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Liposomal siRNA nanocarriers for cancer therapy ☆

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ABSTRACT

Small interfering RNAs (siRNA) have recently emerged as a new class of therapeutics with a great potential to revolutionize the treatment of cancer and other diseases. A specifically designed siRNA binds and induces post-transcriptional silencing of target genes (mRNA). Clinical applications of siRNA-based therapeutics have been limited by their rapid degradation, poor cellular uptake, and rapid renal clearance following systemic administration. A variety of synthetic and natural nanoparticles composed of lipids, polymers, and metals have been developed for siRNA delivery, with different efficacy and safety profiles. Liposomal nanoparticles have proven effective in delivering siRNA into tumor tissues by improving stability and bioavailability. While providing high transfection efficiency and a capacity to form complexes with negatively charged siRNA, cationic lipids/liposomes are highly toxic. Negatively charged liposomes, on the other hand, are rapidly cleared from circulation. To overcome these problems we developed highly safe and effective neutral lipid-based nanoliposomes that provide robust gene silencing in tumors following systemic (intravenous) administration. This delivery system demonstrated remarkable antitumor efficacy in various orthotopic human cancer models in animals. Here, we briefly overview this and other lipid-based approaches with preclinical applications in different tumor models for cancer therapy and potential applications as siRNA-nanotherapeutics in human cancers.

Contents

1. Introduction

1.1. Gene silencing by small-interfering RNA

The discovery of RNA interference (RNAi), including micro RNA (miRNA) and small-interfering RNA (siRNA) mediated gene silencing, is considered one of the most important advancements in biology in the last decade [1–3]. siRNA is now commonly used as a powerful tool for silencing post-transcriptional gene expression and investigating gene. More importantly, potential applications of siRNA have led to a great interest in harnessing this technology for therapeutic use in cancer and other diseases. A specifically designed siRNA can bind the target gene (mRNA) in a sequence specific manner and induce degradation of mRNA translation [3]. These short double-stranded (ds) RNAs are cleaved into fragments called siRNA (21-base pairs) by DICER protein.

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The target mRNA is bound by the antisense strand after forming a complex with proteins, designated as the RNA-Induced Silencing Complex (RISC). An RNA endonuclease (Argonaute 2) within the complex cleaves the target mRNA and leads to its degradation, shutting down protein expression (Fig. 1). For therapeutic applications, synthetic siRNA is used for targeting oncogenes and genes that are involved in cancer cell proliferation, survival, invasion, angiogenesis, metastasis, and resistance to chemotherapy or radiotherapy in cancer and for targeting disease-causing genes in other pathologies [4,5].

1.2. Obstacles for systemic use of siRNA-based therapeutics

The broad therapeutic applications of siRNA-based therapeutics in cancer are largely dependent on the development of rationally designed systemic delivery systems that can efficiently deliver the siRNA molecules into tumors and target cells [6,7]. The major limitations of the systemic use of siRNA-based therapies include rapid degradation by nucleases (half-life ~15 min in serum) and renal clearance following systemic administration [8]. Thus earlier studies with siRNA-based therapies entered into clinical trials relied on the local administration, including the intravitreal or intranasal routes [7,8]. To enhance the stability of various siRNA chemical modifications, such as backbone (phosphorothioate, boranophosphate) and sugar modifications (2′ modifications to the sugar ring, namely 2′-OMe, 2′-fluoro, and 2′-*O*-methoxyethyl (2′-MOE)), have been used [7]. However, poor cellular uptake remains an important issue due to negatively charged cell membranes preventing efficient intracellular uptake of siRNA molecules, which also have a negatively charged backbone, leading to electrostatic repulsion, requiring a carrier to increase the uptake into cancer cells. Rationally designed specific siRNA for the exclusion of partially complementary sequences and certain motifs that induce immune response and the use of the minimum effective dose of siRNA may also enhance unwanted side effects [4]. Overall, developments of safe, stable, effective and tumor-specific delivery systems for systemic administration are important goals for translation of siRNA-based therapeutics into successful clinical applications. Nanotechnology holds promise for widespread clinical applications of siRNA-therapeutics. Nanocarriers also have great potential to reduce siRNA related toxicities and prevent off-target effects in normal tissues (reviewed in detail by Jackson and Linsley, 2010) [50].

2. Nanocarriers for systemic siRNA delivery

Nanocarriers (submicron size particles ranging from 1 to 1000 nm) can overcome most hurdles that prevent the systemic use of siRNA [9,10]. Nanoparticles have been shown to carry and deliver desired cargos or payloads, such as chemotherapeutic agents, oligonucleotides, drugs, peptides, and imaging agents in *in vivo* systems. In general, the ideal nanocarrier is expected to be safe, non-toxic, biocompatible, biodegradable, and non-immunogenic, and to be able to bypass rapid hepatic or renal clearance. Furthermore, an ideal delivery system should be able to preferentially target siRNA into the tumor or preferred tissues, and allow escape of the siRNA from endosomal capsulation, releasing the payload into cytoplasm for maximal efficacy.

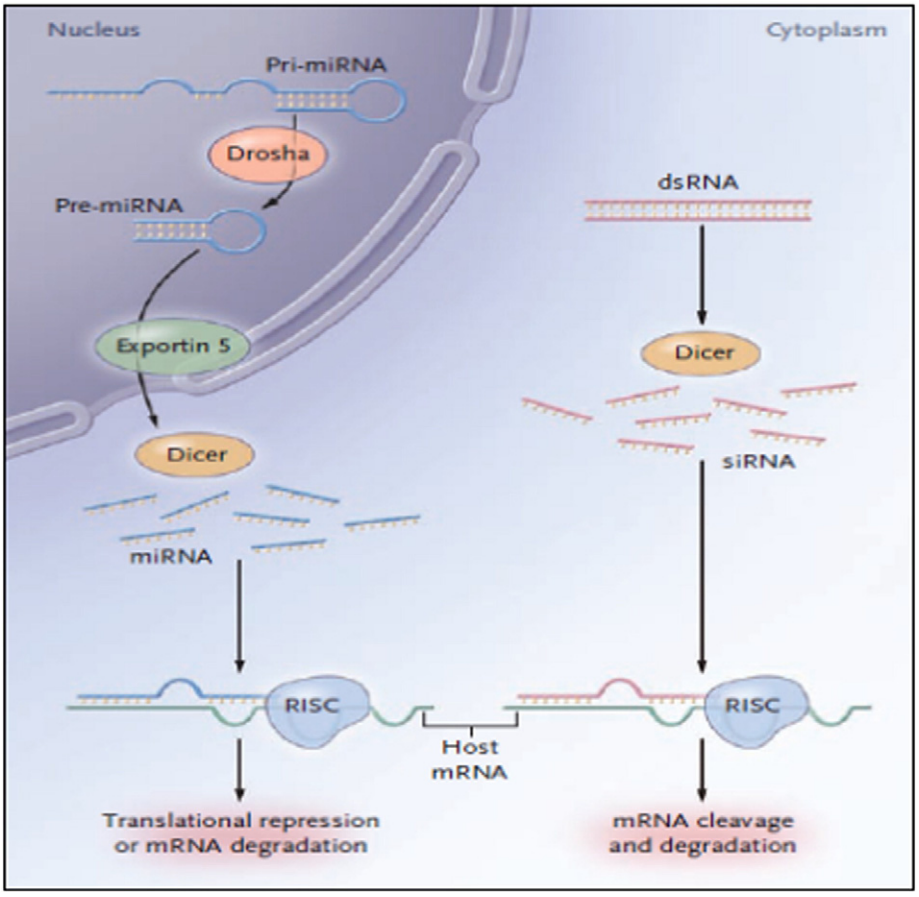

Fig. 1. The process of RNA-interference in eukaryotic cells. Long precursor microRNA (miRNA), called pri-miRNA, is cleaved by RNase III endonuclease (Drosha) into pieces of approximately 70 nucleotides each (called pre-miRNA) in the nucleus. Following transportation into the cytoplasm by exportin 5 another RNase III endonuclease (Dicer) cleaves it into mature miRNA segments. Degradation of messenger RNA (mRNA) and translational repression occurs after miRNA binds to the RNA-induced silencing complex (RISC). Cytoplasmic long double-stranded RNA (dsRNA) is cleaved by Dicer into small interfering RNA (siRNA), which is incorporated into RISC, resulting in the cleavage and degradation of specific target mRNA. Synthetic double-stranded siRNA is not processed by Dicer and directly incorporated by the RISC.
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