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Editorial

What is the Future of SiRNA Therapeutics?

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INTRODUCTION

RNA interference (RNAi) – gene silencing by double-stranded RNA, is a Nobel Prize winning discovery by Fire and Mellow [1]. Since RNAi was found to occur in mammalian cells, it has been intensively investigated as a new therapeutic strategy. RNAi can be triggered by the introduction of synthetic sequence-specific small interfering RNA (siRNA), which is able to target and cleave complementary messenger RNA (mRNA) to achieve specific gene silencing. SiRNA is short double-stranded RNA molecule (21-25 base pairs) with a characteristic 2 nucleotide 3' overhang that allows it to be recognized by the machinery of RNAi that eventually leads to degradation of target mRNA. The mechanism involving siRNA in order to achieve RNAi has been extensively reviewed [2-4]. According to the database of clinical trials (*ClinicalTrials.gov*), over 30 siRNA-based therapeutics have reached the clinical trial stage for the treatment of a wide variety of diseases including cancers, infections, cardiovascular diseases and genetic disorders [5-7]. Despite the huge therapeutic potential, siRNA-based therapeutics is yet to be approved by the Food and Drug Administration (FDA). To move siRNA therapeutics into the clinic, two major bottlenecks must be overcome: abrogation of off-target silencing effects and efficient delivery of siRNA.

Off-target silencing effect

The specificity of RNAi is not as robust as it was initially thought to be. Introduction of siRNA can result in off-target effect, i.e. the suppression of genes other than the desired gene target, leading to dangerous mutations of gene expression and unexpected consequences. The majority of the off-target gene silencing of siRNA is due to the partial sequence homology, especially within the 3'untranslated region (3'UTR), exists with mRNAs other than the intended target mRNA [8]. This mechanism is similar to the microRNA (miRNA) gene silencing effect. The off-target effect can also be a result of the immune response. RNA is recognized by immunoreceptors such as Toll-like receptors (TLRs) [9], leading to the release of cytokines and changes in gene expression. Although the sequence dependence of the immune response is not fully understood, some immunostimulatory motifs have been identified [10] and they should be avoided. Chemical modification of siRNA, such as 2'-O-methylation of the lead siRNA strand can also taper the miRNA-like off-target effects as well as the immunostimulatory activity without losing silencing effect of the target gene [9]. Overall, therapeutic siRNA must be carefully designed. A combination of computer algorithms and empirical testing is also encouraged to allow effective design of potent siRNA sequences and minimize off-target effect.

SiRNA delivery

Getting siRNA into the target cells is another big challenge of siRNA therapy. SiRNA are susceptible to nuclease degradation and cannot be systemically administered. Chemical modification of siRNA can improve its resistance against nucleases [11]. In addition, siRNA is negatively charged, hydrophilic macromolecule with poor membrane permeability, a delivery agent is therefore required to facilitate the cellular uptake of siRNA and to protect the siRNA from premature degradation. Virus-based delivery systems are notorious for immunogenic responses and other safety concerns such as insertional mutagenesis. Focus has been shifted to the use of non-viral vectors for siRNA delivery. Cationic polymers (e.g. chitosan, polyethylenimine, poly(lactic-co-glycolic acid) and lipid-based nanoparticles are the most frequently studied non-viral siRNA delivery systems, some of which have reached the clinical studies stage, especially for/in the treatment of specific cancers [12]. However, there *in vivo* transfection efficiency is generally poor, and toxicity may also be a problem when they are used at high concentrations, limiting their therapeutic potential.

The eyes and lung are two of the very few sites in the body where successful RNAi could be achieved by local administration of naked siRNA (unmodified or modified), i.e. without the need of a delivery agent. The mechanism of how naked siRNA gains entry into cells to initiate RNAi is not clear. Nevertheless, the ease of formulation of naked siRNA makes this an attractive approach for the treatment of ocular and respiratory diseases. For ocular delivery, naked siRNA is usually administered topically to the anterior segment or by intravitreal injection to posterior segment [13]. Several siRNA therapeutics for ocular disorders has already reached clinical trials with some promising results. PF-655, which is a siRNA targeting the expression of RPT801 (proprietary target of Quark Pharmaceuticals) by intravitreal injection is currently being evaluated in Phase II studies for the treatment of age-related macular degeneration (AMD) and diabetic macular edema (DME). SYL040012, which is the siRNA targeting β2 adrenergic receptor developed by Sylentis as an eye drop formulation for the treatment of glaucoma, is also

currently in the Phase II clinical trial. Both of the candidates are well tolerated in the Phase I/IIa study. However, in other clinical studies, siRNA targeting vascular endothelial growth factor (VEGF) receptor (siRNA-027 developed by Allergen) and VEGF (Bevasiranib developed by Opko Health), both for the treatment of AMD, were terminated prematurely at Phase II and III trials respectively. In both cases the decision was made due to the lack of efficacy demonstrated in order to meet the primary endpoints required. Despite these initial positive results, and that there was no overall safety concerns these trials were halted [13]. Nevertheless, the prospects of siRNA therapeutics for ocular diseases are still encouraging.

For pulmonary delivery, naked siRNA was effective in achieving RNAi following intranasal or intratracheal administration in animals. SiRNA is investigated for the treatment of a number of lung diseases including lung cancer, viral infections and asthma [14, 15]. At least two siRNA-based therapeutics targeting respiratory diseases have reached the clinical trials. ALN-RSV01 developed by Alnylam is a siRNA targeting viral nucleocapsid protein for the treatment of respiratory syncytial virus infection. Naked siRNA is administered to the lungs by nasal spray or nebulization. The Phase II trial has been completed and the results showed effective antiviral activity and good safety [16, 17]. The company is currently seeking a partner to continue to advance this program. SiRNA therapeutics, Excel lair™, which targets the spleen tyrosine kinase (STK), is developed by ZeBeCor for the treatment of asthma. Currently in the Phase II clinical trials, Excel lair™ is administered by inhalation and the results from Phase I showed good safety profile with improvement of breathing or reduced inhaler usage in patients, although it is unclear whether a delivery agent is employed [15].

FUTURE PROSPECTS AND CONCLUSION

Substantial advances have been made in the development of siRNA therapeutics in the past decades. There is no doubt that siRNA has a huge potential in the treatment of a wide range of diseases including the currently untreatable diseases. Recent progress in clinical trials of siRNA therapy is promising, with the majority of those studies to date focusing on the treatment of cancers and ocular conditions. Safety is still the primary concern for any new therapeutics. While the off-target effect of siRNA is a major issue that needs to be addressed by improving the knowledge in this area, the long-term safety of siRNA is still not clear. Delivery, especially systemically administered siRNA, is another important barrier to be overcome. Although new materials and delivery systems are being investigated to enhance the delivery efficiency, approval procedures could be hindered by the complicated formulation. On the other hand, eyes and lungs are promising tissues for local delivery of naked siRNA, especially the former, which is reflected by the high number of clinical trial studies targeting this site. It is not surprising to see the first siRNA therapeutics to be approved is for ocular therapy in the very near future.

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