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Review

Therapeutic potentials of gene silencing by RNA interference: Principles, challenges, and new strategies

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ABSTRACT

During recent decades there have been remarkable advances in biology, in which one of the most important discoveries is RNA interference (RNAi). RNAi is a specific post-transcriptional regulatory pathway that can result in silencing gene functions. Efforts have been done to translate this new discovery into clinical applications for disease treatment. However, technical difficulties restrict the development of RNAi, including stability, off-target effects, immunostimulation and delivery problems. Researchers have attempted to surmount these barriers and improve the bioavailability and safety of RNAi-based therapeutics by optimizing the chemistry and structure of these molecules. This paper aimed to describe the principles of RNA interference, review the therapeutic potential in various diseases and discuss the new strategies for in vivo delivery of RNAi to overcome the challenges.

Contents

Abbreviations: AAV, adenovirus-associated vectors; AGO, argonaute; AGT, angiotensinogen; AMD, age-related macular degeneration; APP, amyloid precursor protein; AV, adenovirus vectors; BACE1, β-site APP cleavage enzyme 1; bFGF, basic fibroblast growth factor; CCR5RZ, T cell CCR5 cytokine receptor; CD, cyclodextrins; Clu, clusterin; FOXO1, forkhead box protein O1; GnT-V, N-acetylglucosaminyltransferase V; HAART, highly active antiretroviral therapy; Her2, human epidermal growth factor receptor 2; IFN, interferon; LV, lentivirus vectors; MID, middle; miRNA, microRNA; MMP-9, metallopeptidase 9; MRP1, multidrug resistance-associated protein-1; NOX, NADPH oxidase; PAZ, Piwi–Argonaute–Zwille; PCSK9, proprotein convertase subtilisin/kexin type 9; PDAC, pancreatic ductal adenocarcinoma; PDX-1, pancreatic duodenal homebox-1; PEG, polyethylene glycol; PEI, polyethylenimine; PIWI, P-element induced wimpy testis; PKR, protein kinase receptor; PVN, paraventricular nucleus; RISC, RNA-induced silencing complex; RNAi, RNA interference; RV, retrovirus vector; shRNA, short hairpin RNA; siRNA, small interfering RNA; TGFbetaR2, transforming growth factor-beta receptor 2; TLRs, Toll-like receptors; VEGF, vascular endothelial growth factor; XPO-5, Exportin-5.

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1. Introduction

In 1998, double stranded RNAs were firstly discovered to be the silencing effectors to regulate gene functions in *Caenorhabditis elegans* (Fire et al., 1998). Shortly thereafter, similar phenomena were found also in plants and mammals (Hamilton and Baulcombe, 1999), which uncovered the new world of RNA interference (RNAi) and heralded a revolution in RNA regulation. Since then, RNAi has rapidly become one of the most powerful and widely used tools for the study of gene function. In addition, it has been developed as a novel therapeutic tool to target disease genes for treatment. Fig. 1 summarizes the timeline of RNAi development and application.

1.1. RNAi machinery

RNAi is defined as a mechanism of specific post-transcriptional gene-silencing mediated by small RNAs, including endogenous microRNA (miRNA) and exogenous small interfering RNA (siRNA) or short hairpin RNA (shRNA) (Wang et al., 2011). Double-stranded small RNAs incorporate into the RNA-induced silencing complex (RISC), where the strands are separated, and one strand guides RISC to the complementary or near-complementary region of target mRNA, suppressing the gene expression either by degrading mRNA or blocking mRNA translation (Fig. 2) (J. Martinez et al., 2002; Zamore et al., 2000).

The heart of RISC is the Argonaute (AGO) proteins. In humans there are 8 AGO proteins, 4 from AGO clade (AGO1–4) and 4 from P-element induced wimpy testis (PIWI) clade (PIWI 1–4) (Hutvagner and Simard, 2008). Not all AGO proteins are cleavage competent. Only AGO2 is the executer that accomplishes siRNA-induced silencing. AGO2 has three functional domains, Piwi–Argonaute–Zwille (PAZ), MID (middle) and PIWI, and PIWI has an RNase H fold that harbors "slicer" activity for cleavage of target RNA substrates. Guide-strand 5′ monophosphate group tucks in between the MID and PIWI domains, binding to a magnesium ion. Meanwhile, PAZ domain specifically recognizes the guide-strand 3′ dinucleotide overhang. This positioning exposes the guide-strand 2 nt to 8 nt (counting from the 5′ end), the "seed region" to complementary target mRNA for base pairing. Next base pairing at 10 nt–11 nt correctly orients the scissile phosphate between them for cleavage by PIWI domain (Hutvagner and Simard, 2008; Liu et al., 2004; Song et al., 2004).

For siRNA, it has a well-defined synthesized structure, a short (usually 21-bp) double-stranded RNA with phosphorylated 5′ ends and hydroxylated 3′ ends with two overhanging nucleotides. This type of small RNA directly incorporates into RISC, where its guide-strand binds to and cleaves the complementary mRNA with a perfect match. When the cleaved mRNA is released, the guide-strand bound RISC binds to another mRNA and starts a new round of cleavage (Elbashir et al., 2001; Robb et al., 2005).

For shRNA, since the half-life of siRNA is short, shRNA has been developed as an alternative RNA molecule. ShRNA is transcribed in the nucleus from an external expression vector bearing a short double-stranded DNA sequence with a hairpin loop by RNA polymerase II or III. The shRNA transcript is then processed by Drosha, an RNase III endonuclease. The resulting pre-shRNA is exported to cytoplasm, where it is processed by Dicer (another RNase III enzyme) and incorporated into RISC, followed by the same cytoplasmic RNAi process as in siRNA (Yu et al., 2002). Comparing with siRNA, shRNA is constantly synthesized in host cells, leading to more durable gene silencing. Moreover, a shRNA expression vector cost less than the bulk manufacturing of siRNA (Aagaard and Rossi, 2007).

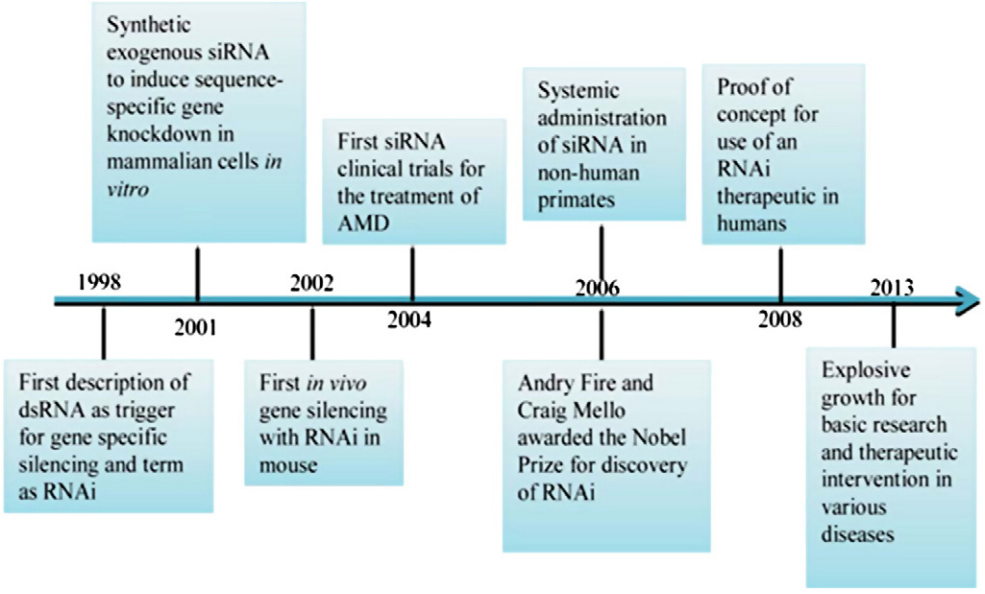

Fig. 1. Timeline of RNAi development and applications.