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Imaging-guided delivery of RNAi for anticancer treatment☆

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ABSTRACT

The RNA interference (RNAi) technique is a new modality for cancer therapy, and several candidates are being tested clinically. In the development of RNAi-based therapeutics, imaging methods can provide a visible and quantitative way to investigate the therapeutic effect at anatomical, cellular, and molecular level; to noninvasively trace the distribution; to and study the biological processes in preclinical and clinical stages. Their abilities are important not only for therapeutic optimization and evaluation but also for shortening of the time of drug development to market. Typically, imaging-functionalized RNAi therapeutics delivery that combines nanovehicles and imaging techniques to study and improve their biodistribution and accumulation in tumor site has been progressively integrated into anticancer drug discovery and development processes. This review presents an overview of the current status of translating the RNAi cancer therapeutics in the clinic, a brief description of the biological barriers in drug delivery, and the roles of imaging in aspects of administration route, systemic circulation, and cellular barriers for the clinical translation of RNAi cancer therapeutics, and with partial content for discussing the safety concerns. Finally, we focus on imaging-guided delivery of RNAi therapeutics in preclinical development, including the basic principles of different imaging modalities, and their advantages and limitations for biological imaging. With growing number of RNAi therapeutics entering the clinic, various imaging methods will play an important role in facilitating the translation of RNAi cancer therapeutics from bench to bedside.

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1. Introduction

Since it was discovered that double-stranded RNA (dsRNA) can induce RNA interference (RNAi) in *Caenorhabditis elegans* [1], the capability of RNAi that can suppress the expression of target genes with high specificity, and efficacy has been confirmed with small RNA sequences (siRNA/miRNA) [2]. Since then, much effort has been directed to develop RNAi therapeutics into clinic as a potential disruptive tool for new conservative and personalized treatment of a broad range of diseases [3], including viral infection [4], eye disorder [5], inflammatory disease [6], cardiovascular disease [7], genetic disorder [8], and cancer [9–11].

Currently, both naked siRNAs and nanoparticle-mediated delivery of RNA fragments have been investigated for cancer therapy. The administration route for naked siRNA is critically reliant on its accessibility to the target sites inside the body. Most of the naked siRNA fragments have been administered topically, which has the advantages of high bioavailability in target organs and low adverse reactions compared to systemic administration. Because the systemic administration of siRNA fragments requires them to reach the right organs or tissues after dispersing in the body and passing through the biological barriers, which is virtually impossible for naked small RNAs. The systemic delivery of siRNAs or other small therapeutic RNAs to target sites is impeded by many factors including nuclease degradation and rapid systemic elimination of "naked" small RNAs in biological systems [12]; intrinsic negative charge nature on the surface of small RNAs make them very difficult to transfer into cells that also with negatively charged surface of bilayer phospholipid membrane [13]. Therefore, a variety of delivery systems have been engineered to address above issues and deliver small RNAs specifically to target sites, including tumors and inflammatory foci, for instance, lipid nanoparticles made with cationic lipids; lipidol and other lipid materials [14–17]; polymeric nanoparticles made with polyethylenimine (PEI); dendrimers; membrane-disruptive and cyclodextrin-containing polymers [18–20]; membrane-disruptive peptides [21,22]; aptamer-siRNA chimeras [23]; single-chain fragmented antibodies [24]; supramolecular assemblies [25–27]; inorganic nanoparticles, including multishell nanoparticles, mesoporous nanoparticles, and carbon-based nanomaterials [28]; and so on [29–33]. However, the entrapment of nano-sized carriers in the reticuloendothelial system (RES), the vascular endothelial resistance in tissues, the barriers of extracellular matrix, and the intracellular release from endosomal uptake [34,35] are the major challenges for the systemic delivery of RNAi therapeutics. In addition, the siRNA-based therapeutics encounter safety concerns owing to unexpected adverse effects, including off-target effects, innate immune responses, proinflammatory cytokine induction, and spleen toxicity, which were found in the early studies of those candidates [8,36–38].

There are three major aspects of scientific challenges in developing RNAi therapeutics for cancer therapy (Fig. 1): (1) target gene selection for RNAi and gene vector development for either systemic or local administration; (2) product screening and preclinical evaluation including the relative efficacy, biodistribution, pharmacokinetics, and toxicity; and (3) clinical study of the efficacy, safety, pharmacokinetics, and optimal dose. It has been proven that noninvasive imaging methods and biomarker detection could speed up the development of RNAi therapeutics [39–41]. Developing powerful imaging techniques and methods is important for providing valuable information by visualizing, characterizing, and quantifying the biological processes of RNAi therapeutics and monitoring their therapeutic effects and factors that are crucial for the optimization of RNAi cancer therapeutics.

Generally, *in vivo* imaging of RNAi refers to the utilization of a variety of imaging modalities, including bioluminescence imaging (BLI) [42], photoluminescence imaging [43,44], magnetic resonance imaging (MRI) [45], positron emission tomography (PET) [46], single-photon emission computed tomography (SPECT) [47], and ultrasound [48], to quantitatively and/or qualitatively visualize the *in vivo* behavior of the RNAi therapeutics. Many imaging modalities have been applied for RNAi researches. The therapeutic genes have been directly or indirectly marked with a variety of imaging contrast agents to make them detectable in biological systems with clinically relevant imaging equipment. These probe-labeled systems with target delivery function hold high promise for studying the pharmacological properties of RNAi therapeutics in clinical translation. This review highlights the current status of RNAi-based cancer therapeutics in clinical trials with discussion of selected cases. Before addressing the current progresses of applying

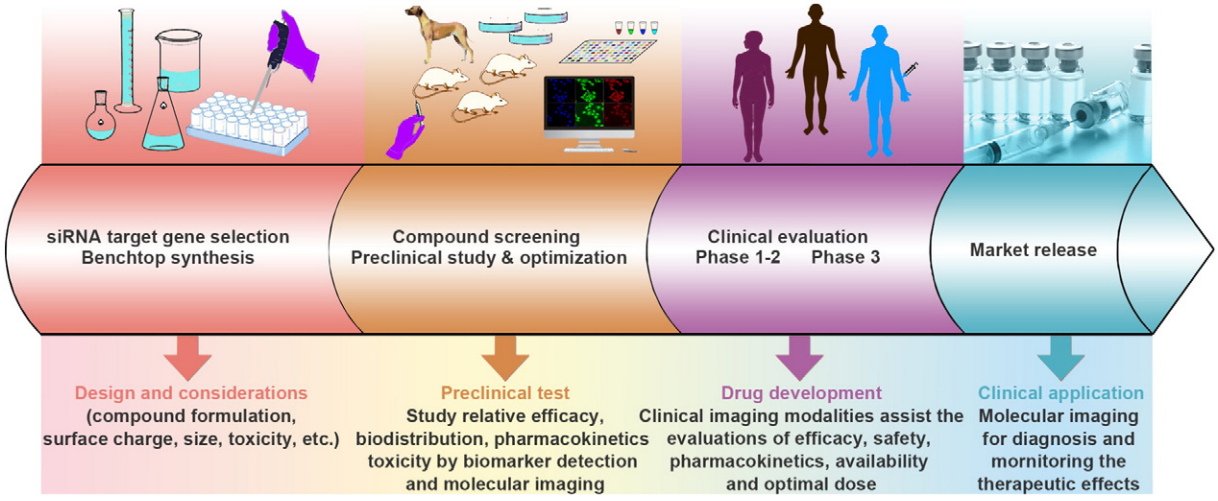

Fig. 1. The workflow of developing RNAi therapeutics and translating them for clinical applications. There are mainly four steps for this, including targeting gene selection, compound screening, clinical evaluation, and market release. Imaging methods could assist the development of RNAi therapeutics at every step in the RNAi therapeutics development processes, including marking for gene selection, tracing the small RNA sequences for pharmacokinetics study, evaluating the gene-silencing efficacy, and diagnosing tumors and monitoring the therapeutic effects.