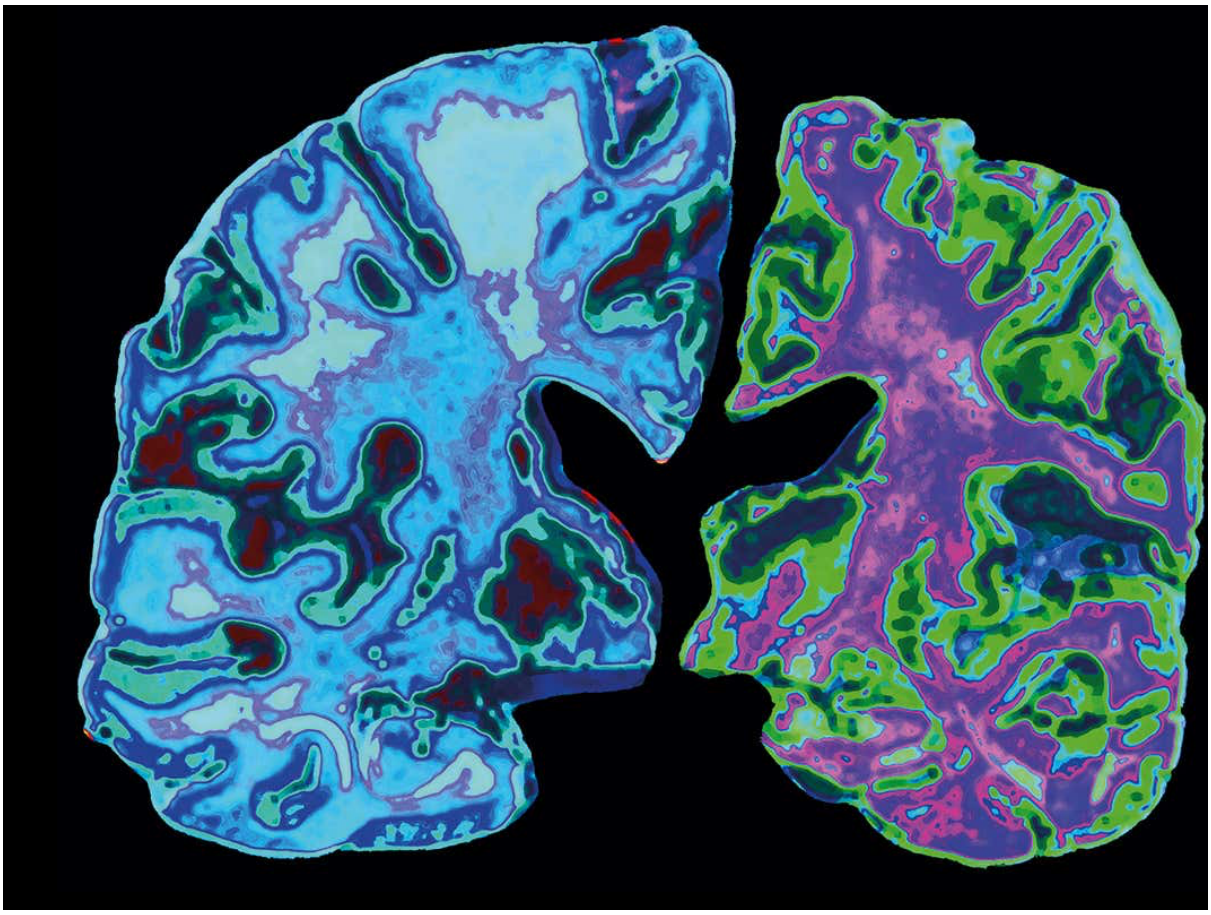
RNAi as a tool of silencing Alzheimer's disease genes



A Literature Review

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Abstract

Alzheimer's disease (AD) is the common neurodegenerative disorder and the most frequent cause of dementia. The disease accounts around 80% of all dementia diagnoses. The main reason of developing AD is amyloid cascade which starts from missense mutations in APP (amyloid precursor protein), Rho-associated coiled-coil kinase isoforms 2 (*ROCK-II*) genes and the presence of senile plaques and neurofibrillary tangles (NFTs) composed of Tau microtubule protein in the hippocampus leading to widespread neuronal disfunctions and cell death resulting in dementia. To control AD, the RNA interference (RNAi) may potentially be a mechanism that can inhibit the protein gene expression of a specific genes by directly blocking gene expression with high specificity. RNAi has major implications for basic and biomedical research that may lead to a number of clinical applications. Therefore, the selective silencing of mutant alleles have a potential to treat the inherited dementia. In this review, the role of RNAi in silencing of the mutant genes that cause AD will be defined and potential treatment of gene silencing technique will be determined.

Keywords: RNAi, tau, amyloid prosecutor protein, ROCK-II, siRNA, Alzheimer's disease, RNA-based therapeutics

Introduction

Alzheimer's disease (AD) is the common neurodegenerative disorder and the most frequent cause of dementia characterized by memory, cognitive dysfunction, changes in behavior and personality. AD is also associated with inflammatory responses, synaptic damage, changes in hormonal levels, mitochondrial structural and functional abnormalities. Nevertheless, the molecular events leading to synaptic loss in AD are unknown [1]. In general, disease accounts for up to 80% of all dementia diagnoses [1,2]. The strongest risk factors for Alzheimer's disease are people in their mid 60s or later. Nevertheless, the disease can also affect people in their 30s or 40s, however a very small amount of people has a disease in early-onset form. It is still unexplored what triggers the start of AD [3]. It is suspected that the main reason of developing AD is amyloid cascade which starts from missense mutations in amyloid precursor protein (APP), presenilin 1 protein (PS1) or presenilin 2 protein (PS2), Rho-associated coiled-coil kinase isoforms 2 (*ROCK-II*) genes and the presence of senile plaques and neurofibrillary tangles (NFTs) composed of Tau microtubule protein in the hippocampus leading to widespread neuronal disfunctions and cell death resulting in dementia. However, the detail mechanism of the disease is still unexplored due to the variety of factors that might affect. Nowadays, some of the therapeutics for AD provide treatment only for symptoms. Therefore, there is no effective treatment do delay the progression of the disease [4-6]. To determine the functions of the factor and how they work together, RNA interference might be an excellent tool to investigate the disease and potential treat. RNAi is extremely important in this case because it regulates the expression of genes by controlling the synthesis of protein with a posttranscriptional gene-silencing mechanism. RNAi is triggered by the presence of long pieces of double-strand RNA, which cleave into the fragment known as small interfering RNA (siRNA) [4]. RNAi is well suited to probe the biological function of individual genes, genes in pathways, genes known to be associated with diseases (including inherited genetic diseases) and viral pathogens. In addition, RNAi may be used to discover new genes or proteins that are essential in pathogenic processes, also RNAi has major implications for basic and biomedical research that may lead to a number of clinical applications. Compared with other therapeutics, the main advantage of RNAi is that all targets – including those that are usually unable to be targeted with drugs – are able to be drugged using RNAi. This is because, any transcript that encodes a protein that

causes or contributes to a disease can be targeted by RNAi. Furthermore, RNA- based therapeutics have the potential to control the disease pathways by reducing brain accumulation of β-amyloid or phosphorylated tau by siRNA that selectively silences mutant alleles and maintaining expression of normal alleles [4,5]. Currently RNAi has been widely used in basic bioscience – including in the study of AD, which may be caused by different genes and proteins – and such usage may lead to novel therapies in the future. The purpose of the review is to investigate the effect of different RNAi nanocomplexes on the mutant genes of Alzheimer's disease.

siRNA nanocomplexes in gene silencing

The Alzheimer's disease neuropathology is described by the presence of extracellular Aβ deposits and intracellular NFTs (neurofibrillary tangles) comprised of misfolded, hyperphosphorylated tau. Thus, the spread of tau combines and correlates with cognitive decline in AD, meaning that spread of NFTs contributes to synapse loss. The tau is meant to interact with native tau in the cytosol, drive misfolding of tau, and ultimately generate neurofibrillary pathology [2,8]. RNA interference suggests a potential therapy for human disease, it allows the direct investigation of the role of these pathways in AD models. However, it is difficult to predict the siRNA concentration required for therapeutics effect in vivo due to the fact that siRNA therapy has been limited by its low stability and rapid degradation in presence of nucleases. By using different nanocomplexes of siRNA, the disease protein is expressed at much lower levels and knockdown of mutant protein levels is highly achievable with modest intracellular concentrations of siRNA. Furthermore, several target sites and designs must be created and tested before optimal silencing can be achieved.

Tau protein, APP and *ROCK-II* – target genes and proteins

One of the Tau main functions is to modulate the stability of axonal microtubules whereas microtubules are naturally unstable and require interaction with Tau protein to maintain their structure. Tau protein hyperphosphorylation results in disruption of microtubule organization [1,9,10]. In a normal brain, there is a balance between phosphorylation and dephosphorylation of the Tau protein (fig. 1). However, accumulation of hyperphosphorylated Tau in neurons leads to protein misfold and aggregation in NFTs, reducing their affinity for microtubules. Recent studies revealed that efficient way to create siRNA against gene of interest is to produce short RNA duplexes complementary to the target gene in in vitro transcription reactions employing T7 RNA polymerase. By the immunological staining amyloid

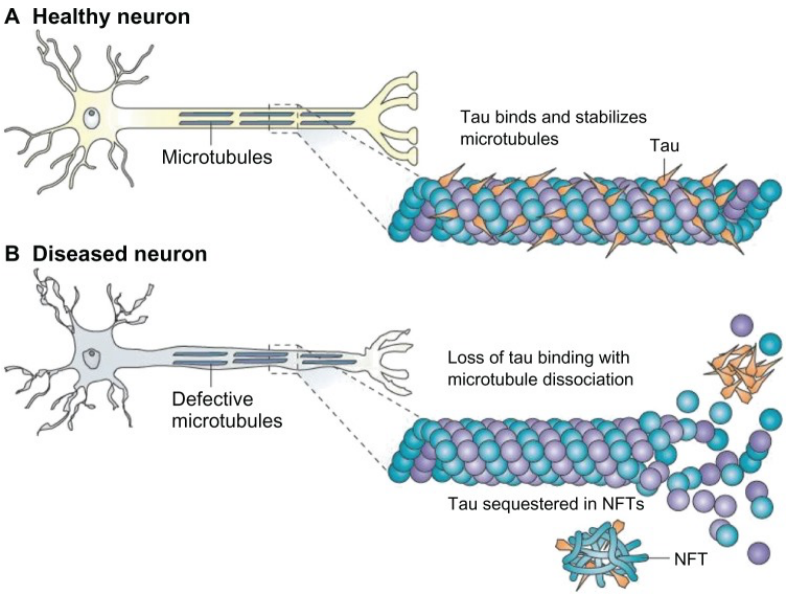

FIGURE 1. (A) TAU FACILITATES MICROTUBULE STABILIZATION WITHIN CELLS AND IS PARTICULARLY ABUNDANT IN NEURONS. (B) TAU FUNCTION IS COMPROMISED IN ALZHEIMER'S DISEASE AND OTHER TAUOPATHIES [4].

plaques can be found. In the brain of AD patients there are intracellular neurofibrillary tangles, which are composed of hyperphosphorylation of tau (p-tau). In order to silence hyperphosphorylation of tau, different siRNA nanocomplexes can be used to determine the effect of knocking down the gene. *SUT-2* is a gene required for tau neurotoxicity, the RNAi knockdown of *MSUT2* (mammalian *SUT-2*) overexpressing tau causes a marked decrease in tau protein aggregation [4]. Researchers found that knocking down endogenous tau fully prevented the neurotic changes, while overexpressing human tau accelerated them [10].

The APP gene is located on chromosome 21 in humans with three major isoforms arising from alternative splicing [13,14]. The metabolism of APP mediated by a series of enzymes termed "secretases" (á, ß, and ã). Cleavage of APP by á-secretase occurs in the middle of the peptide, generating non-amyloidogenic APP fragments (fig. 2). The common isoforms are fragments of 40 or 42 amino acids called Aß40 and Aß42. Hence, the Aß40 form is the more common of the two, however Aß42 is primarily responsible for neuronal damage. Aâ forms insoluble filaments which constitute one of the main components of senile plaque, the primary hallmark of AD. Using an RNAi approach, the APP results in a great reduction and increasing the APP á-secretase, the cleavage is considered a therapeutic approach for AD [14].

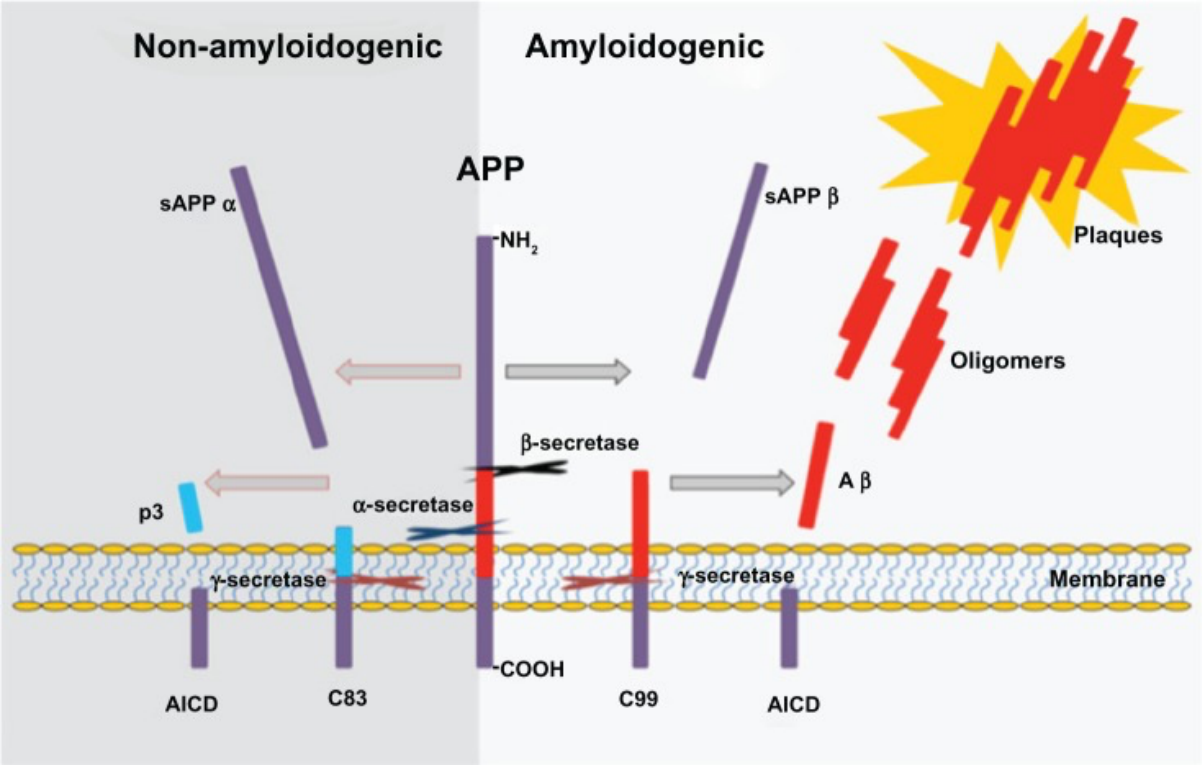

FIGURE 2. PROTEOLYTIC PROCESSING OF APP BY Á-, ß-, AND Ã-SECRETASE. SEQUENTIAL APP CLEAVAGE BY ß- AND Ã-SECRETASE IS REFERRED TO AS THE "AMYLOIDOGENIC PATHWAY" AND GENERATES Aß. BETA-SECRETASE CLEAVAGE OCCURS WITHIN THE ECTODOMAIN OF APP CLOSE TO THE TRANSMEMBRANE DOMAIN, RESULTING IN THE SHEDDING OF THE MEMBRANE-BOUND.

There are many nanocomplexes that can silence target genes, where target genes vary. For instance, *PEG–PEI/ROCK-II*-siRNA complexes are also highly efficient to knockdown Rho-associated coiled-coil kinase isoforms (*ROCK-II*), where the gene that promotes the occurrence of Alzheimer's disease [8]. *ROCK-II* phosphorylates the cytosolic domain of APP and that this action influences Aβ generation. By

knocking down *ROCK-II*, the Aβ levels are decreased, meaning that β-site APP cleaving enzyme is suppressed, and process of phosphorylation will not happen [11,12].

The therapeutic potential of RNAi in AD has been demonstrated through allele-specific gene silencing by short-hairpin RNA (shRNA) to selectively suppress mutant forms of the mutant genes [4,14].

Conclusion

RNAi holds promise as a potential therapy for human diseases, however there is no effective therapy that can stop the progression of AD. There are genes such as APP, *ROCK-II* gene and tau that are the best candidates for RNAi for targeting because of their central role in inherited and acquired forms of age-related dementia [3-5]. Alzheimer's disease is characterized by two major pathological hallmarks: senile plaques, which contain beta-amyloid (Aβ) derived from cleavage of APP; and neurofibrillary tangles, which contain filamentous tau protein. Although abnormal deposition of tau and the APP cleavage product Aβ are central to AD pathogenesis, the precise roles of these proteins in the brain remain to be unexplored. By applying siRNA therapy to the nervous system, effective delivery of siRNA nanocomplexes to the correct target cells in the brain the silencing of the mutant genes can be achieved. Nevertheless, functions of many proteins that are involved in metabolic pathway is still unknown and it is impossible to predict how effective the gene knockdown will be. Specific silencing of mutant alleles extends the potential utility of the approach to genes with important or essential functions [1,15]. RNAi has become a valuable research tool to help to understand disease and to provide an efficient method for clinical applications. It is believed that RNAi technology to knockdown genes will be highly helpful in addressing unresolved questions concerning AD via in vitro and in vivo approaches, but there is no guarantee that AD will be cured.

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