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BRAIN RESEARCH

Review

RNAi therapeutics for CNS disorders

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ARTICLE INFO

Article history:
Accepted 15 March 2010
Available online 20 March 2010

Keywords:
RNAi
Therapy
Neurodegenerative
Huntington's
Alzheimer's
Parkinson's
Ataxias

ABSTRACT

RNA interference (RNAi) is a process of sequence-specific gene silencing and serves as a powerful molecular tool to manipulate gene expression *in vitro* and *in vivo*. RNAi technologies have been applied to study gene function and validate drug targets. Researchers are investigating RNAi-based compounds as novel therapeutics to treat a variety of human diseases that are currently lacking sufficient treatment. To date, numerous studies support that RNAi therapeutics can improve disease phenotypes in various rodent models of human disease. Here, we focus on the development of RNAi-based therapies aimed at treating neurological disorders for which reduction of mutant or toxic gene expression may provide clinical benefit. We review RNAi-based gene-silencing strategies, proof-of-concept studies testing therapeutic RNAi for CNS disorders, and highlight the most recent research aimed at transitioning RNAi-based therapeutics toward clinical trials.

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1. Introduction

Treatment of neurological diseases affecting the central nervous system (CNS) has proven to be a major challenge for clinicians. As average life span continues to increase among the human population, so does the societal burden caused by age-related neurodegenerative conditions; the two most prominent being Alzheimer's disease (AD) and Parkinson's disease (PD). These

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doi:10.1016/j.brainres.2010.03.038

diseases, among others, may have known genetic components or may appear sporadically with unknown etiology. By contrast, some neurological disorders [e.g., Huntington's disease (HD) and several spinocerebellar ataxias (SCAs)] are solely caused by the inheritance of genetic mutations. In recent years, there has been considerable progress made in elucidating the pathogenic mechanisms underlying these various neurological diseases; however, there are currently no cures, and therapies are largely symptomatic. Thus, researchers are investigating innovative therapeutic strategies to treat these diseases. One such approach is to silence (i.e., turn off or reduce) the expression of genes that cause or contribute to disease phenotypes. For neurological conditions with more complex origins, the candidate target genes are often less clear; however, gene-silencing strategies may be employed to inhibit cellular pathways that contribute to disease manifestation. For several autosomal dominant neurodegenerative diseases, gene mapping has identified the disease-causing mutations, facilitating candidate target gene selection for therapeutic silencing. Notably, in some cases, researchers have validated therapeutic target genes using tetracycline-regulated transgenic mouse models of dominant neurodegenerative diseases. These inducible models—in which the expression of mutant genes can be turned on or off—serve as powerful tools for assessing the reversibility of neurological conditions and evaluating disease-causing genes as therapeutic targets. For example, using these models, independent groups working on HD and SCA1 demonstrated that neuropathological and abnormal behavioral features of disease developed over time when the respective mutant proteins were expressed (Yamamoto et al., 1984; Zu et al., 2004). However, when transgene expression was turned off in affected mice, disease progression halted and pathological and behavioral features improved. Together, these experiments serve as proof-of-principle studies supporting the notion that inhibiting the expression of disease-causing genes may provide therapeutic benefit in patients already exhibiting disease phenotypes. In recent years, scientists have been rigorously investigating a variety of strategies to selectively inhibit gene expression with high specificity. To date, RNA interference (RNAi), which is capable of gene-specific targeting of messenger RNAs (mRNAs), has shown beneficial effects in cell and animal models.

2. RNAi overview

RNAi is a natural cellular process that serves to regulate gene expression and provide an innate defense mechanism against viral invasion and transposable elements (McManus and Sharp, 2002). The identification of the RNAi process has been recognized among the most significant contributions to cell biology. In 2006, the Nobel Prize in Physiology or Medicine was awarded to researchers Craig Mellow and Andrew Fire for their crucial role in the discovery of RNAi (Fire et al., 1998). Having been observed first as a confusing experimental result in plant and worm studies, RNAi is now a well-characterized process of gene regulation. RNAi mediates sequence-specific gene silencing by double-stranded RNA (dsRNA), which is processed into functional small inhibitory RNAs (~21nt) (Provost et al., 2002). Small inhibitory RNAs are capable of base pairing with specific target mRNAs; this binding may induce gene silencing by causing transcript degradation or translational inhibition depending on the degree of complementarity (Lee et al., 2004b). Transcript degradation generally requires a high degree of complementarity, whereas translational repression occurs when small RNAs base pair imperfectly to target mRNAs (primarily in the 3′UTR). Indeed for the latter, stretches of homology as short as 6–7 nt may be sufficient to cause gene silencing (Lewis et al., 2005).

In recent years, numerous studies have characterized the cellular pathways controlling endogenous small RNA biogenesis and RNAi-mediated gene silencing. In nature, RNAi for regulation of gene expression occurs primarily via small RNAs known as microRNAs (miRNAs). miRNAs are first transcribed from the genome as larger primary miRNA transcripts (pri-miRNAs), which form intramolecular stem-loop (i.e. hairpin) structures (Fig. 1) (Cai et al., 2004; Lee et al., 2004a). Subsequently, pri-miRNAs undergo a series of processing events, catalyzed by several proteins, to generate the mature miRNA (~19–25 nucleotides in length). Most pri-miRNAs are first processed in the nucleus by Drosha-DGCR8, the microprocessor complex, to an approximately 60–70 nucleotide pre-miRNA stem-loop (Gregory et al., 2004; Lee et al., 2003; Zeng et al., 2005). Pre-miRNAs are then transported by Exportin-5 to the cytoplasm (Lund et al., 2004; Yi et al., 2003), where the mature miRNA duplex is generated via Dicer cleavage, which removes the loop region (Forstemann et al., 2005; Provost et al., 2002; Saito et al., 2005). A single strand (the antisense "guide" strand) of the resulting duplex is then incorporated into the RNA-induced silencing complex (RISC), thus producing a functional complex that is capable of seeking out and silencing target transcripts (Khvorova et al., 2003; Schwarz et al., 2003).

3. Tools for RNAi

With a better understanding of endogenous miRNA biogenesis and gene silencing processes, scientists have devised strategies to co-opt the RNAi machinery to specifically silence various genes of interest. In this way, RNAi serves as a powerful molecular tool used to study gene function in biological processes and provides a novel strategy to treat a variety of diseases (e.g., dominant genetic disorders, cancer, and viral invasion, among others). The application of RNAi as a biological or therapeutic tool is primarily limited by our ability to introduce inhibitory RNAs into target cells or tissues. Inhibitory RNAs can be designed to mimic primary miRNA stem-loops (artificial miRNAs), processed pre-miRNAs (short-hairpin RNAs or shRNAs), or mature miRNAs with perfect complementarity to their targets (small interfering RNAs or siRNAs) (Fig. 1). SiRNAs and shRNAs were among the first to be utilized in cell-free, cell culture, and animal systems. SiRNAs are double-stranded effector molecules that are typically produced by chemical synthesis *in vitro*. Following delivery into cells, siRNAs may be processed by Dicer or loaded directly into the RISC (Elbashir et al., 2001; Kim et al., 2005). ShRNAs are generally expressed from vector systems, most often with strong, constitutive Pol-III promoters (Paul et al., 2002; Sui et al., 2002). To mimic pre-miRNAs, shRNAs are transcribed as sense and antisense sequences connected by a loop of unpaired nucleotides. Following transcription, shRNAs are