REVIEW

Formulation Approaches to Short Interfering RNA and MicroRNA: Challenges and Implications

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ABSTRACT: RNA interference has emerged as a potentially powerful tool in the treatment of genetic and acquired diseases by delivering short interfering RNA (siRNA) or microRNA (miRNA) to target genes, resulting in their silencing. However, many physicochemical and biological barriers have to be overcome to obtain efficient *in vivo* delivery of siRNA and miRNA molecules to the organ/tissue of interest, thereby enabling their effective clinical therapy. This review discusses the challenges associated with the use of siRNA and miRNA and describes the nonviral delivery strategies used in overcoming these barriers. More specifically, emphasis has been placed on those technologies that have progressed to clinical trials for both local and systemic siRNA and miRNA delivery. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

The therapeutic application of short interfering RNS (siRNA) and micro RNA (miRNA) as an alternative approach in the treatment of many genetic diseases has been ongoing since Fire and Mello first discovered that the RNA interference (RNAi) could silence target gene expression in *Caenorhabditis elegans* by using long, double-stranded RNAs (dsRNAs).[1,2]

Because of the potential shown for RNAi to inhibit gene expression, the use of siRNA and miRNA has now been extensively studied in mammalian cells and is expected to be an important therapeutic drug class applicable for diseases such as cancer,[3] viral infections, and both genetic and nongenetic diseases. RNAi possess attractive characteristics such as high sequence specificity and capability to induce a robust and potent knockdown of the targeted genes.[1,4,5]

Despite the identification of promising molecular and disease targets, RNAi therapeutics have shown significant challenges including extra- and intracellular barriers that limit their knockdown efficacy. Multiple approaches have been developed to overcome all of these challenges; some of them have successfully reached their target and progressed to clinical trials. In this review, before addressing the challenges identified for the RNAi effector oligonucleotide delivery, a brief introduction on the mechanisms and target sites of the therapeutic molecules is provided.

MECHANISM OF RNAi FOR siRNA AND miRNA

The salient mechanistic features of RNAi will be briefly presented here to provide the context of how and where these molecules need to be delivered. Extensive reviews of RNAi mechanisms can be found elsewhere.

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RNA interference has been described as a conserved posttranscriptional mechanism of gene silencing present in species such as protozoa, fungi, plants, and animals, which act as innate immune defense machinery protecting them against harmful effects or invasive nucleic acids from pathogens.[6] The capacity of either siRNA or miRNA to silence gene expression is related to their similarity in terms of the physicochemical and molecular characteristics; however, they differ in the way they recognize their target genes and interact to trigger RNAi.[7] For example, both siRNA and miRNA are 21–25 bp oligonucleotides that negatively regulate gene expression; siRNA and many miRNA induce target degradation when associated with the RNA-induced silencing complex (siRISC for siRNA and miRISC for miRNA, respectively). On the contrary, although siRNA is exogenous in nature (no siRNA in mammals; only plants), miRNA represent a class of endogenous gene regulators.[2] Another difference between miRNA and siRNA is that the latter is directed to a particular messenger RNA (mRNA) target via perfect complementarity, whereas single miRNA can regulate multiple mRNA targets via binding with a number of mismatches.[7]

RNA interference can be triggered either by siRNA or miRNA when they are transcribed from various vectors, by direct introduction of synthetic (or *in vitro* transcribed) RNA into cells by injection or transfection, and "naturally"—when miRNAs are processed from stem-loops precursors encoded within the host genome.[6]

Mechanistically, when dsRNAs are introduced into the cytoplasm, they are recognized and digested by the enzyme Dicer, a member of the RNase III family of specific ribonucleases, which converts them into siRNAs of 21–25 nucleotides in length with complementary sequences to the transcript that they regulate. When short 21 bp siRNAs are introduced into cells, they can directly engage with the RNAi machinery, without any processing steps. Once in the cytoplasm, the siRNA duplex is incorporated into a siRISC, which unwinds the duplex into two strands: the passenger strand, which is cleaved and expelled by Argonaute-2 (Ago2), a protein residing within the RISC, and the guide strand, which recognizes the complementary mRNA sequences triggering the silencing of the homologous mRNA also by Ago2 protein.[8–10] After the silencing process, the cleaved fragments are degraded and the siRISC is free to bind to another mRNA target[10] (see Fig. 1).

The RNAi mechanism of miRNA initially involves the transcription of miRNAs by RNA polymerase II to produce a long primary miRNA (pri-miRNA) transcript containing the mature miRNA sequence, which is embedded in one arm of the stem-loop structure. This pri-miRNA then undergoes cleavage by the Drosha nuclease along with Pasha in the nucleus to produce the corresponding precursor of miRNA (pre-miRNA) stem loop of approximately 65 nucleotides in length.[11–14] The pre-miRNA is actively transported from the nucleus to the cytoplasm by Exportin-5. Here, the pre-miRNA is further processed by the RNase III Dicer into approximately 21 nucleotide mature duplexes, which are subsequently loaded into the miRISC, in a similar way to siRNA, that bind the complementary mRNAs and either suppress translation or induce its degradation (see Fig. 1).

The degree of complementarity between miRNA and mRNA has been shown to be a determinant in the miRISC-mediated gene inhibition. Site-specific cleavage, the mechanism described above, appears to be restricted to miRNAs with a perfect or near-perfect complementarity to the target RNA. Nevertheless, processes such as mismatched miRNA/target sequences have also been associated with mRNA degradation and translational inhibition. The combination of these processes is described as noncleavage repression and has been reported as the default mechanism by which miRNA represses gene expression.[15,16]

Abnormal expression (overexpression or underexpression) of miRNAs has been associated with a variety of diseases including cancer. This inappropriate expression of miRNA is implicated with promoting proliferation and tumor metastasis. In some cases, reduced expression of miRNA has been found. For example, the miRNA miR-145 is downregulated in colon cancer.[17] Let-7 is normally expressed in differentiated tissue but it is lost in lung cancer. miR34, which acts like a tumor suppressor, is also lost in lung cancer. On the contrary, miRNA overexpression has been found for miR-21, miR-155, and miR-206 in breast cancer,[18] and miR-132 is overexpressed in ovarian cancer.[19]

In the past few years, two therapeutic strategies related to the expression of miRNA have been developed aiming at restoring or antagonizing the miRNA function.[20]

In case of restoring the miRNA function (i.e., "miRNA replacement therapy"), miRNA mimics (synthetic duplexes) are introduced into the cell to activate pathways that interfere with the oncogenic activity. Another way to increase the levels of miRNA is by using adeno-associated viruses.[21]

After systemic and local administration of miR-145 complexed with polyethylenimine (PEI) in a mouse xenograft tumor model, reduced tumor proliferation (60%) and increased apoptosis were reported. Also, adenovirus-mediated delivery of let-7 led to tumor reduction in a nonsmall cell lung carcinoma (NSCLC) mouse model.[22] Consistent with these studies, the systemic delivery of miR-34a and let-7b mimics in mouse models of NSCLC also showed lower tumor burden, reduced proliferation, and higher levels miR-34a and let-7b.[23]