Research Progress and Challenges of Lipid Nanocarriers Mediated Sirna Delivery for Cancer Therapy

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Abstract. Cancer remains a major global health burden, and traditional treatment methods have limitations. Precision medicine, especially the use of small interfering RNA (siRNA), offers new hope. siRNA can specifically silence cancer - related genes, but its clinical application is hindered by multiple challenges, such as instability, poor cellular uptake, and potential off - target effects. Lipid - based delivery systems, including cationic liposomes, anionic and neutral liposomes, stable nucleic acid - lipid particles (SNALPs), and lipid nanoparticles (LNPs), have emerged as promising solutions. This review focuses on the role of siRNA in cancer treatment, its mechanism, and potential targets. It also comprehensively analyzes the lipid - based delivery systems, including their delivery strategies, advantages, and disadvantages. By exploring these aspects, this paper aims to provide valuable insights for future research and clinical applications in siRNA - based cancer therapies.

Keywords: Small interfering RNA (siRNA); Cancer treatment; Gene silencing; Delivery system; Lipid nanocarriers.

1. Introduction

Cancer is a complex and life - threatening disease that has long plagued the medical community. The data from the National Cancer Center indicates that there are over 100 identified types of cancer [1]. The metastasis of malignant tumor cells through the blood - lymphatic system to various tissues and organs not only complicates treatment but also significantly contributes to the high mortality rate associated with cancer [2]. This metastatic ability is a major obstacle in effectively combating the disease.

Traditional cancer treatment methods, such as surgical resection, radiotherapy, and chemotherapy, have been the cornerstones of cancer management for a long time. Surgical resection can precisely remove non - metastatic tumors with the help of advanced surgical and imaging technologies. However, it is ineffective against metastatic cells, which may have already spread to distant sites in the body. Radiotherapy uses high - energy radiation to target and kill tumor cells. Still, it often causes damage to healthy tissues surrounding the tumor, resulting in a range of side effects that can impact the patient's quality of life. Chemotherapy, which works by inhibiting cell proliferation, unfortunately, also affects normal proliferating cells. This can lead to various adverse effects like hair loss, nausea, and a weakened immune system. Moreover, cancer cells can develop drug resistance over time, rendering chemotherapy less effective [3][4].

The emergence of precision medicine in 2015 marked a significant milestone in cancer treatment. Leveraging advancements in genome sequencing and bioinformatics, it enables personalized treatment strategies. Small interfering RNA (siRNA) has become a promising option within this framework. siRNA can specifically silence disease - related genes through base - complement pairing. Its high specificity ensures that it can target genes accurately with minimal off - target effects. Operating solely in the cytoplasm, siRNA avoids entering the nucleus and integrating into the genome, thus reducing the risk of causing host gene mutations. Even a small amount of siRNA can trigger a significant gene - silencing effect. With the continuous progress in molecular biology and whole - genome sequencing, the potential targets for siRNA have become more diverse and numerous [5].

In summary, the development of precision medicine has brought new hope for cancer treatment, among which siRNA has shown great potential, but many problems hinder its clinical application. Going forward, a deeper understanding of the mechanism of action and potential targets of siRNA

therapy for cancer will help explore more effective solutions and lay the foundation for subsequent research.

2. Mechanism of siRNA and Potential Targets for Cancer Therapy

Small interfering RNA (siRNA) holds great promise in cancer treatment, and understanding its mechanism and potential targets is crucial for advancing cancer therapy (Figure 1).

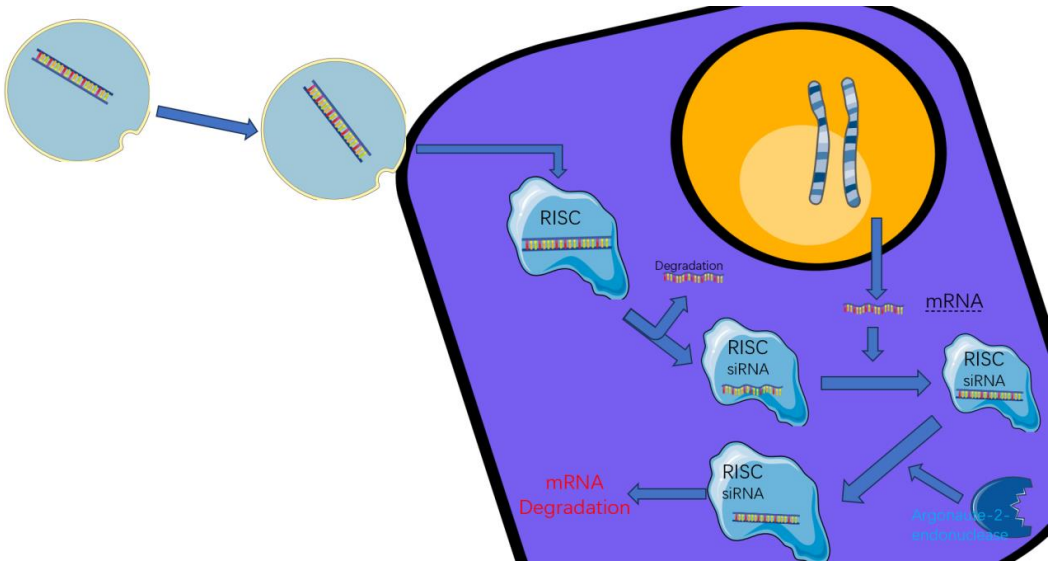

Figure 1: siRNA mechanism diagram.

The mechanism of SirNa-based cancer therapy stems from the principle of RNA interference (RNAi). When siRNA enters the cell, it binds to the RNA-induced silencing complex (RISC). The RISC-siRNA complex then recognizes and binds complementary sequences on the target mRNA by base pairing. This combination prompts the Argonaute-2 endonuclide in RISC to cut the target mRNA, blocking the translation process of mRNA to protein, and finally silencing the expression of the target gene [6]. This highly specific gene - silencing mechanism offers a powerful approach to target cancer - related genes.

There are numerous potential targets for siRNA in cancer therapy. For example, genes involved in cell cycle regulation, such as polo - like kinases (Plks), are promising targets. Plks play a crucial role in mitosis, and their overexpression is often associated with cancer progression. Silencing Plk genes using siRNA can disrupt the cell cycle of cancer cells, inhibiting their proliferation [7]. Another important target is the hypoxia - inducible factor - 2α (HIF - 2α). In many cancers, HIF - 2α is overexpressed under hypoxic conditions, promoting tumor growth, angiogenesis, and metastasis. Targeting HIF - 2α with siRNA can potentially block these processes and slow down cancer development [8].

Moreover, genes related to cancer metabolism, like glucose transporters (GLUTs), are also potential siRNA targets. GLUTs are responsible for the increased glucose uptake in cancer cells, which is essential for their rapid growth. By silencing GLUT genes with siRNA, it may be possible to starve cancer cells of energy, thereby inhibiting their proliferation [9].

It can be seen that the action mechanism of siRNA is precise and the potential targets are abundant, which opens up a new path for cancer treatment. However, to translate this into effective clinical therapies, an efficient delivery system is essential. The following will explore lipid-based siRNA delivery systems in detail and analyze how they address the challenges of siRNA delivery (Figure 2).

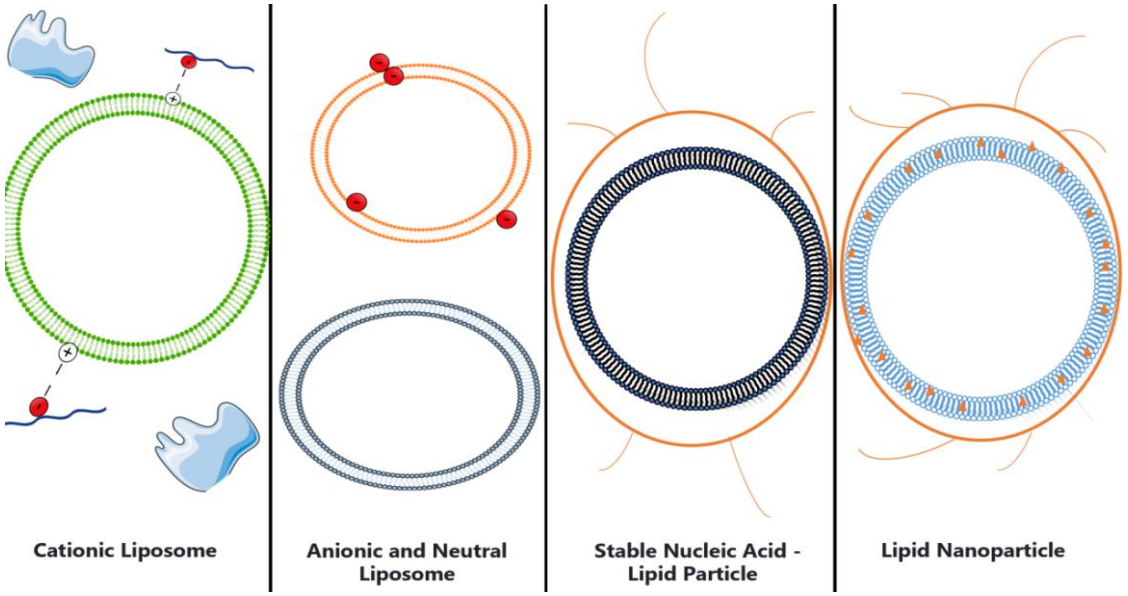

Figure 2: Lipid-Based siRNA Delivery Systems.

3. Lipid - Based siRNA Delivery Systems

In the pursuit of effective siRNA - based therapies, lipid - based delivery systems have emerged as a cornerstone, offering a promising solution to the numerous challenges associated with siRNA delivery. These challenges include the instability of siRNA in physiological environments, its poor cellular uptake, and the need for efficient endosomal escape. Lipid - based delivery systems have the potential to overcome these barriers, making them a subject of intense research in recent years. This section will comprehensively explore the various lipid - based siRNA delivery strategies, their origins, detailed mechanisms, differences, and comparative advantages and disadvantages.

3.1. Cationic Liposomes

Cationic liposomes have long been recognized as a popular choice for siRNA delivery. Among them, commercial Lipofectamine stands out due to its remarkable transformation efficiency at the cellular level with RNAiMAX [10]. The underlying mechanism of cationic liposomal mediated siRNA delivery is based on electrostatic interactions. Because cationic lipids, such as dioleyl-phosphatidylethanolamine and 1, 2-dioleyl-3-trimethylpropane (DOTAP), have a positive charge, negatively charged siRNA molecules are able to form electrostatic complexes with them. This complex can then interact with the negatively charged cell membrane, ultimately leading to the cell phagocytosis of siRNA [11].

For example, Sorensen et al. successfully used DOTAP liposomes to deliver TNF-α-siRNA and effectively inhibited bacterial lipopolysaccharide (LPS) -induced death in a mouse model of sepsis [12]. In addition, a cationic lipid carrier system developed by Balgobind et al., consisting of a cholesterol cell effector protein and the neutral helper lipid dioleyl phosphatidyl ethanolamine (DOPE), showed efficient transfection and gene silencing in cells overexpressing HER2/neu[13]. Jarallah et al. used Genzyme lipid 67 (GL67), DC-Chol and DOPE lipids to design a highly efficient siRNA nanoparticle liposome carrier, which significantly improved the metabolic activity and uptake rate of A549 cells [14].

However, cationic liposomes are not without their drawbacks. Their cationic nature makes them less biocompatible compared to anionic or neutral liposomes. The positive charge on cationic liposomes can lead to non - specific interactions with plasma proteins and cells, resulting in their rapid clearance by the mononuclear phagocyte system (MPS), especially in organs like the liver and spleen [18]. This reduces their circulation time in the bloodstream, limiting their ability to reach target tissues effectively. Moreover, excessive positive charge can cause cytotoxicity, as it may disrupt the

cell membrane integrity and intracellular processes. This cytotoxicity can lead to unwanted side effects and limit the clinical applications of cationic liposomes. To mitigate these issues, strict control of the charge of cationic liposomes is required, typically by maintaining an appropriate complex phosphorus ratio (N/P) of around 2 to 3 [15].

3.2. Anionic and Neutral Liposomes

Because the negatively charged nature of biofilm is conducive to the compatibility of anionic or neutral liposomes with physiological environment, anions and neutral liposomes with better biocompatibility compared with cationic liposomes have attracted attention. For example, 1, 2-dioleyl-SN-glycerol-3-phosphatidylcholine (DOPC), which is widely used to improve the encapsulation efficiency of siRNA, is a significantly neutral lipid.

In 2005, Landen et al. developed SIRNA-neutral liposomes containing the targeted oncoprotein EphA2 (siRNA-EphA2-DOPC). In a mouse model of in-situ ovarian cancer, EphA2 expression was significantly down-regulated within 48 hours after administration [16]. siRNA-EphA2-DOPC is currently in Phase I clinical trials at md Anderson Cancer Center (MDA), showing potential as a therapeutic agent.

Recently, Sahu et al. constructed a DOPC based nanocore system (NL-mTOR siRNA), which can effectively and successfully deliver mTOR siRNA. In vitro and in vivo experiments have confirmed that NL-mTOR-siRNA can inhibit the growth and invasion of breast cancer cells, reshape tumor morphology, and enhance the enrichment of siRNA in breast cancer tissues. This not only optimizes the distribution of siRNA within the tumor, but also enhances its anti-tumor efficacy [17].

Ramos-Gonzalez et al. implemented respiratory delivery using a novel neutral DOPC liposome siRNA system (L-siRNA). This strategy successfully silenced the WT1 gene in vitro in B16F10 melanoma cells and in a mouse model of lung metastatic melanoma. L-siRNA not only inhibited cell proliferation, greatly reduced tumor weight, but also delayed the death of experimental animals [18].

However, anionic and neutral liposomes also have limitations. Their transfection efficiency is generally lower compared to cationic liposomes. Since they lack the strong electrostatic interaction with cell membranes that cationic liposomes possess, it is more difficult for them to deliver siRNA into cells. This lower transfection efficiency may limit their effectiveness in certain therapeutic applications where high - level gene silencing is required.

3.3. Stable Nucleic Acid - Lipid Particles (SNALPs)

Stable Nucleic Acid - Lipid Particles (SNALPs) are a well - known and highly promising class of lipid - based siRNA delivery vectors. The FDA - approved siRNA drug for Ebola treatment is delivered by SNALPs, highlighting their clinical significance [19]. SNALPs are lipid nanoparticles with a characteristic structure. They have a lipid bilayer composed of a mixture of cationic lipids and pro - fusion lipids on the outer layer, which helps in membrane fusion and cellular entry. The siRNA is encapsulated within the inner layer, protecting it from degradation. The surface of SNALPs is modified with polyethylene glycol (PEG), which confers several advantages. PEGylation allows SNALPs to evade the immune system and achieve prolonged circulation in the bloodstream. It also enables passive enrichment at the tumor sites through the enhanced permeability and retention (EPR) effect, as tumor blood vessels are often leaky, allowing the nanoparticles to accumulate in the tumor tissue [19].

Alnylam Pharmaceuticals created the formulation of ALN-VSP02 by simultaneously encapsulating two SiRnas (VEGF siRNA and KSP siRNA) in SNALPs and entered Phase I clinical trials in advanced solid tumors in April 2009. The initial 28 patients were well tolerated and showed antitumor activity after receiving six doses of the highest dose (1.25mg/kg). In particular, one patient with endometrial cancer achieved complete remission, strongly confirming the potential of SNALP-based siRNA delivery in cancer therapy [20].

SNALP - Plk1 siRNA (TKM080301) was developed by Tekmira Pharmaceuticals and is now in the process of phase I and II clinical trials targeting diverse solid tumors and lymphomas [21]. A

stable SNALP formulation was created by Abdel - Bar and his colleagues. This formulation has the ability to co - transport Dox and siCD47. Notably, it can synergistically boost the tumor immunogenic cell death (ICD). After optimization, the SNALPs achieved an encapsulation efficiency of more than 65% for both siRNA and dox, and also saw an improvement in serum stability. In a tumor challenge model [22], the combination of siCD47 and SNALPs loaded with Dox manifested powerful anti - tumor activity.

Despite their successes, SNALPs face challenges. The manufacturing process of SNALPs is complex and requires precise control over lipid composition, particle size, and encapsulation efficiency. This complexity can lead to batch - to - batch variability, which is a major concern for large - scale production and clinical applications. Additionally, the long - term effects of PEGylation on the body, such as potential immunogenicity and accumulation in certain tissues, are still being investigated.

3.4. Lipid Nanoparticles (LNPs)

As another key lipid siRNA delivery carrier, lipid nanoparticles (LNPs) are composed of lipids, cholesterol and pegylated lipids. Similar in structure to SNALPs, LNPS have a lipid bilayer structure and pegylated surface, which enclose siRNA in its core [20]. Akinc et al. pioneered a method for rapid chemical synthesis of lipid-like libraries, from which they screened the most efficient lipid-like nanoparticles for siRNA delivery. Among them, 98N12-5 is one of the most promising lipid nanoparticles. When using 98N12-5 encapsulated siRNA with ApoB or FVII factor, 75% to 90% gene silencing was achieved in non-human primate hepatocytes, fully demonstrating its high efficiency in siRNA delivery [23].

LNPs offer several advantages. They can protect siRNA from degradation by nucleases in the bloodstream and extracellular environment. The lipid bilayer provides a physical barrier that shields the siRNA. LNPs also enhance the cellular uptake of siRNA. The PEGylation on their surface improves their biocompatibility and reduces non - specific interactions with plasma proteins and cells. This results in a longer circulation time in the bloodstream, increasing the chances of the nanoparticles reaching the target tissues. Moreover, LNPs can be easily modified with targeting ligands to achieve active targeting, further improving their delivery specificity.

However, LNPs also have limitations. Similar to SNALPs, the manufacturing of LNPs on a large scale is challenging. The optimization of lipid composition, particle size, and surface properties requires a high level of technical expertise and resources. The cost of production is relatively high, which may limit their widespread use in clinical settings, especially in resource - constrained regions. Additionally, the long - term safety of LNPs, particularly with regard to potential lipid accumulation in the body and its consequences, needs to be further investigated.

In general, each lipid-based siRNA delivery system has advantages and disadvantages. The current issues point the way for further research, and further research is needed to address these issues in the journey to optimize delivery systems and move SirNa-based cancer therapies from the laboratory to the clinic. In the following, these research directions are prospected to explore how to further explore the potential of lipid-based delivery systems in the future.

4. Conclusion

In conclusion, siRNA has great potential in cancer treatment due to its specific gene - silencing ability. However, its successful clinical translation is currently restricted by challenges in delivery and potential side - effects. Lipid - based delivery systems present a variety of options, each with its own set of characteristics. Cationic liposomes offer high transfection efficiency but suffer from biocompatibility and cytotoxicity issues. Anionic and neutral liposomes have better biocompatibility but lower transfection efficiency. SNALPs and LNPs show promise in clinical applications, yet face difficulties in large - scale manufacturing and cost - effectiveness. Future research efforts should be directed towards optimizing these delivery systems. This includes enhancing their stability,

improving transfection efficiency, increasing biocompatibility, and reducing production costs. By addressing these key issues, lipid - based siRNA delivery systems can be further developed, enabling the full realization of the potential of siRNA - based therapies in combating cancer and other diseases.

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