the application of short electrical pulses that induce transient pores in the cell membrane, facilitating the entry of nucleic acids. This technique can be applied both in vitro and in vivo and is particularly effective for muscle tissue, tumor cells, and skin. Depending on the pulse parameters, electroporation can be reversible or irreversible, the latter being used for tumor ablation [11,12].

5.3.2. Magnetofection

Magnetofection uses magnetic nanoparticles associated with nucleic acids and guided by an external magnetic field toward target cells or tissues. This method enhances sedimentation and cellular uptake, particularly in vitro, and has shown promise for localized gene delivery in vivo [11,12].

5.3.3. Ballistic DNA Delivery

Also known as gene gun delivery, this method involves the propulsion of DNA-coated metallic microparticles into tissues at high velocity. Although precise, its application remains largely limited to experimental and localized gene delivery contexts.

5.3.4. Sonoporation and Direct Injection

Sonoporation employs ultrasound waves to increase membrane permeability, often in combination with microbubbles that enhance nucleic acid delivery. Direct injection of naked DNA represents the simplest delivery method but is associated with low efficiency due to rapid degradation and clearance.

6. Modes Of Administration Of Non-Viral Vectors

The route of administration of non-viral vectors plays a crucial role in the efficiency, specificity, and safety of gene therapy. Depending on the therapeutic objective and the target tissue, gene delivery may be performed locally or systemically. Each approach presents distinct advantages and limitations that must be carefully considered [11, 26,31].

6.1. Local Administration

Local administration involves the direct delivery of therapeutic nucleic acids or vector–gene complexes to a specific tissue or organ. This approach allows for higher local concentrations of the genetic material while limiting systemic exposure and reducing the risk of off-target effects [6,7].

6.1.1. Intratumoral Injection

Intratumoral administration consists of injecting the gene delivery system directly into the tumor mass. This method ensures precise targeting of tumor cells and the surrounding microenvironment, including stromal and endothelial cells. By confining gene expression to the tumor site, intratumoral injection minimizes systemic toxicity and enhances therapeutic efficacy.

Non-viral vectors such as lipid nanoparticles and cationic polymers are commonly used in this context. This strategy has been widely investigated in cancer gene therapy, particularly for the delivery of suicide genes, immunomodulatory genes, or genes inducing apoptosis [24–28].

6.1.2. Intramuscular Injection

Intramuscular delivery is frequently employed in DNA vaccination and gene therapy targeting muscle-related disorders. Muscle tissue is easily

accessible and well vascularized, making it a suitable target for gene delivery. Moreover, muscle cells are capable of long-term protein expression, which is advantageous for sustained therapeutic effects.

This route may involve the administration of naked plasmid DNA, polymer-based complexes, or gene delivery combined with physical enhancement methods such as electroporation, which significantly increases transfection efficiency.

6.1.3. Intra-Articular Injection

Intra-articular administration involves the direct injection of therapeutic genes into joint cavities. This approach is particularly relevant for the treatment of inflammatory and degenerative joint diseases such as rheumatoid arthritis and osteoarthritis [53–62].

By targeting synovial cells and chondrocytes, intra-articular gene therapy enables localized expression of anti-inflammatory cytokines, growth factors, or apoptosis-inducing proteins. Non-viral vectors such as polymeric nanoparticles and lipid-based carriers have shown promising results in preclinical models [11, 26,31].

6.2. Systemic Administration

Systemic administration refers to the delivery of gene therapy vectors via intravenous or other general routes, allowing distribution throughout the body. This approach is essential when multiple organs or widespread cell populations must be targeted.

Systemic delivery offers the advantage of treating disseminated diseases; however, it is associated with several challenges. These include rapid clearance of vectors from the circulation, non-specific distribution, reduced bioavailability at the target site, and potential toxicity. Moreover, achieving efficient and selective targeting of specific tissues remains a major hurdle.

Non-viral vectors used for systemic administration must exhibit prolonged circulation time, resistance to serum nucleases, and the ability to escape immune recognition. Despite these challenges, ongoing research aims to improve targeting strategies and enhance the therapeutic potential of systemic non-viral gene delivery [11, 26,31].

7. Clinical Applications Of Gene Therapy

Gene therapy has been investigated in a wide range of diseases where conventional treatments remain insufficient or palliative. Its applications are particularly well established in genetic disorders, chronic inflammatory diseases, and cancer. Advances in non-viral delivery systems have further expanded the therapeutic scope of gene-based strategies [24–28].

7.1. Genetic Diseases

Genetic diseases constitute one of the primary fields of application for gene therapy, as they often result from well-characterized mutations affecting a single gene. The objective of gene therapy in this context is to restore the expression of a functional protein or to correct aberrant gene expression responsible for disease pathology.

7.1.1. Cystic Fibrosis

Cystic fibrosis is a monogenic autosomal recessive disorder caused by mutations in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene. The resulting dysfunctional chloride channel leads to dehydration of epithelial surfaces and the production of abnormally viscous mucus, particularly affecting the respiratory and digestive systems [39,43–45].

Gene therapy strategies for cystic fibrosis primarily focus on restoring functional CFTR expression in airway epithelial cells. Given the high turnover rate of these cells and their limited accessibility, in vivo gene delivery is the preferred approach. Aerosolization and nebulization of gene delivery systems via the respiratory tract represent the most suitable administration routes. Non-viral vectors have been extensively explored in this context due to their favorable safety profile, although biological barriers such as thick mucus layers, chronic inflammation, and immune defenses significantly limit transfection efficiency [39,43–45].

7.1.2. Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is a severe X-linked recessive disorder caused by mutations in the dystrophin gene, leading to progressive muscle degeneration. The absence of functional dystrophin compromises muscle fiber stability and results in irreversible muscle damage. Gene therapy approaches for DMD include gene addition and exon-skipping strategies. Exon skipping, mediated by antisense oligonucleotides, enables the production of a truncated yet partially functional dystrophin protein, resembling the milder Becker muscular dystrophy phenotype. Although this strategy

does not fully restore dystrophin function, it significantly improves muscle integrity and disease progression [12, 46,47].

7.1.3. Sickle Cell Disease

Sickle cell disease is a hereditary hemoglobinopathy caused by a point mutation in the β-globin gene, resulting in the production of abnormal hemoglobin S. Under hypoxic conditions, hemoglobin S polymerizes, causing red blood cells to assume a rigid, sickle shape. This leads to vaso-occlusive crises, chronic anemia, and progressive organ damage [48–52].

Gene therapy strategies aim to correct or compensate for the defective β-globin gene, often through ex vivo modification of hematopoietic stem cells. Although viral vectors are frequently employed, non-viral approaches are under investigation to reduce safety concerns and improve accessibility. The ultimate goal is to achieve long-term expression of functional hemoglobin and eliminate disease-related complications [11].

7.2. Inflammatory Diseases

Chronic inflammatory diseases represent another important field of application for gene therapy, particularly when conventional pharmacological treatments provide limited efficacy or are associated with significant adverse effects. Gene-based strategies offer the possibility of long-term modulation of inflammatory processes through localized or sustained expression of therapeutic proteins.

7.2.1. Rheumatoid Arthritis

Rheumatoid arthritis is a chronic autoimmune inflammatory disease characterized by persistent synovial inflammation, joint destruction, and progressive disability. The pathophysiology of the disease involves excessive production of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), which promote synovial hyperplasia, cartilage degradation, and bone erosion [53–62].

Gene therapy approaches for rheumatoid arthritis aim to locally suppress inflammation or eliminate pathogenic synovial cells. Intra-articular administration of therapeutic genes represents a particularly relevant strategy, as it allows direct targeting of the affected joints while minimizing systemic exposure. Genes encoding anti-inflammatory cytokines, such as IL-10 and IL-4, or inhibitors of pro-inflammatory pathways, such as soluble TNF receptors, have demonstrated significant anti-arthritic effects in preclinical models [53–62].

Another strategy involves the induction of apoptosis in synovial cells through the transfer of suicide genes, including thymidine kinase, followed by systemic administration of a prodrug. This approach leads to selective destruction of proliferating synovial tissue, resulting in reduced joint inflammation and structural damage.

Non-viral vectors offer attractive advantages for intra-articular gene delivery due to their reduced immunogenicity and improved safety compared to viral vectors. Although challenges related to transfection efficiency and duration of gene expression persist, experimental studies support the feasibility of gene therapy as a complementary or alternative approach in the management of inflammatory joint diseases [11, 26,31].

7.3. Cancer

Cancer is characterized by uncontrolled proliferation, survival, and dissemination of abnormal cells resulting from genetic and epigenetic alterations. Because tumor development is directly linked to deregulation of gene expression, cancer represents a particularly relevant target for gene therapy strategies. Over the past decades, gene therapy has been extensively investigated in oncology, with numerous preclinical studies and clinical trials demonstrating encouraging results [24–28].

Gene therapy in cancer primarily relies on three complementary approaches: stimulation of antitumor immunity, induction of apoptosis in malignant cells, and sensitization of tumors to chemotherapeutic agents. One of the most advanced strategies involves the genetic modification of immune cells, particularly T lymphocytes, to enhance their ability to recognize and eliminate cancer cells. In this context, autologous T cells are genetically engineered to express chimeric antigen receptors (CARs) capable of binding tumor-associated antigens. Once reinfused into the patient, these modified cells selectively target and destroy cancer cells [24–28].

Another widely explored approach is suicide gene therapy, which involves the introduction of genes encoding enzymes that convert non-toxic prodrugs into cytotoxic compounds within tumor cells. This strategy enables localized tumor cell killing while limiting systemic toxicity. Additionally, gene therapy can be used to inhibit immune checkpoint pathways, such as PD-1

signaling, thereby restoring antitumor immune responses.

The use of oncolytic viruses represents another gene-based strategy in cancer therapy; however, non-viral approaches remain particularly attractive due to their improved safety profile and lower immunogenicity. Despite promising outcomes, the clinical application of cancer gene therapy remains limited by challenges related to delivery efficiency, tumor heterogeneity, and immune interactions. Nevertheless, ongoing advances in vector design and targeting strategies continue to expand the therapeutic potential of gene therapy in oncology [24–28].

8. Challenges, Limitations, And Ethical Considerations

Despite its considerable therapeutic potential, gene therapy faces numerous scientific and clinical challenges. Ensuring safe and efficient gene delivery remains one of the most critical obstacles, particularly in the case of in vivo administration. Poor targeting specificity, limited persistence of gene expression, and unintended genetic modifications may compromise both efficacy and safety [11,12].

Biological barriers represent a major limitation to successful gene transfer. Therapeutic nucleic acids must overcome extracellular degradation, cellular membrane impermeability, endosomal sequestration, cytosolic degradation, and nuclear entry. In addition, immune responses against vectors or transgene products may lead to reduced therapeutic efficacy or adverse effects, particularly after repeated administrations [6,7]. The major biological barriers limiting gene delivery efficiency are summarized in Figure 6.

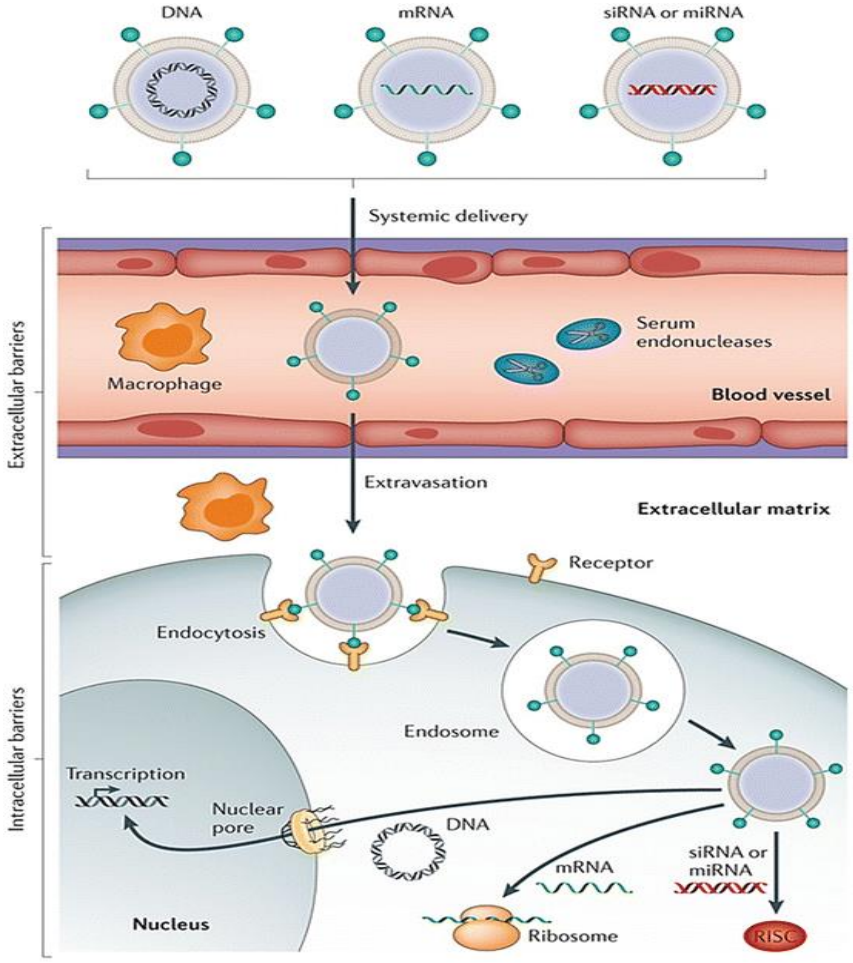

Figure 6. *Biological barriers to non-viral gene delivery*

After administration, therapeutic nucleic acids must overcome multiple barriers, including extracellular degradation, cellular uptake, endosomal escape, cytosolic trafficking, and nuclear entry, which collectively limit gene transfer efficiency.

Ethical considerations also play a central role in the development of gene therapy. While somatic gene therapy is widely accepted, germline modification raises profound ethical, social, and regulatory concerns due to its heritable nature. These issues necessitate strict oversight and the

establishment of robust regulatory frameworks to ensure responsible development and clinical application [8].

9. Conclusion

Gene therapy represents one of the most promising therapeutic innovations of modern medicine, offering the potential to treat diseases at their genetic origin rather than managing symptoms alone. Considerable progress has been made in understanding gene regulation, developing delivery systems, and translating gene-based strategies into clinical applications. Non-viral vectors have emerged as particularly attractive tools due to their favorable safety profiles, flexibility, and scalability. Although challenges related to delivery efficiency, targeting specificity, and sustained expression persist, continuous technological advances and interdisciplinary collaboration are driving steady progress in the field. Ultimately, gene therapy holds significant promise for the treatment of genetic disorders, chronic inflammatory diseases, and cancer. Continued research efforts, coupled with rigorous ethical oversight and regulatory adaptation, are essential to fully realize the clinical potential of gene-based therapies and ensure their safe integration into future medical practice.

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