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Current preclinical small interfering RNA (siRNA)-based conjugate systems for RNA therapeutics☆

Soo Hyeon Lee [b], Yoon Young Kang [a], Hyo-Eun Jang [a], Hyejung Mok *,[a]

[a] Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea
[b] Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology Zurich (ETHZ), Zurich, Switzerland

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ABSTRACT

Recent promising clinical results of RNA therapeutics have drawn big attention of academia and industries to RNA therapeutics and their carrier systems. To improve their feasibility in clinics, systemic evaluations of currently available carrier systems under clinical trials and preclinical studies are needed. In this review, we focus on recent noticeable preclinical studies and clinical results regarding siRNA-based conjugates for clinical translations. Advantages and drawbacks of siRNA-based conjugates are discussed, compared to particle-based delivery systems. Then, representative siRNA-based conjugates with aptamers, peptides, carbohydrates, lipids, polymers, and nanostructured materials are introduced. To improve feasibility of siRNA conjugates in preclinical studies, several considerations for the rational design of siRNA conjugates in terms of cleavability, immune responses, multivalent conjugations, and mechanism of action are also presented. Lastly, we discuss lessons from previous preclinical and clinical studies related to siRNA conjugates and perspectives of their clinical applications.

Contents

1. Introduction

Small interfering RNAs (siRNA), symmetric or asymmetric double stranded RNAs with around 20 base pairs, have long been used as molecular tools to regulate the expression of genes of interest in basic research [1,2]. After attachment to complementary target mRNAs, siRNAs

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* Corresponding author. Tel.: +82 2 450 0448.
E-mail addresses: hjmok@konkuk.ac.kr, hyejungmok@gmail.com (H. Mok).

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allow any target genes to be suppressed specifically. Currently, many researchers have vigorously examined the feasibility of siRNAs as biotherapeutics to silence disease-causing genes which have not been regulated with conventional therapeutics [3–6]. Because RNA therapeutics are still at an early stage, many unexpected obstacles e.g., off-target effect and immune response via activation of toll-like receptor (TLR), have hampered preclinical and clinical studies in big pharmaceutical industries [3,7,8]. To overcome these obstacles, a variety of approaches, including modification of RNAs, development and optimization of carrier systems, and proper in vivo administration, have been exploited intensively [3,9–13]. Moreover, several recent promising clinical results still boost developments of RNAi-based therapeutics in biotech industries [14–16]. To derive more successful clinical translations, faced issues including poor delivery efficiency and off-target effects should be clearly understood and addressed. In addition, currently available carrier systems under clinical trials, including cationic liposomes, anionic liposomes, polymeric carriers (cyclodextrin-based nanoparticles), and siRNA conjugates, should be comparatively examined and evaluated in terms of feasibility in clinics [5,17].

Bioconjugation techniques for a link between active molecules have been well established as delivery systems of biotherapeutics. A wide range of conjugate systems for drug delivery, such as antibody–drug conjugates and polymer–drug conjugates, have been already in clinics and lots of them are under clinical trials [18]. Since the first polyethylene glycol (PEG)–protein conjugate, Adagen® (pegademase bovine), was approved by the US Food and Drug Administration (FDA) in 1990, around ten FDA-approved PEG–protein conjugates are available currently [19,20]. More recently, antibody–small molecule drug conjugates, Adcetris® (brentuximab vedotin) and Kadcyla® (ado-trastuzumab emtansine), were approved by FDA in 2011 and in 2013, respectively, which demonstrated the improved therapeutic effects by targeted drug delivery compared to unmodified small molecule drugs [21]. In addition, one of the leading candidates in siRNA-based drugs under clinical trial is *N*-acetylgalactosamine-siRNA conjugates (GalNAc–siRNA conjugates), developed by Alnylam Pharmaceuticals [15,22]. Previously, siRNA conjugate systems were discussed regarding type of chemical modifications and detailed synthetic schemes for conjugation (e.g., solid-phase synthesis, carbodiimide-mediated coupling reaction, and Michael addition reaction) [23,24]. However, only few studies have presented comparative evaluations of siRNA conjugates to other delivery systems, considerations in terms of the preclinical and clinical studies of siRNA conjugates, and current status in their preclinical developments.

In this review, we introduce recent noticeable works regarding siRNA conjugates for clinical translations, and focus on the considerations for the rational design of siRNA conjugates to improve biological benefits in preclinical and clinical studies. First, pros and cons of siRNA conjugates as therapeutics in terms of the physicochemical properties of siRNA, targeted delivery, therapeutic efficacy, and other biological benefits are described. Second, we introduce various siRNA conjugates with aptamers, peptides, carbohydrates, lipids, polymers, and nanostructured materials in terms of in vitro and in vivo efficacy in detail. Lastly, remaining challenges and perspectives regarding siRNA conjugates for clinical application are discussed.

2. siRNA conjugates: pros and cons as therapeutics compared with other delivery strategies

Many research groups in industries and academia have paid attention to siRNA conjugates to endow favorable physicochemical properties and biological benefits for clinical translation. As shown in Fig. 1, various functional molecules could be incorporated into siRNA conjugates to enhance their delivery efficiency. To date, a wide range of molecules have been attached to the ends of siRNAs to improve biological half-life and modify pharmacokinetics, which are crucial for in vivo therapeutic efficiency [25–27]. For example, PEG conjugation to siRNAs improved their physicochemical stability against enzymatic digestion and extended their blood half-life in vivo due to increase of hydrodynamic volume [26,28]. In addition, conjugation of targeting ligands like peptides and carbohydrates have greatly improved accumulation of siRNAs in target cells and tissues, which could reduce the siRNA dose required for in vivo therapeutic effects [29,30]. These molecules could be linked to siRNA via various linkages, such as cleavable bonds, noncleavable bonds, and biological bonds, using different conjugation strategies. Cleavable bonds like reducible disulfide bonds or acid–labile hydrazine bonds can be cleaved in a reductive environment (e.g., cytosol) or in an acidic environment (e.g., endosomal lumen), respectively, and free siRNAs can be dissociated from conjugates. After bond cleavage, free siRNAs can be dissociated from conjugates without DICER processing in cells [31]. SiRNA conjugates with noncleavable

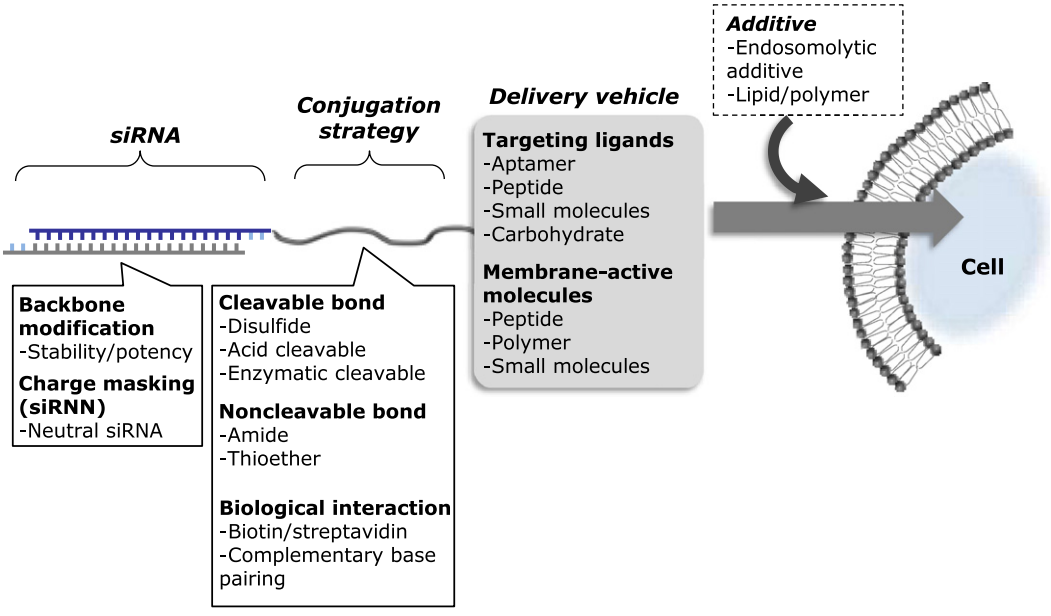

Fig. 1. Strategy of siRNA conjugates design for efficient delivery.