Recent Advances in siRNA Delivery System and Drugs

Wenjie Huang[1] and Yichen Wu[2]
[1]*Medicinal Chemistry, China Pharmaceutical University, Nanjing, China*
[2]*Kunshan KangChiao School, Kunshan, China*

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Abstract: siRNA drugs are an emerging class of nucleic acid drugs that can treat many diseases at the genetic level, especially for rare diseases. siRNA has the characteristics of poor lipid solubility, high immunogenicity, poor stability, and difficulty in in vivo delivery, which makes the development of related drugs full of challenges. However, the continuous innovation of molecular modification methods and increasingly sophisticated delivery strategies have brought hope to the development of siRNA drugs. This article reviews the mechanism of action, modification, delivery technology, and research and development history of siRNA drugs, aiming to provide feasible research and development strategies for potential siRNA drugs and continuously expand the clinical application of siRNA drugs.

1 INTRODUCTION

siRNA drugs have opened up a new avenue for innovative gene-based therapies. Since the approval of the world's first siRNA drug, Patisiran, in 2018, siRNA-based therapeutics have demonstrated immense potential. siRNA drugs have opened up new avenues for innovative gene therapy. Since the approval of the world's first siRNA drug, Patisiran, in 2018, siRNA-based therapies have shown great potential. Small nucleic acid drugs represented by siRNA drugs have set off the third wave of drug research and development after small molecule drugs and antibody drugs. After siRNA drugs are made into drugs, they have many advantages such as long-term effectiveness and precise targeting, which makes them have huge market potential. With the rich R&D experience accumulated over the years and the excellent sales results facing the market, it is proved that siRNA drugs have withstood the test in all aspects and have become a successful example of new drug research and development.

1.1 Background of siRNA

In 1998, Fire et al. discovered in their study on *Caenorhabditis elegans* that long double-stranded RNA (dsRNA) could silence the expression of specific genes. They termed this phenomenon RNA interference (RNAi), opening the door to potent and specific gene silencing through dsRNA (Fire et al.,1998). siRNA was first identified by David Baulcombe's team in plants as part of the post-transcriptional gene silencing (PTGS) mechanism (Hamilton and Baulcombe,1999). Building upon these studies, Thomas Tuschl et al. successfully induced RNAi in mammalian cells using synthetic small interfering RNAs (siRNAs) in 2001, without triggering an immune response typically associated with long dsRNA (Tuschl,2001). These groundbreaking discoveries paved the way for the application of siRNA in both research and therapeutic fields.

1.2 Introduction to RNAi

RNAi refers to the process of introducing exogenous or endogenous double stranded RNA (dsRNA) into cells and processing it through Dicer, a member of the RNase III-like enzyme family, to form 21~23 nucleotide (nt) short dsRNAs (Agrawal et al.,2003). These small dsRNAs are called small interfering RNAs (siRNAs). They form RNA-induced silencing complex (RISC) under the action of Argonaute (Ago) protein. At the same time, dsRNA is unzipped into two single strands: Guide Strand and Passenger Strand. Guide Strand exists in RISC, while Passenger Strand is degraded. Subsequently, under the action of the cleavage enzyme argonaute-2, RISC recognizes and cuts the target sequence in mRNA through the principle of base complementary pairing, promoting the cleavage or degradation of target mRNA, thereby

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interrupting the translation process of mRNA and downregulating the translation level of specific proteins (Elbashir et al.,2001). This discovery is an important milestone in the field of gene therapy. It provides feasible solutions for drug development in multiple fields.

1.3 Advantages of siRNA

SiRNA, as a new type of gene therapy, has its unique advantages. First, siRNA is specific. Since the positive chain only binds to the mRNA that is completely complementary to it, siRNA can highly specifically identify any target gene in the human body, with low off-target efficiency and high silencing efficiency. Second, siRNA drugs are safe. The gene silencing effect produced is transient and will not be integrated into the genome, thus avoiding the risk of host gene mutation. In addition, siRNA drugs are long-lasting. Small interfering RNA (siRNA) drugs have significantly improved stability and resistance to nuclease degradation due to their chemical modification, thereby extending the biological half-life and achieving long-term treatment with dosing every few months or even half a year (Huang et al.,2025). For instance, Inclisiran can successfully lower levels of low-density lipoprotein cholesterol (LDL-C) by targeting PCSK9. It only needs to be administered twice a year, which significantly improves patient compliance. siRNA drugs have shown the advantages of long-lasting efficacy and low frequency of use in the treatment of chronic diseases, providing important support for precision medicine (Zhang and Li,2022).

2 SIRNA DELIVERY

Small interfering RNA (siRNA) has shown huge potential in gene therapy and precision medicine because it can specifically silence the expression of target genes. However, siRNA itself has problems such as easy degradation, low cellular uptake efficiency and difficulty in crossing biological barriers, which makes its clinical transformation face challenges. In order to overcome the above problems, researchers have developed corresponding vectors to protect siRNA and deliver it to target cells.

2.1 Viral Vectors

Viral vectors achieve targeted delivery of siRNA by simulating the natural infection mechanism of viruses, mainly including adenovirus, lentivirus and adeno-associated virus (AAV). Its core advantages are high transfection efficiency and genetic stability, but potential immunogenicity, insertion mutation risk and production cost limit its clinical application (Kaczmarek et al.,2017).

In recent years, AAV has become a research hotspot due to its low pathogenicity and persistent expression characteristics. By engineering the AAV capsid protein (such as targeted receptor modification), its tissue specificity can be significantly improved and its immunogenicity can be reduced (Srivastavaet al.,2021). However, AAV still faces problems such as limited drug loading capacity.

2.2 Lipid Carriers

Lipid nanoparticles (LNPs) are currently the only FDA-approved siRNA delivery system (such as Patisiran). They form a core-shell structure through the self-assembly of cationic lipids and siRNA, which protects nucleic acids while promoting endosomal escape. The success of LNPs stems from four major characteristics: (1) modular design facilitates functional modification; (2) pH-sensitive lipid-mediated endosomal escape; (3) PEGylated surface prolongs circulation half-life; and (4) standardized process that can be industrialized (Kulkarni et al.,2019).

Although LNPs have been clinically applied, their targeting specificity and long-term toxicity still need to be optimized. The latest progress includes: (1) the development of tissue-specific ligands (such as GalNAc targeting hepatocytes); (2) the design of ionizable lipids to reduce cytotoxicity; and (3) the construction of stimulus-responsive carriers (such as tumor microenvironment-triggered release). It is worth noting that the nonspecific binding of cationic lipids to serum proteins remains a major challenge for systemic drug delivery, which has prompted researchers to develop novel components such as zwitterionic lipids (Hou et al.,2021).

2.3 Polymer Carriers

Polymer carriers achieve siRNA loading through electrostatic adsorption or covalent coupling, and their performance depends on molecular weight, branching degree and surface charge. Common systems include: Polyethyleneimine (PEI): High cationic density gives it strong nucleic acid binding ability, but high molecular weight PEI (>25 kDa) produces cytotoxicity due to the proton sponge effect. Solutions include: (1) coupling low molecular weight

PEI with hydrophobic groups; (2) constructing a degradable cross-linked structure (Wang et al.,2010).

PLGA: FDA-approved biodegradable material that encapsulates siRNA through the double emulsion method. Although it has excellent biocompatibility, the negatively charged surface leads to low cellular uptake rate, which needs to be improved by cationic polymer (such as chitosan) coating or targeting ligand (such as folic acid) modification (Danhier et al.,2012).

Emerging strategies focus on the development of smart responsive polymers, for example: temperature-sensitive Pluronic copolymers can enhance tumor penetration, while reduction-sensitive disulfide bond polymers can achieve intracellular specific release.

2.4 Inorganic Non-Metallic Carriers

Inorganic non-metallic materials used for siRNA drug delivery include carbon nanomaterials, calcium phosphate, silica, graphene, etc. WEI Bo and his research team used four techniques including cell transfection, fluorescence imaging, MTT method and hemolysis experiment to confirm that silica can effectively deliver siRNA to primary cells of mammals, showing excellent delivery ability, and no obvious cytotoxicity and physiological hemolysis were found. This provides an innovative delivery tool for subsequent drug research (Wei et al.,2025).

2.5 Metal Nanocarriers

Metal nanocarriers achieve efficient siRNA delivery through controllable surface modification, of which two types of systems are the most representative: Gold nanoparticles (AuNPs). The surface of AuNPs can be thiolated to load dense siRNA chains (about 30-60 per 10 nm particle) to form a spherical nucleic acid (SNA) structure. This structure is efficiently endocytosed through the scavenger receptor SR-A1 and directly penetrates the cell membrane, avoiding the endosomal escape barrier of traditional carriers (Giljohann et al.,2010). However, the particle size must be controlled at 20-50 nm to balance tumor targeting (EPR effect) and in vivo retention time. Iron oxide nanoparticles (IONPs). IONPs can achieve deep tissue targeting under the guidance of an external magnetic field. For example, the Fe3O4@SiO2 core-shell structure can increase the drug loading to 200μg siRNA/mg particle, and realize tumor microenvironment-triggered release through pH-responsive materials.

3 MODIFICATION OF SIRNA

Unmodified small nucleic acids are easily degraded when used directly in vivo, with poor effects and easy off-target effects. Modification of siRNA can improve its resistance to enzymatic degradation, maintain sequence stability and prolong half-life, while increasing the lipid solubility of the drug and reducing immunogenicity. Modification has become one of the key directions of siRNA drug research and development.

3.1 Chemical Modification

The basic skeleton of siRNA is composed of phosphate, ribose and base. By chemically modifying each part, its stability, nuclease resistance and gene silencing efficiency can be increased, pharmacokinetics and pharmacodynamics can be improved, the interaction between nucleic acid and protein can be strengthened, off-target effects can be reduced, and the efficiency of small nucleic acid drugs can be improved. The chemical modification of siRNA mainly includes phosphate skeleton modification, ribose modification and base modification.

3.1.1 Phosphate Group Modification

Nuclease hydrolyzes nucleic acid by acting on the phosphodiester bond on the nucleotide phosphate skeleton. The center of nuclease attack is the phosphorus atom in the phosphodiester bond. Therefore, whether nucleic acid is resistant to enzymatic hydrolysis depends on the change of phosphorus atom structure. The most widely used method is thio modification (phosphorothioate, PS). The sulfur atom replaces a non-bridging oxygen atom of the phosphate group to form a thiophosphate bond. This method can make siRNA resistant to nucleases, prolong the drug's action period in the body, increase the specific recognition of small nucleic acids and target cells, promote their aggregation in the cell nucleus, and improve the drug's efficacy.

3.1.2 Ribose Modification

Nuclease acts on the 2' hydroxyl group in ribose to achieve cleavage. Therefore, modification of the 2' hydroxyl group can protect nucleic acids from attack by nucleases. The current methods for ribose modification applied to siRNA include locked nucleic acid (LNA) and methoxy modification. LNA uses aminomethylene bridges, thiomethylene bridges or

oxymethylene bridges to connect the 2'-O and 4' positions on the ribose into a ring, thereby forming a restricted nucleic acid conformation. Compared with unmodified siRNA, the use of LNA-siRNA to treat SARS is more effective, and some LNA-siRNA drugs have entered the clinical stage (Elmen et al.,2005). Methoxy modification is the most commonly used nucleic acid modification technology. The 2'-OH group of the nucleotide is changed to 2'-OCH3. This method can significantly enhance the affinity of siRNA for the target gene and improve its ability to resist nucleases (Li et al.,2014), while having little effect on the activity of siRNA. The first siRNA drug approved by the FDA, patisiran, significantly improves its stability and therapeutic effect by modifying the nucleosides on the sense and antisense strands from 2'-OH to 2'-OCH3.

3.1.3 Base Modification

The pharmacological mechanism of small nucleic acid drugs is based on the precise recognition of specific hydrogen bonds between them and the target sequence. In the molecular structure optimization strategy, base modification technology effectively regulates drug activity and metabolic characteristics by reconstructing the intermolecular forces between nucleotide bases. From the analysis of chemical modification types, base modification of small molecule non-coding nucleic acids mainly involves structural substitution or introduction of functional groups, among which the structural modification of NTP has become an important technical path to expand molecular functions and optimize chemical selectivity. When selecting spatial modification sites, the C5 position of the pyrimidine ring and the C2 position of the purine ring are commonly used targets due to their good chemical modification properties. Experimental results show that the thermodynamic stability of RNA molecules is significantly improved by adding modified nucleotides such as pseudouracil, 2-thiouracil, N6-methyladenosine (m6A) and 5-methylcytosine, and the activation of TLR-mediated innate immune response is also effectively inhibited. It is worth noting that m6A modification has been proven to have dual regulatory functions: it can promote the maturation and processing of pri-miRNA and enhance the epigenetic regulation of X chromosome inactivation specific transcript (XIST). In terms of clinical application optimization, nucleotide site-directed modification shows unique advantages. Zhang's team's research confirmed that the introduction of 5-nitroindole modified nucleotides at the 15th site of the siRNA sense strand can reduce the off-target effect mediated by the sense strand by 82% while completely retaining the targeting activity of the antisense strand (Zhang,2012). More noteworthy is the new cytosine analog 6'-phenylpyrrolidine (PhpC), which not only maintains the ability to accurately pair with natural bases, but also has high thermal stability (Tm value increased by 4.2℃) and strong fluorescence properties (quantum yield of 0.68). The gene silencing efficiency of the siRNA complex constructed based on PhpC is comparable to that of the prototype molecule (IC50=12nM), and its endogenous fluorescent labeling function provides an innovative research method for real-time monitoring of drug intracellular transport and distribution.

4 SIRNA DRUGS: A CHALLENGING RESEARCH AND DEVELOPMENT PATH

Since the RNAi phenomenon won the Nobel Prize in Physiology or Medicine in 2006, people have begun to pay attention to the potential of siRNA drugs in the field of precision treatment of diseases, and major pharmaceutical companies have begun to develop siRNA drugs. However, due to the difficulty of siRNA drug development and the lack of delivery technology, the results of the initial research and development were not ideal. From 2008 to 2012, two siRNA drugs for the treatment of blinding choroidal neovascularization were interrupted in Phase III studies due to immunotoxicity and unclear efficacy. Ultimately, the first generation of small interfering RNA drugs were terminated due to safety concerns and lack of efficacy (Setten et al.,2019). With the improvement of siRNA chemical modification technology and the continuous innovation of siRNA delivery strategies, from 2013 to 2014, a number of chemically modified siRNA drugs combined with targeted delivery systems entered clinical research one after another, among which the most representative is the N-acetylgalactosamine (GalNAc) delivery system. GalNAc-modified siRNA drugs can specifically bind to the asialoglycoprotein receptor (ASGPR) highly expressed on the surface of hepatocytes, deliver siRNA to hepatocytes and exert pharmacological activity. However, in 2016, the development of small interfering RNA drugs based on early chemical modification and delivery technology failed again. Revusiran is the first siRNA drug combined with a GalNAc delivery system and administered subcutaneously. It is designed to treat

hereditary thyroxine-mediated amyloidosis polyneuropathy (hATTR). However, in the Phase II study, the drug failed to achieve the clinical preset outcome and the study was forced to be terminated early (Maraganore et al.,2022).

Fortunately, in 2018, the first siRNA drug Patisiran was approved by the FDA for marketing, marking its first successful application in siRNA drug development. Patisiran is used to treat hATTR. It contains 19 base pairs and uses lipid nanoparticles (LNP) as a delivery system. By modifying the 2'-OH of the nucleosides on the forward and reverse chains to 2'-OCH3, the stability and therapeutic effect are significantly improved. hATTR is caused by a gene mutation that causes impaired function of transthyretin (TTR) in the body. TTR protein is mainly produced in the liver and is a carrier of vitamin A. When TTR mutates, abnormal amyloid TTR (ATTR) accumulates in the human body, causing damage to human organs and tissues (such as peripheral nerves and heart), and causing difficult-to-treat peripheral sensory neuropathy, autonomic neuropathy or cardiomyopathy. Patisiran can specifically bind to the conserved gene sequence of TTR mRNA, so it can specifically silence the expression of TTR and inhibit the production of TTR protein, thereby reducing the deposition of amyloid protein in peripheral nerves and avoiding organ and tissue damage. So far, the efficiency of siRNA drug development has advanced by leaps and bounds. Six siRNA medications have received FDA approval for commercialization as of 2023.

Another well-known siRNA drug is Inclisiran. It is used as an adjunct to diet to treat adult patients with heterozygous familial hypercholesterolemia (HeFH) or clinical atherosclerotic cardiovascular disease (ASCVD). Cardiovascular disease (CVD) has always been the leading cause of death worldwide, and ASCVD, including hypercholesterolemia, is the main CVD. Furthermore, dyslipidemia, which is defined by increased LDL-C or total cholesterol (TC), is a significant risk factor for ASCVD and is one of the major causes of ASCVD. Therefore, controlling LDL-C is crucial for patients with heart disease and non-heart disease. Inclisiran is a GalNAc-coupled siRNA drug (GalNac-siRNA) that targets liver distribution and can directly act on the mRNA encoding PCSK9 protein. It uses RNA interference mechanism to induce PCSK9 mRNA degradation, increase the expression and circulation of LDL-R on the surface of hepatocytes, and then increase the uptake of LDL-C and reduce the level of LDL-C in the circulation. The medication has long-term persistence, requires just two injections per year, and is effective for six months following a single administration.

Nedosiran, the most recent siRNA medication, received FDA approval in 2023. It was created to treat primary hyperoxaluria by Dicerna Pharmaceuticals, a Novo Nordisk affiliate. The rare autosomal recessive hereditary condition known as primary hyperoxaluria (PH) is typified by the liver's overproduction of oxalate. The kidneys ordinarily eliminate oxalate, but when it builds up too much, it forms crystals of insoluble calcium oxalate. Nephrocalcinosis and kidney stones are caused by this buildup, which can progress to chronic kidney disease. Nedosiran is a synthetic double-stranded siRNA that is attached to a N-acetyl-D-galactosamine (GalNAc) amino sugar residue. Hepatocytes preferentially absorb it through the succinate glycoprotein receptor (ASGPR) following subcutaneous injection. It is loaded into the RNA-induced silencing complex (RISC) inside the cell, where it breaks down LDHA mRNA by RNA interference, lowering the synthesis of LDH and preventing the buildup of oxalate.

5 CONCLUSION

As siRNA research continues to advance, siRNA medicine development has advanced significantly and has been crucial in the prevention and management of a number of illnesses. Since 2018, the FDA has authorized and marketed six siRNA medications. At present, many siRNA drugs in different clinical stages provide new treatment options for genetic diseases, blood diseases, eye diseases and tumors. Continuous innovations in chemical modification and delivery technologies have given siRNA drugs broad prospects in clinical applications and are expected to make important contributions to human health.

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