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Review

Delivery systems for siRNA drug development in cancer therapy

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ABSTRACT

Since the discovery of the Nobel prize-winning mechanism of RNA interference (RNAi) ten years ago, it has become a promising drug target for the treatment of multiple diseases, including cancer. There have already been some successful applications of siRNA drugs in the treatment of age-related macular degeneration and respiratory syncytial virus infection. However, significant barriers still exist on the road to clinical applications of siRNA drugs, including poor cellular uptake, instability under physiological conditions, off-target effects and possible immunogenicity. The successful application of siRNA for cancer therapy requires the development of clinically suitable, safe and effective drug delivery systems. Herein, we review the design criteria for siRNA delivery systems and potential siRNA drug delivery systems for cancer therapy, including chemical modifications, lipid-based nanovectors, polymer-mediated delivery systems, conjugate delivery systems, and others.

1. Introduction

RNA interference (RNAi) was first discovered in plants, but it was not widely noted in animals until Fire and Mello demonstrated that double-stranded RNA (dsRNA) can cause greater suppression of gene expression than single-stranded RNA (ssRNA) in *Caenorhabditis elegans* [1]. Due to the excellent gene silencing potential of RNAi, it has attracted broad attention in terms of how to harness the capabilities of RNAi. In 2001, Tuschl et al. first transferred dsRNA into mammalian cells and solved the interferon effect of dsRNA transfection in these cells, which broadened the therapeutic use of Rania [2]. In 2010, Davis et al. reported the first targeted siRNA delivery nanoparticle in humans via systemic injection, which provided a reference and a solid foundation for siRNA clinical use [3]. In recent years, RNAi has become more and more important in gene silencing and drug development because of its high specificity, significant effect, minor side effects and ease of synthesis.

Naturally, RNAi is an important defense mechanism by which eukaryotic cells can degrade exogenous genes, like viruses. When dsRNA enters the cell, it is first cleaved into short double stranded fragments of ~20 nucleotide siRNAs by

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the enzyme Dicer. Then, each double stranded siRNA is split into the passenger strand and the guide strand. After that, the guide strand is incorporated into the RNA-induced silencing complex (RISC), while the passenger strand is degraded. In the RISC, the guide strand of siRNA pairs with a complementary sequence in a messenger RNA molecule and induces cleavage by Argonaute, which causes post-transcriptional gene silencing. There are three strategies for RNAi: short hairpin RNA (shRNA), endogenous microRNA (miRNA) and small interfering RNA (siRNA). siRNA is more suitable for drug use because it does not require genome integration and can be easily synthesized. Since rational design of siRNA can specifically inhibit endogenous and heterologous gene expression, it can modulate any disease-related gene expression. For example, most cancer is caused by oncogene overexpression or gene mutation, so it may be possible to cure cancer by disease-related gene suppression via rational siRNA design. Owing to its great potential in biological research and drug development, RNAi was awarded the Nobel Prize for medicine in 2006. Since then, billions of dollars have been invested in the therapeutic application of RNAi in humans. At least 22 RNAi-based drugs have entered clinical trials (Table 1).

Among these clinical trials, most siRNAs were administered by local delivery, typically via the intravitreal or intranasal routes. However, local delivery may not be appropriate for all diseases. Under some circumstances, systemic drug administration by intravenous (i.v.) injection is needed, and delivery systems will be necessary to administer the siRNA payload. For example, PRO-040201 (ApoB-SNALP) administrated by i.v. injection was developed by Tekmira with a stable nucleic acid lipid particle (SNALP) system. It was developed for the treatment of hypercholesterolemia by targeting ApoB, which is produced by hepatocytes. In July 2009, Tekmira initiated a Phase I clinical trial for PRO-040201. Seventeen subjects received a single dose at one of seven different dosing levels and six subjects received a placebo. The results revealed that ApoB siRNA was delivered into hepatocytes efficiently and resulted in a significant reduction of LDL and triglycerides in blood. However, Tekmira terminated the clinical trial in January 2010 because one of the two subjects treated with the highest dose experienced flu-like symptoms consistent with stimulation of the immune system caused by the ApoB siRNA payload [4]. Calando Pharmaceuticals (Pasadena, California, USA) has developed an siRNA therapeutic (CALAA-01), which is a cyclodextrin-based polymeric nanoparticle containing the M2 subunit of ribonucleotide reductase (RRM2) targeted siRNA. CALAA-01 was modified with the human transferrin (TF) protein and polyethylene glycol (PEG) to improve its stability [3]. Unfortunately, its phase I clinical trial has been terminated in 2013 according to U.S. Food and Drug Administration (FDA). In addition to the abovementioned siRNA drugs, many more are in the developmental pipeline.

2. Advantages of siRNA and barriers to siRNA in cancer therapeutics

Compared to chemotherapeutic anti-cancer drugs, there are a lot of advantages of siRNA drug. Due to the special mechanism of siRNA, it has four advantages as a potential cancer therapeutic strategy. The first is its high degree of safety. siRNA acts on the post-translational stage of gene expression, so it does not interact with DNA and thereby avoids the mutation and teratogenicity risks of gene therapy. The second advantage of siRNA is its high efficacy. In a single cancer cell, siRNA can cause dramatic suppression of gene expression with just several copies. Compared to other small molecule drugs or antibody-based drugs, the greatest advantages of siRNA are the unrestricted choice of targets and specificity determined by the principle of complementary base pairing. This strategy also benefits from rapid developments in molecular biology and whole-genome sequencing. In addition, comprehensive nucleotide sequence databases have been established, including human genomic databases, cDNA databases and disease gene databases, which have laid a solid foundation for siRNA drug development. The basic strategy of an siRNA drug is to treat cancer by silencing the specific cancer-promoting gene with rationally designed siRNA. Of course, it is also possible to design effective siRNA drug targeting for any disease gene according to the mRNA sequence.

However, several barriers still exist on the road to siRNA clinical use for cancer therapy (Fig. 1). Firstly, siRNA is unstable under physiological conditions. When siRNA traffics through the blood, it is easily digested by nucleases in the serum. The intracellular trafficking of siRNA delivered by different reagents generally begins in early endosomes. These early endosomes subsequently fuse with sorting endosomes, which in turn transfer their contents into late endosomes. The endosomal compartments of cell are significantly acidic (pH 5.0~6.2), while the cytosol or intracellular space is neutral (pH $\approx$ 7.4) [5]. Endosome is then relocated to the lysosomes, which are further acidified (pH $\approx$ 4.5) and contain various nucleases that promote the degradation of siRNA [6]. The ideal administration route of siRNA is systemic injection, so that siRNA can reach cancer cells more efficiently. After injection into the blood, siRNA is easily enzymatically degraded by endogenous nucleases, filtered by the kidney, taken up by phagocytes and aggregated with serum proteins [7]. One of the first biological barriers encountered by administered siRNA is the nuclease activity in plasma and tissues. The major nuclease in plasma is a 3′ exonuclease; however, cleavage of internucleotide bonds can also take place. The reported half-life for unmodified siRNA in serum ranges from several minutes to 1 h [8]. In addition, the kidney plays a key role in siRNA clearance; several studies in animals have reported that the biodistribution of siRNA shows the highest uptake in the kidney [9]. In addition to circulating nuclease degradation and renal clearance, a major barrier to *in vivo* delivery of siRNA is uptake by the reticuloendothelial system (RES). The RES is composed of phagocytic cells, including circulating monocytes and tissue macrophages, the physiological function of which is to clear foreign pathogens and to remove cellular debris and apoptotic cells [10]. Tissue macrophages are most abundant in the liver (where they are called Kupffer cells) and the spleen, tissues that also receive high blood flow and exhibit a fenestrated vasculature. Thus, it is not surprising that these organs accumulate high concentrations of siRNA following systemic administration. siRNA uptake after standard i.v. tail vein injection or intraperitoneal (i.p.) injection