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Review of the research and development status of siRNA drugs for gene therapy

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Abstract. Gene therapy is a new kind of therapy technology, which can modify or manipulate gene expression, gene silencing, and other functions, or change the biological characteristics of cells, so as to achieve the goal of curing disease at the gene level. It is not restricted by the drug-forming property of the target protein and has obvious advantages compared with traditional protein drug therapy. To develop small interfering ribonucleic acid (siRNA) drugs for gene therapy, the mechanism of ribonucleic acid interference (RNAi), which was discovered more than 30 years ago, can be taken as a basis. Later, scientists' unremitting exploration made siRNA drugs develop rapidly. So far, there are five kinds of siRNA drugs listed, with good application prospects. This paper aims to summarize the existing siRNA drugs and explore their shortcomings, as well as some existing improvement measures, thereby pointing out the direction of drug development and providing some help for the better application of siRNA drugs. According to the analysis, it is found that there are still some dilemmas in the research and development of siRNA drugs in terms of stability, targeting accuracy, long-term effectiveness, efficiency, and safety. Meanwhile, the performance of the drug delivery system also determines whether the drug can play an effective role. Some improvements have been proposed in this paper, such as modifying drug molecules or improving delivery systems to help drugs function better, which could be the direction of future drug progress.

Keywords: RNA interference, small interfering RNA, delivery technology, challenges.

1. Introduction

At present, drug therapy is a commonly used method to treat diseases. According to different pathogenesis, drug targets and modes of action are also different. Among them, the vast majority of targets are receptors, enzymes, and other proteins, and drugs acting as agonists or inhibitors of proteins are most common. However, they cannot prevent the translation of abnormal proteins, in other words, they cannot eliminate the effect of abnormal proteins from the root. In addition, there are many disease-related proteins that cannot be targeted by drug molecules. As a result, certain medication restrictions do exist. Thus, non-protein-targeted drugs are needed to address this conundrum, particularly ribonucleic acid (RNA)-based gene therapies that can serve as potential therapies to specifically target and silence any target gene[1]. Compared with traditional protein-based drugs, RNA-based gene therapies have obvious advantages such as high specificity, high efficiency, and long-term effect, having broad development and application prospects. RNA interference (RNAi) is an important strategy of RNA-based gene therapy. The specific process is that when double-stranded RNA

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molecules are present in a cell due to viral infection or other causes, these double-stranded RNA molecules are recognized and cleaved by an RNA enzyme called Dicer. Small interfering RNA (siRNA) is a double-stranded RNA containing about 20 base pairs. It is divided into two strands by the core AGO2 protease of the RNA-induced silencing complex (RISC) multiprotein complex. One is the passenger strand, which is degraded, and the other is the guide strand, which serves as a template for messenger RNA (mRNA) alignment and binds to the RISC complex, target mRNAs with complementary nucleotide sequences are specifically recognized and cleaved, thus interfering with the expression of specific genes and reducing the production of aberrant proteins.

siRNA drugs are based on the development of RNAi drugs. For eukaryotic cells, siRNAs enter the cytoplasm through different ways. One is through the endogenous pathway, and the long precursor is the Dicer enzyme cleaved into mature siRNA. The other is through an exogenous pathway, where synthetic siRNA will enter the vesicle via endosomes and be released into the cytoplasm [2]. siRNA drugs act as gene silencers in the cytoplasm.

In this paper, the author reviews the discovery process of the RNAi mechanism and summarizes the application of different types of siRNA drugs based on this mechanism. In addition, the current challenges and corresponding solutions to the research and development of siRNA drugs are also discussed. As a very new drug therapy, siRNA drugs are bound to have bottlenecks at this stage. This paper can give some enlightenment on the further improvement of siRNA drugs and point out a way for their future development.

2. The discovery of the RNAi mechanism

RNAi was first observed in Petunia flowers in the early 1990s, when the introduction of the purple pigment gene turned out to be the opposite of the expected dark purple flowers, a phenomenon known as “Co-inhibition”, that is, the activity of both the introduced gene and the homologous endogenous gene were inhibited. In 1998, Fire et al. investigated a similar effect in Caenorhabditis elegans and demonstrated that it was the double-stranded RNA, produced when sense RNA was transcribed in vitro, that really played a role, so the effect was formally named RNAi [3]. In 2001, the team of Elbashir was the first team to successfully synthesize siRNAs and demonstrate siRNA-mediated gene silencing in mammalian cells, confirming the RNAi mechanism in mammals [4]. The use of exogenous siRNAs holds great promise for the analysis of gene function in human cell culture and the development of gene-specific therapies. Since then, RNAi has become an experimental method of molecular biology, and it is also expected to have a greater application in the field of biomedical research and drug development.

3. The application of different types of siRNA drugs

Since the discovery of the RNAi mechanism and the subsequent confirmation of its availability in mammalian cells, siRNA therapy has made significant progress and become a powerful tool for the treatment of various diseases. So far, five siRNA drugs have been approved.

3.1. Patisiran

The first kind of siRNA drug, ONPATTRO (Patisiran), was approved by the U.S. Food and Drug Administration (FDA) on August 10, 2018. It is used to treat peripheral neuropathy (polyneuropathy) in adult patients with Hereditary Transthyretin Amyloidosis (hATTR), a fatal progressive disease caused by mutations in the transthyretin (TTR) gene, leading to abnormal accumulation of amyloid in peripheral nerves, hearts, kidneys, gastrointestinal tracts and other organs, interfering with their normal function. Patisiran aims to target sequences in TTR mRNA to prevent the production of aberrant forms of TTR RNA and reduce the accumulation of amyloid deposits, thereby improving symptoms. Prior to this agent, treatment options typically focused on symptom relief, orthotopic liver transplantation, and TTR tetramer stabilizers to increase the stability of circulating TTR tetramers [5]. RNAi therapy provides a good opportunity to eradicate the disease.

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The efficient delivery and precise targeting of siRNA is an important step and guarantee for the action of siRNA drugs. Patisiran employs lipid nanoparticles (LNP) delivery technology to encapsulate siRNA in lipid nanoparticles to avoid degradation. After cellular uptake, as ionizable cationic lipids are important components of LNP, they induce the escape of RNA from endosomes due to the charge interaction between lipids and endosomal membranes [6], thus playing a role in gene silencing.

However, the technology still faces some key challenges, such as tissue targeting. As it is already known, the disease occurs in several parts of the body, however, at present, the tissue targeting of LNP is mainly confined to the liver, and after intravenous injection, almost 90% of LNP will enter the liver, and eventually be absorbed by hepatocytes. Therefore, how to achieve the non-liver tissue targeting of LNP after systemic administration is a difficult problem to be solved.

What is more, the carrier molecule can trigger its own immune response. Patients must therefore take steroids, paracetamol, and antihistamines before taking Patisiran, which is given intravenously, to reduce the chance of an immune response. Other studies are underway to design a drug delivery system that can reach the target tissue without triggering an immune response.

In other words, while LNP technology is realizing the potential for gene silencing, it is noticeable that disadvantages exist. The experience gained from the clinical development and the use of the first LNP-siRNA drug product has provided valuable insights for improving technologies for future applications [7].

3.2. Givosiran

In 2019, the second siRNA drug approved for market by the FDA was Givlaari (Givosiran), which is the first kind of siRNA drug to be marketed with GalNAc coupling technology. The target is aminolevulinic acid synthase (ALAS), which is used to treat adult acute hepatic porphyria.

Acute hepatic porphyria (AHP) is a genetic disorder of heme biosynthesis. It is characterized by a life-threatening acute neurovisceral attack triggered by the factors that up-regulate hepatic 5-ALAS1 activity. Induction of hepatic ALAS1 leads to the accumulation of porphyrin precursors, in particular 5-aminolevulinic acid (AlA), which is considered to be a neurotoxic mediator leading to acute onset symptoms such as severe abdominal pain and autonomic dysfunction.

Repeated high intravenous (IV) doses are required to produce favorable RNAi-mediated therapeutic effects, therefore, robust subcutaneous delivery siRNA conjugates need to be developed [8].

Another delivery system called Galnac (n-acetyl 2-amino-2-deoxy-D-galactopyranose) was developed. It recognizes and binds to the cell surface protein asialoprotein receptor (ASGPR), which transports GalNAc from the cell surface to the cytoplasm via endocytosis. The receptor is specifically expressed in hepatocytes and is rare in other cells, so it has limitations in the delivery range and is effective only for liver targeting. Givosiran is a subcutaneously administered Galnac-conjugated small-molecule interfering RNA, targeting ALAS1, that is taken up almost exclusively by hepatocytes through the asialoglycoprotein receptor [9].

The main side effect of GalNAc-conjugated drugs is its hepatotoxicity. This is mainly due to the accumulation of its oligonucleotides and metabolites in the cell and the off-target effect it triggers [10]. With the improvement of the effectiveness of GalNAc conjugates, how to reduce their side effects has become a hot research topic.

3.3. Other drugs

More and more siRNA drugs are making progress and breakthroughs. In 2020, the third kind of siRNA drug approved by the FDA was Oxlumo (Lumasiran), which targets the glycolate oxidase HAO1 for the treatment of type 1 primary hyperoxaluria. In 2020, the fourth kind of siRNA drug approved by the FDA was Leqvio (Inclisiran), which targets PCSK9 for the treatment of hypercholesterolemia in adults. In 2022, the fifth kind of siRNA drug approved by the FDA was AMVUTTRA (Vutrisiran), an updated version of Patisiran that also targets the TTR and uses ESC-GalNAc conjugation. Such chemical modification of siRNA makes it more stable. Compared to Patisiran, which is delivered with a lipid complex, Vutrisiran is not effective enough, but patients' compliance has improved dramatically. Nearly

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100 additional GalNAc-siRNA-based therapies from Alnylam are undergoing clinical trials [11]. The great majority of these drugs use ESC-GalNAc conjugation, as the pharmaceutical company recognizes this as a promising delivery system.

4. Current challenges in the research and development of siRNA drugs

Although the mechanism of siRNA shows great potential in clinical applications and the pharmaceutical industry, its delivery in vivo still faces many challenges that cannot be ignored [12]. According to present drugs, various existing challenges can be seen, mainly in the limitations of targeting, off-target effect, immunogenicity of siRNA in large doses, as well as the toxicity, hindering its further application in vivo. Besides, the delivery system is the key to many problems, and the barrier and low delivery efficiency of siRNA in vivo have also become a bottleneck to the development of siRNA drugs. Currently, the following problems in detail are waiting to be solved: (1) siRNA cannot pass through the biofilm freely or target accurately, thus they must depend on a suitable delivery system, but there is not a perfect one to avoid all these problems; (2) the stability of siRNA drug transport in vivo after intravenous injection is poor, it is easy to be degraded by enzymes in blood and tissue, and also easy to be cleared by livers and kidneys, which results in an insufficient amount of drug reaching target tissue; (3) siRNA drugs may be recognized by the auto-immune system and produce immunogenicity; (4) siRNA drugs can only produce effects if they undergo efficient intracellular and exocytosis processes and maintain the integrity of the double-stranded siRNA structure, but some have difficulty with this.

5. Corresponding solutions to the problems of siRNA drugs

Therefore, in order to achieve the effective and safe therapeutic effect of siRNA on diseases, it is necessary to carry out stable structural modification and design a rational gene delivery system.

There might be different measures to solve some problems at present. According to present research, the stability and utilization of siRNA can be greatly improved by the modification of siRNA in vivo, including the modification of glycosyl, backbone, terminal, and base.

As for the siRNA delivery system, its quality should include strong stability, strong targeting, and good safety. The siRNA delivery system protects siRNA from being captured and degraded by nuclease and lysosome, prolonging the circulation time in the body; the siRNA delivery system can effectively reach the target site and can avoid causing the body immune response; the siRNA delivery system can quickly release siRNA into cytoplasm so that it can play a gene silencing role in the cytoplasm.

Although viral vectors have the advantage of transfection efficiency in nucleic acid delivery, they have potential safety risks such as immunogenicity and mutagenesis during clinical treatment. Therefore, the development of safe and efficient siRNA non-viral vectors has become the current research hot issue. In addition to the two carriers mentioned above, there are cationic polymers, biomimetic carriers, calcium phosphate nanoparticles, and so on.

6. Conclusion

RNAi technology can specifically silence the expression of specific genes and has irreplaceable advantages in exploring gene function and implementing gene therapy for multiple diseases. The delivery of these drugs is the main influencing factor but it still faces many technical challenges. How to further improve the delivery efficiency, reduce the toxicity of the delivery system, and achieve the delivery of other liver tissues and organs will be the focus of this field. This paper illustrates the discovery of RNAi, which was proven to function in human bodies, contributing to the treatment of diseases at the RNA level. After studying and summarizing the existing drugs, bottlenecks are found, such as the inability to target the whole body and the problem of triggering an immune response caused by Patisiran, or rather, its delivery system, which are the main current challenges of the research and development of siRNA drugs. Finally, the improvement measures for these problems are summarized, especially the selection and optimization of delivery vectors, so as to provide direction for the future development of drugs. It is believed that with the progress of gene sequencing and targeted delivery technology in recent years and in the future, especially the development of new nano-targeting carriers,

siRNA drugs will be developed rapidly and then steadily, with a growing number of siRNA drugs entering phase II or even phase III clinical trials. The successful development of more siRNA drugs is expected so that new treatments for some difficult-to-treat indications can be found and planned.

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