Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 3
BLSME 2024

Outside-in: Overview of Delivery Method in Sirna Therapy

Kaijun Yuan *

Department of Cellular and Molecular Biology, Duke Kunshan University, Suzhou, China

* Corresponding Author Email: ky101@duke.edu

Abstract. RNA interference (RNAi) is a Universal biological system based on double-strand small interference RNA (siRNA). RNAi efficiently inhibit the expression of genes based on a guiding strand. SiRNA can be applied against a broad spectrum of diseases but is limited by insufficient siRNA delivery methods. Delivery of siRNA is a challenging direction of modern molecular biology and pharmacy study; scientists aim to improve the efficiency and accuracy of siRNA-based treatment. Many approaches have been suggested to overcome challenges in effectively and safely delivering siRNA to the necessary tissues, cells, and organisms, Furthermore, endeavors have been concentrated on augmenting the effectiveness of siRNA concerning its stability, activity, specificity, and minimizing side effects. This review overviews the barriers to siRNA entrance and the challenge of siRNA delivery vectors, introduces and categorizes the currently available siRNA delivery systems including conjugate-based vectors, Lipid nanoparticle vectors, and Dendrimer vectors. And discuss the latest progress in the development of siRNA therapy. This review aims to offer a concise overview of the present advancements in siRNA delivery research and suggests potential avenues for future exploration.

Keywords: siRNA; systematic delivery; siRNA delivery vectors.

1. Introduction

Fire and Mello first observed modest gene silencing effects in the Caenorhabditis elegans induced by double-strand RNA molecules in 1998 [1]. Subsequently, the intricacies of the RNAi mechanism have undergone comprehensive examination.

RNAi is a highly conserved eukaryote immune process against viruses and other foreign genetic material. The mechanism of how the RNAi carries out gene expression manipulation starts after a double-strand siRNA molecule enters the cytoplasm. The endoribonuclease Dicer, a helicase with a PAZ (Piwi/Argonaute/Zwille) domain, together with two RNase III and two double-stranded RNA-binding domains (DUF283 and dsRBD) [2] will cleave and convert long double-stranded RNA and short-hairpin RNA into short, fully formed double-stranded siRNA molecules. The mature siRNA is then loaded into the Argonaute protein and form the RNA-induced silencing complex (RISC) that includes Argonaute-2 and the siRNA molecule. Following this, the protein Argonaute selects the less thermodynamically stable 5' end strand, this selected strand, referred to as the guide strand, is responsible for targeting mRNA for enzymatic degradation, while the remaining strand, known as the passenger strand, is eliminated and broken down by the RISC complex. After siRNA activation by attaching the guide strand, the RISC complex can bind to the target messenger RNA [1]. The siRNA-loaded RISC can bind to mRNA molecules multiple times and mediate the degradation of the target siRNA. Consequently, gene manipulation can be efficiently induced with siRNA concentrations as low as in the picomolar range.

In theory, obtaining the correct nucleotide sequence along the target mRNA would enable scientists to potentially target any gene of interest using siRNA. Therefore, siRNA therapy shows substantial potential as an innovative method for addressing a range of genetic disorders and diseases by precisely targeting and manipulating the specific genes accountable for the condition. This therapy offers a highly specific and efficient way to modulate gene expression, potentially providing more effective and targeted treatments with fewer side effects. For instance, siRNA therapy has shown potential in addressing conditions like hypercholesterolemia by targeting genes involved in cholesterol

metabolism [3], as well as in treating certain types of cancer by suppressing the expression of oncogenes responsible for tumor growth and suppressing tumor growth sites with precision. Additionally, siRNA therapy holds the potential to treat neurodegenerative disorders, like Parkinson's disease, for they can selectively silence specific genes contributing to disease progression.

Although siRNA holds a promising future for therapeutics, its efficiency is greatly limited by intracellular and extracellular barriers, which greatly limits its therapeutic capabilities. Naked and unmodified siRNA have limitations: (1) the potential off-target effects, (2) unsatisfactory stability and poor pharmacokinetic behavior. Upon systemic administration into the circulation, endonucleases or exonucleases present throughout the body rapidly degrade siRNA into fragments, thereby impeding the accumulation of siRNA at specific sites. The phosphodiester bond within siRNA is susceptible to degradation by RNases and phosphatase activity. The siRNA can also activate the Toll-like receptor 3 (TLR3), [4] which will affect the blood system and trigger an immune response.

To enhance treatment efficacy and mitigate potential side effects associated with siRNA, extensive efforts have been dedicated to the exploration of diverse chemical modification strategies and innovate delivery vectors. A wide range of delivery materials, such as virus, polymers, lipid nanoparticles, peptides, inorganic nanoparticles, exosomes, and others, have been carefully engineered and effectively integrated into both preclinical and clinical studies. A spectrum of modification patterns has been proposed and rigorously studied about their impact on therapeutic activity, stability, specificity, and safety. This review overviews the siRNA vector system and related issues including RNAi delivery, barriers, a brief overview of the three most well-studied categories of siRNA delivery vectors, and some new insight into siRNA delivery technique.

2. Overview of the Process of siRNA Entrance

To suppress the gene expression inside the intercellular matrix, the siRNA molecules need to go through several processes that help them overcome biological barriers. However, Due to stability, accuracy, efficiency, and other issues, a specific vector is needed to assist RNA molecules in going through the following process to enter the cytoplasm:

2.1. RNA Extracellular Trafficking:

RNA molecules require vectors to guide them to the target cell type. This vector serves two major tasks: protect the RNA cargo from degradation and help facilitate their movement through the extracellular matrix. In this process, Surface modifications on the vectors can enable specific interactions with target cells, increasing their binding affinity and cellular uptake, while the chemical modification or encapsulation protects the RNA molecule from immune recognition and enzymatic activity. This phase is pivotal for achieving precise targeting, efficient siRNA delivery to the desired cells, and reducing off-target effects to irrelevant tissue.

2.2. RNA Internalization:

Upon reaching the cell membrane, RNA particles need to be internalized into the cell. Receptor-mediated endocytosis involves binding specific receptors on the cell surface to ligands present in the delivery system, triggering engulfment and formation of endocytic vesicles. For example, certain RNA viruses can directly merge with the cell membrane, discharging their genetic material into the cytoplasm. Specific non-viral vectors have evolved to exploit cellular processes, such as cell penetration peptide to break free from endosomes.

2.3. RNA Intercellular Trafficking and Endosomal Escape:

After entering the cell, the RNA molecules are trafficked within the intricate network of intracellular vesicles. The enveloped RNA cargo might be transported toward the nucleus for transcriptional regulation or to specific organelles for localized translation. Additionally, carriers like exosomes, which are small membrane vesicles secreted by cells, can facilitate intercellular RNA transfer,

allowing genetic information to be exchanged between neighboring or distant cells. Once inside endocytic vesicles, the RNA particles face the challenge of escaping these compartments to avoid lysosomal degradations. The vesicles may be combined with lysosomes, which are acidic and contain degradative enzymes. Effective endosomal escape is essential to ensure the RNA molecules reach the cytoplasm where translation occurs. Some delivery systems leverage pH-responsive materials or incorporate membrane-disrupting agents to trigger the release of RNA from endosomes.

3. Challenges of siRNA Therapy

Because siRNA is a relatively big molecule with a complex biological nature, it can be affected by many in vivo processes, making it somewhat unstable. In vivo processes like RNase activity, immune response to significant biomolecules, glomerular filtration, charged properties of RNA molecules, and lysosome-mediated hydrolysis after entering the cell all limit the effect of RNAi.

3.1. Size issue:

Small interfering RNAs (siRNAs) are duplexes of 21–23 nucleotides, approximately 7.5 nm in length and 2 nm in diameter [5]. Their size, although too large to cross the cytomembrane, allows them to be readily filtered into urine [6] as they are smaller than 8 nm. As a result, siRNA tends to accumulate in the bladder. The siRNA can also accumulate in the liver since the liver receives a significant portion of the body's blood supply due to its role in detoxification and metabolism. [5] This high blood flow rate allows for efficient distribution of substances, including siRNA, delivered through the bloodstream. As a result, siRNA molecules delivered systemically have a higher chance of encountering and being filtered up by liver cells. The porous structure of endothelial cells and Kupffer Cells and Phagocytosis effectively capture the siRNA molecule in the circulation: Kupffer cells, specialized macrophages present in the liver can engulf particles like nanoparticles or siRNA delivery complexes. As a result, instead of accumulating in the target site, siRNA molecules may be more likely to accumulate in the liver and bladder naturally.

3.2. In vivo environmental issues:

There are RNase activity and immune activity in vivo, which can cause RNA lysis. The siRNA molecule with a size larger than 30 nucleotides can trigger the activation of interferons [7]. The pattern recognition receptors (PRRs) will recognize the dsRNA, particularly those belonging to the RIG-I-like receptor (RLR) family. When long nucleotides are present in the cytoplasm of a cell, RLRs like Retinoic acid-inducible gene I detect them and undergo a conformational change, this change activates RIG-I, enabling it to interact with another protein called MAVS (Mitochondrial Antiviral Signaling protein) [8]. This interaction triggers a signaling cascade, which ultimately triggers the production and release of Type I interferon, which initiates a series of cellular responses that bolster the immune defense. These responses include the upregulation of antiviral genes, activation of immune cells, and establishment of an antiviral state within the cells.

3.3. RNA intracellular escaping:

After entering the cell, the RNA molecule is present in the lysosome, and the siRNA needs to escape the lysis process. Efficient escape of siRNA from endosomes into the cytoplasmic matrix is necessary before the antisense strand of the siRNA can be loaded into the RISC complex.

Many delivery systems, utilize pH-responsive components with a “proton sponge effect”, which makes them react to H+ fluctuations. These components take in protons (H+) and exhibit a positive charge on their surface. As a result, there's an elevation in osmotic pressure within endosomes or lysosomes, prompting the inflow of water (H2O) and chloride ions (Cl-), which eventually disrupt membranes of lysosomes, leading to membrane instability and membrane expansion. Arginine-rich cationic peptides like TAT peptide can permeabilize endosomal membranes. Linking multiple TAT peptides helps substances escape endosomes. This can be achieved by connecting peptides to proteins

or using a synthetic framework. Recent research shows that constructs with 2 or 3 TAT branches are better at escaping endosomes than those with just 1 TAT unit [9]. Amphiphilic peptides with a mix of hydrophobic and hydrophilic parts can also disrupt endosomal membranes, an instance is PTD4 (YARAAAARQARA), and CM18 (KWKLFKKIGAVLKVLTTG). The hydrophobic property makes them connect with membranes and disrupt membranes nonselectively, which might harm the cell.

Small molecules called lysosomotropic agents can break membranes in cells' storage compartments, for example, L-leucyl-L-leucine O-methyl ester can accumulate and make lysosomes swell and leak. Another molecule, chloroquine can cause osmotic swelling. These molecules can facilitate cytosolic penetration but are rather toxic [10]. Photosensitizers are molecules that react to light and damage cell membranes by generation of reactive oxygen. By changing these molecules to be more water-friendly, they can enter cells and damage specific compartments when exposed to light.

Although the rate of observed release in cells is relatively high, only a small fraction of total internalized siRNA can be released into the cytoplasm, within a limited period after internalization [5]. Both enhancing the rate of endosomal escape and diminishing the toxicity of endosomal escape agents, are essential requirements for the advancement of siRNA therapy.

4. Overview of Current siRNA therapy technique:

4.1. Local delivery

4.1.1. Naked siRNA therapy (SKIN, respiratory system)

First-generation siRNA therapy drugs usually use Naked siRNA without delivery vectors. Since siRNA naturally accumulate in the kidney, it can be employed for gene silencing in kidneys and the treatment of renal diseases for therapeutic purpose. It can also be used to target skin and respiratory system tissue that can be directly reached by the drug and target hermetic organs like eyeballs.

For example, QPI-1002(RNA I5NP) is a blunt-ended 19-base-pair RNA duplex with methoxy modification on the 2′ positions of the ribose sugar to prevent nuclease. After loading to RISC, the RISC-I5NP complexes can degrade p53 mRNA, thereby delaying the pro-apoptotic activity of p53 following renal injury. This delay provides damaged cells with an opportunity for recovery. Study in living tissues shows that QPI-1002 can very efficiently accumulate in the liver during a relatively short period, effectively suppressing the p53 expression at a low concentration [11].

Injection based method is another method of naked siRNA delivery, it directly injects siRNA molecules into the target location, like eyeball organisms. ‘Fomivirsen’ is a 21-base phosphorothioate oligodeoxynucleotide [12], this is a highly effective and specifically targeted antiviral treatment for cytomegalovirus retinitis. It is a complement to the mRNA transcript of the major immediate-early region (IE2), the 'Fomivirsen' molecules operate in a sequence-specific manner, impeding CMV immediate-early gene expression. Study reveals that ‘Fomivirsen’ can inhibit the growth of CMV, The Ec50 for Fomivirsen was 0.37 μmol/L against the human CMV. Since the Human eye is a relatively hermetic organ, the study found no ‘Fomivirsen’ in plasma circulation.

4.2. Systematical delivery

Although some targets can be reached by local delivery mechanisms like injections, most therapeutics target other organisms, where RNA can’t be naturally accumulated. In this case, siRNA delivery vectors are needed to protect and guide the siRNA molecules through the intracellular matrix, internalized into the target cell type, and inhibit certain gene expressions.

4.2.1. Vira siRNA vector

Virus membranes can shield the viral genetic material and deliver it accurately to the cytoplasm and the nucleus of specific kinds of cells to internalize and amplify the viral gene materials inside the host

cell. These properties make the virus and virus-like particles(virosome) a potential tool for siRNA molecule precision delivery, retrovirus, Adeno-associated viruses, Moloney Murine Leukemia Virus, HIV, and HVJ all Have the potential to become viral vectors of siRNA [6].

4.2.2. Non-viral siRNA vector

Although viral siRNA vectors have high efficiency and are easy to synthesize, it has many concerns; The viral-based vector may have safety-related issues since the viral antigens might trigger the immune system and even trigger a pre-existing immune response, some viral vectors have the potential to integrate their genetic material into the host genome, which could potentially lead to insertional mutagenesis and disrupt normal cellular functions. On the other hand, synthesized non-viral vectors have higher Customization flexibility: they are safer, less likely to trigger an immune response, and can target cells that can't be reached by viral vectors. Nanocarriers require exceptional serum stability, enabling them to evade clearance by the liver's reticuloendothelial system and the kidney's renal clearance function, while also releasing their cargo promptly at the intended target site [5]. Currently, Lipid-based, conjugated, and polymer-based siRNA delivery vectors are the most used non-viral siRNA delivery method.

1) Lipid nanoparticles

The utilization of lipid nanoparticle based (LNP) vectors as siRNA delivery systems has seen a growing trend. The lipid nanoparticle encapsulation can protect siRNA cargo while transporting and releasing the siRNA molecule to the target tissue. The common siRNA-LNP vectors are comprised of assistance components including cationic lipids, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and polyethylene glycol (PEG).

Cationic lipids, owing to their positive charge, can engage in electrostatic interactions with the negative charge of RNA molecules, resulting in the formation of lipoplexes. These negatively charged lipid vesicles are drawn towards the nucleic acid molecules and organize themselves into a multilayered lipid bilayer structure. Only the liposome is not enough to deliver siRNA, the liposome needs to integrate helper molecules to become a functional delivery vehicle. For example, fusogenic phospholipids can destabilize the cell membrane and enhance transfection efficiency. Polyethylene glycol (PEG) lipids can decrease the immune response, shielding LNPs from macrophage engulfment [13] and controlling the liposome size. Cholesterol nanoparticles help stabilize the lipid and enhance cationic lipid activity. Lipids with different charged natures can also be used to modify to vector's charge to target different cell types.

The earliest lipid nanoparticles were assembled through self-assembly during formulation, which is susceptible to disruption within endosomes. This disruption degrades RNA molecules within the endosomal environment instead of facilitating their release into the cytosol. Consequently, this challenge prompted the creation of pH-sensitive LNPs. These advanced LNPs exhibit alterations in their charge within endosomes, resulting in enhanced RNA escape following cellular entry.

Lipid nanoparticle-based vectors can enable greater flexibility for siRNA delivery, Currently, multiple vectors have been established to target specific cell types and diseases, like cancer, viral infection, and rare inheritance diseases that can't be easily reached by common medications: For example, A type of biodegradable lipid molecule L101 was used to create an effective and biodegradable LNP vector combined with helper lipids. These formulations contained a special siRNA molecule that targets a protein called PCSK9 protein, a liver protein that regulates the behavior of LDL particles, commonly known as "bad" cholesterol. When PCSK9 binds to LDL receptors, it interferes with their ability to eliminate LDL particles, which causes heart disease related cholesterol increase. Studies show that the LNP vector based on L101 with the siRNA targeting PCSK9 was very successful in reducing the protein's levels by more than 90% in non-human primates after being injected into their veins. This formulation also showed it's safe because the body clears it effectively from the liver. [14]

LNP can also aid RNAi-based siRNA treatment against cancer progression: A protein called PLK1 is important to target in cancer treatment because it's often too active in cancer cells, leading to excessive cell division. Blocking PLK1 can stop cell division and potentially cause cell apoptosis. A Corporation called Arbutus Biopharma has finished the initial phase of LNP based siRNA drug development and is now in the second phase with patients who have different types of tumors, including gastrointestinal neuroendocrine tumors, and adrenocortical carcinoma. The first phase of testing showed that higher doses of the treatment had better effects, with five out of six patients experiencing stabilized disease, as well as a 19.3% reduction in tumor size. LNPs work well in this situation because they have a higher specificity to neuroendocrine tumors, making structures like the adrenal cortex potential targets. [15]

2) Conjugate nanoparticles

Conjugates encompass individual chemical entities in which fully modified siRNA is covalently joined with targeting ligands. These specialized ligands serve as navigational guides, leading the conjugates to precise cells or tissues within the body and attaching to specific receptors on the membrane. This intricate strategy resembles a sophisticated "lock and key" arrangement, where the lock symbolizes the receptor located on the targeted cell, and the ligand, acting as the key, is fastened to the siRNA, enabling precise recognition and interaction. Antibodies, aptamers, Peptides, Small molecules with high affinity for specific cell receptors, Transferrin, are all Ligands for Endocytosis that can be used as a siRNA delivery vectors.

For example, N-acetylgalactosamine, or GalNAc conjugates are widely used conjugate-based siRNA delivery. The GalNAc conjugate represents a simple, compact, and precisely composed method for delivering substances to the liver. Hepatic binding protein and the Ashwell–Morell receptor (ASGPR) expressed on liver hepatocytes have a high affinity to the GalNAc molecule. GalNAc-siRNA conjugates vector can bind ASGPR and go through rapid internalizations into clathrin-coated endosomes [16]. The GalNAc-siRNA can then be released from ASGPR binding by the low PH environment in the endosome. ASGPR undergoes recycling to return to the cytomembrane surface, while the siRNA is released through the action of endosomal glycosidases and stays in the endosome's lumen, leaving the conjugated vector naturally degraded. ‘Inclisiran’ [17] is an FDA-approved GalNAc-siRNA conjugate medicine that targets PCSK9 proteins in liver tissue. These proteins impact both overall and local cholesterol regulation. ‘Inclisiran’ can effectively reduce unhealthy Low-Density Lipoprotein Cholesterol (LDL-C) levels. In studies like the ORION trials, when ‘Inclisiran’ is used alongside the highest tolerable statin dose, LDL-C levels dropped by an impressive 150%.

Inorganic nanoparticle conjugates are another category of siRNA vector that has been widely studied. Inorganic nanoparticles like gold nanoparticles, silver nanoparticles, Silica Nanoparticles, and Copper Sulfide Nanoparticles can help cellular absorption and selectively target cells. Some conjugates like Magnetic and oxidized Iron Nanoparticles can be used for both siRNA delivery and imaging. They can be guided to target cells using external magnetic fields.

Mesoporous silica nanoparticles (MSNs) are potential conjugate-based siRNA delivery vectors [18]. They have an ordered porous structure that makes them potential vectors for delivering hydrophobic substances, as the drugs are enclosed within the pores and protected by the hydrophilic silica matrix. MSNs are highly modifiable; their size and surface can be manually modified for better cargo interaction and protection. Positively charged groups like amines can be added to enhance interaction with cargo. For example, hyperbranched polymer polyethyleneimine (hbPEI) modifications can increase surface amine groups. PEI-coated MSNs can cap pore entrances, retaining cargo (molecular gatekeeping) and aiding cell entry via endocytosis, PEI can also have a unique "proton-sponge" effect which helps exiting endosomes. PEI and MSN combination vectors give MSNs a positive charge on their surface for loading negatively charged genetic material. Experiments show that PEIs with lower molecular weights showed no toxicity while revealing high nucleic acid affinity. Researchers managed to load siRNA both on the surface and within the pores of magnetic MSNs by treating the particles with PEI. This enhanced vector displayed low toxicity.

Gold nanoparticles are another commonly used siRNA vector. Gold nanoparticles (AuNPs) exhibit a series of unique properties. Its inherent inertness enhances biocompatibility and stability, making it a valuable protective agent against RNase degradation for siRNA with little immune response. The AuNPs also have great modification potential, scientists can precisely modify their size, shape, and specific traits at several levels, and some forms can also displace optical properties. By changing the size and shape of the gold nanoparticles, the researchers can optimize the vector's ability to internalize into the target cell and endosomal escape. Kannan et al, showed how AuNPs - bombesin peptide conjugates can interact with gastrin-releasing peptide receptors with high specificity in a Comparative experiment, in which non-specific accumulation in the liver was significantly reduced while the accumulation in the target organs was increased. [19] The optical characteristics of the siRNA vector incorporating gold nanoparticles can also be harnessed to increase the thermal sensitivity of cancer cells during optical hyperthermia therapy.

3) Polymer and Dendrimer

Biological polymers with cationic properties can readily combine and form nanoparticles by binding to siRNA. The positive charge of these carriers enables them to interact electrostatically with the nucleic acid molecules which have a negative charge, resulting in efficient condensation. These siRNA-polymer complexes, at nanoscale size, effectively protect nucleic acids like siRNA from nonspecific interactions and degradation while circulation throughout body. Polymer vectors are commonly employed for delivering nucleic acids, including low and high-molecular-weight polyethyleneimines, positively charged polysaccharides like chitosan, dendrimers, and polypeptides like poly-L-lysines.

Polyethyleneimine (PEI) is a cationic polymer known for its efficient complexation of nucleic acids due to electrostatic interactions. Because PEI's high positive charge density can lead to cytotoxicity, scientists usually conjugate it with other biocompatible molecules for siRNA delivery purposes. Chitosan is a biocompatible polymer; it has low transfection efficiency for siRNAs. Coupling chitosan with PEI forms chitosan-PEI-siRNA nanoparticles. These nanoparticles exhibited substantial tumor accumulation and target downregulation in vivo, showing potential for therapeutic applications. Hyaluronic Acid (HA)-PEI/PEG nanoparticle is a component of the extracellular matrix (ECM) and has been used for targeting CD44-overexpressing tumors. Combining HA with PEI/PEG forms nanoparticles that carry siRNAs targeting specific proteins. In vivo experiments demonstrated siRNA activity in xenograft tumor models, although accumulation was influenced by factors beyond active targeting. PEI can also combine with Cyclodextrin to form CD-PEI Conjugates. Cyclodextrins are cyclic oligosaccharides used to enhance the pharmacokinetics of drugs. CD-PEI-siRNA complexes exhibited synergistic effects when combined with photothermal therapies [20].

Poly-L-lysine (PLL), another cationic polymer, is being explored for its potential in nucleic acid delivery [21]. Despite better biocompatibility compared to PEI, PLL-siRNA polyplexes can interact with serum proteins, affecting siRNA stability and delivery efficiency. Derivatives of PLL have been engineered to address these challenges: For example: PEG-PCL-PLL Micelles, A polymer developed to form micelles that efficiently deliver siRNAs, demonstrating comparable silencing efficiency to commercial transfection reagents. These micelles were tested in vitro and showed potential for future in vivo applications. Melanin-PLL Nanoparticle is another example, Melanin-PLL nanoparticles combine the photothermal properties of melanin with siRNA delivery capabilities. The resulting nanoparticles effectively inhibited tumor growth both in vitro and in vivo, showcasing their potential therapeutic utility. PLL can also combine with other modification molecules to acquire pH-responsive and proton-responsive behavior, which helps enhance siRNA and drug delivery to specific cell types.

Dendrimers are a contemporary category of cationic polymers that have generated significant attention as carriers for nucleic acids, including siRNA. These highly branched macromolecules possess a precisely defined core and exhibit repetitive attachment of exterior units, providing customizable properties such as size and surface charge. Their special properties, like chemical uniformity, the capacity for generation expansion through repeated chemical group additions, high

density of functional surface groups for ligand attachment, and 3D confirmations, make dendrimers a strong contender for a wide range of biomedical applications. The Dendrimers have greater flexibility to be modified with Surface modification including various polymers and targeting ligands for achieving specific functions in drug and gene delivery, for example, polyethylene glycol (PEG) modification helps the vector escape the reticuloendothelial system capture and extend systemic circulation period. Dendrimers can also be conjugated with other nanoparticles like inorganic gold, iron nanoparticles, and lipids to increase their delivery efficiency and accuracy.

Polycationic dendrimers like polyamide amine (PAMAM) and polypropylene imine (PPI) have been thoroughly investigated for the delivery of drugs and nucleotides. PEI-PAMAM dendrimer is a dendrimer NP with remarkable attributes: they exhibit minimal toxicity, significantly enhance siRNA stability and intracellular transportation, and effectively silence genes in controlled laboratory environments and living organisms. Notably, the amine based dendritic polymer demonstrates an exceptional balance between gene silencing effectiveness and low toxicity when tested in vitro [22].

5. Newly emerged vector

Ultimately, developing new siRNA vectors aims to create siRNA delivery vectors that significantly enhance the therapeutic potential of siRNA-based treatments, making them more effective, secure and can be employed for a broad spectrum of therapeutics conditions, encompassing cancer, genetic disorders, viral infections, and beyond. These vectors aim to bridge the gap between the promising potential of siRNA therapies and their successful clinical implementation. The objective of the newly emerged vectors can be generalized as increasing efficiency and Reducing side effects. Increasing efficiency including Enhanced Targeting and reducing off-target effects, increased cellular Uptake, improved intracellular escape, and prolong the circulation time of siRNA in the bloodstream. Reduce side effects including Minimized Immune Response and cellular toxicity of the vector.

One possible solution for the problems above is utilizing endogenous components to create a programmable siRNA delivery vector for example, SEND (selective endogenous encapsulation for cellular delivery) is a siRNA delivery method based on PEG 10 protein. A considerable percentage of the human genome originated in LTR retroelements that integrated into mammalian genomes. Among LTR-related proteins, the core capsid (CA) domain protein homolog, the LTR retrotransposon protein PEG10, can bind RNA and form capsids. These characteristics give them the potential to transport specific nucleic acids, potentially serving as a programmable endogenous intracellular communication vector. The researchers identified gag-derived genes in both the human genome and mouse genome and found the peg10 gene which originates in LTR retrotransposons and found the CA 1 domain as a conservative functional domain that can assemble into capsid-like spherical particles. The PEG 10 protein capsid contains nucleic acid binding nucleocapsid (NC) domain and reverse transcriptase (RT) domain that can bind several mRNAs inside trophoblast stem cells including its mRNA as other neuron cell’s transcript.

Based on these properties, researchers reprogram the 5 'UTR and 3' UTR of MmPEG10 onto specific RNA sides to achieve packaging, transmission, and translation of other RNA goods. They connect the mRNA of Cre recombinase to the 5 'UTR and 3' UTR of MmPEG10 on both sides and conjugate vesicular stomatitis virus envelope protein (VSVg) present on the cell surface with the PEG10-siRNA complex as a fusion helper. The Co transfection of MmPEG10, cargo (with Cre siRNA), and VSVg into target lox-GFP N2a cells showed that specific RNA goods could be functionally delivered into the cells using MmPEG10 UTR and VSVg, Subsequently, the team optimized the packaging signals of MmPEG10 UTR and HsPEG10 UTR and found that the 3 'proximal 500bp can be used to mediate the transfer of Cre mRNA to the target cell; optimize the cis binding elements in the MmPEG10/HsPEG10 coding sequence that have an impact on the packaging process. The result demonstrates a significant increase in functional transfer into N2a cells. Furthermore, it exhibits approximately much less potency compared to viral-based vectors, as functional titration assays show, and it performs competitively against other mRNA delivery vehicles [23].

6. Conclusion

In conclusion, the past decade has witnessed promising developments in the field of RNA therapies, which reveals the capabilities of siRNA as an effective gene manipulation tool. However, to fully utilize siRNA's capabilities for therapeutic usage, a scientist needs to develop vectors to protect and deliver siRNA to overcome several layers of biological barriers and reach the targets. As scientists understand the intricate interactions between RNA payloads and their delivery vehicles, these interactions provide insight into multiple siRNA delivery vectors that aim to improve targeting precision and tolerability. Unraveling the complex web of how chemical modifications to RNA payloads affect stability, intracellular off-target effects, and immune system responses is imperative. Recent experiments with modified mRNA vectors highlight the potential benefits of tailored chemical modifications to optimize therapeutic outcomes and overcome the limitations of certain vectors. However, therapeutic studies and experiments underscore that issues like cellular toxicity, immune response, and delivery efficiency still limit the performance of siRNA therapy. Exploring novel avenues, such as leveraging naturally occurring systems like PEG10 or extracellular vehicles, holds promise for enhancing extrahepatic delivery.

To summarize, as we continue to deepen our understanding of the intricate interplay between RNA drugs, delivery systems, and the human body, we move closer to unlocking the potential of next-generation gene therapies, offering new hope and improved therapeutics options for patients facing a wide range of diseases.

References

[1] Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature. 1998, 391: 806 – 11.

[2] Iwakawa, H. O., & Tomari, Y. Life of RISC: Formation, action, and degradation of RNA-induced silencing complex. Molecular cell, 2022, 82 (1), 30 – 43.

[3] Chaplin, S. Inclisiran: a siRNA to treat hypercholesterolemia. Prescriber, 2022, 33: 23 - 24.

[4] Kaushal, A. Innate immune regulations and various siRNA modalities. Drug Deliv. and Transl. Res 2023.

[5] Hu, B., Zhong, L., Weng, Y. et al. Therapeutic siRNA: state of the art. Sig Transduct Target Ther 2020, 5, 101.

[6] Ahn, I., Kang, C.S. & Han, J. Where should siRNAs go: applicable organs for siRNA drugs. Exp Mol Med 2023, 55, 1283 – 1292.

[7] Subhan, M. A., & Torchilin, V. P. siRNA-based drug design, quality, delivery, and clinical translation. Nanomedicine: nanotechnology, biology, and medicine, 2020, 29, 102239.

[8] Wu, B., & Hur, S. How RIG-I-like receptors activate MAVS. Current opinion in virology, 2015, 12: 91 – 98.

[9] Kaplan IM, Wadia JS, and Dowdy SF, Cationic TAT peptide transduction domain enters cells by macropinocytosis. Journal of controlled release: official journal of the Controlled Release Society 2005, 102, 247 – 53.

[10] Thiele, D. L., & Lipsky, P. E. Mechanism of L-leucyl-L-leucine methyl ester-mediated killing of cytotoxic lymphocytes: dependence on a lysosomal thiol protease, dipeptidyl peptidase I, that is enriched in these cells. Proceedings of the National Academy of Sciences, 1990, 87 (1): 83 - 87.

[11] Demirjian, S., Ailawadi, G., Polinsky, M., Bitran, D., Silberman, S., Shernan, S. K., Burnier, M., Hamilton, M., Squiers, E., Erlich, S., Rothenstein, D., Khan, S., & Chawla, L. S. Safety and Tolerability Study of an Intravenously Administered Small Interfering Ribonucleic Acid (siRNA) Post On-Pump Cardiothoracic Surgery in Patients at Risk of Acute Kidney Injury. Kidney international reports, 2017, 2 (5): 836 – 843.

[12] Geary, R.S., Henry, S.P. & Grillone, L.R. Fomivirsen. Clin Pharmacokinet 2002, 41: 255 – 260.

[13] Schroeder, A., Levins, C. G., Cortez, C., Langer, R., & Anderson, D. G. Lipid-based nanotherapeutics for siRNA delivery. Journal of internal medicine, 2010, 267 (1): 9 – 21.

[14] Suzuki, Y.; Hyodo, K.; Suzuki, T.; Tanaka, Y.; Kikuchi, H.; Ishihara, H. Biodegradable lipid nanoparticles induce a prolonged RNA interference-mediated protein knockdown and show rapid hepatic clearance in mice and nonhuman primates. Int. J. Pharm. 2017, 519: 34 – 43.

[15] Ramanathan RK, Hamburg SI, Borad MJ, et al. A phase I dose escalation study of TKM-080301, a RNAi therapeutic directed against PLK1, in patients with advanced solid tumors. Cancer Res. 2013, 73 (8): LB - 289.

[16] Springer, A. D., & Dowdy, S. F. GalNAc-siRNA Conjugates: Leading the Way for Delivery of RNAi Therapeutics. Nucleic acid therapeutics, 2018, 28 (3): 109 – 118.

[17] Strat, A. L., Ghiciuc, C. M., Lupuşoru, C. E., & Mitu, F. NEW CLASS OF DRUGS: THERAPEUTIC RNAi INHIBITION OF PCSK9 AS A SPECIFIC LDL-C LOWERING THERAPY. Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi, 2016, 120 (2): 228 – 232.

[18] Slita, A., Egorova, A., Casals, E., Kiselev, A., & Rosenholm, J. M. Characterization of modified mesoporous silica nanoparticles as vectors for siRNA delivery. Asian journal of pharmaceutical sciences, 2018, 13 (6): 592 – 599.

[19] A. Artiga, I. Serrano-Sevilla, L. De Matteis, S. G. Mitchell and J. M. de la Fuente, J. Mater. Current status and future perspectives of gold nanoparticle vectors for siRNA delivery Chem. B, 2019, 7, 876.

[20] Gary, D. J., Puri, N., & Won, Y. Y. Polymer-based siRNA delivery: perspectives on the fundamental and phenomenological distinctions from polymer-based DNA delivery. Journal of Controlled Release, 2007, 121 (1-2): 64 - 73.

[21] Korzhikov-Vlakh, V., Katernuk, I., Pilipenko, I., Lavrentieva, A., Guryanov, I., Sharoyko, V., Manshina, A. A., et al. Photosensitive Poly-l-lysine/Heparin Interpol electrolyte Complexes for Delivery of Genetic Drugs. Polymers, 2020, 12 (5), 1077.

[22] Biswas, S., & Torchilin, V. P. Dendrimers for siRNA Delivery. Pharmaceuticals (Basel, Switzerland), 2013, 6 (2): 161 – 183.

[23] Segel, M., Lash, B., Song, J., Ladha, A., Liu, C. C., Jin, X., ... & Zhang, F. Mammalian retrovirus-like protein PEG10 packages its own mRNA and can be pseudotyped for mRNA delivery. Science, 2021, 373 (6557): 882 - 889.