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siRNA Therapeutics: Future Promise for Neurodegenerative Diseases

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Running head: siRNA therapeutics for neurodegeneration

Abstract

Neurodegenerative diseases (ND), as a group of central nervous system (CNS) and one of the biggest medical problems in the 21st century, are often associated with considerable disability, motor dysfunction and dementia and are more common in the aged population. ND imposes a psychologic, economic and social burden on the patients and their families. Currently, there is no efficient treatment for ND. Since many of ND result from the gain of function of a mutant allele, small interference RNA (siRNA) can be a potential therapeutic agent for the management of ND. siRNA is a powerful tool, based on the RNA interference (RNAi) approach, for modulating the gene expression through gene silencing. However, there are some obstacles in the clinical application of siRNA including unfavorable immune response, off-target effects, instability of naked siRNA, nuclease susceptibility and a need to develop a suitable delivery system. Since there are some issues related to siRNA delivery routes, in this review we focus on the application of siRNA in the management of ND treatment from 2000 to 2020.

Keywords: Central Nervous System; neurodegenerative disorders; siRNA; RNAi; delivery system; antisense technology.

1. Introduction

According to the World Health Organization (WHO) reports, the central nervous system (CNS) related diseases are the main medical problem in the 21st Century. These group of diseases is serious, divergent, numerous and prevalent worldwide. Neurodegenerative diseases (ND) are a large group of CNS disorders. They are often associated with disability, motor dysfunction and dementia (the weakness of mental functions that could affect different intellectual process including language, learning, thinking, calculation, behavior, and memory) due to the progressive deterioration and death of the neurons [1-3]. ND consist of various disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, Multiple Sclerosis and spinal cord injury [4].

Currently, the treatment of these diseases is a big challenge for clinicians and researchers. The currently available medications can only relieve some of the symptoms of these diseases, but they are not capable to stop the progression of these diseases [5]. However, the characterization of the genes and the molecular pathway involved in the pathogenesis of ND as well as the advancement in the gene therapy methods have made some advances towards finding an effective and satisfactory treatment approach for the management of these disorders [6]. One of the most promising approaches to fight ND is the antisense technology due to its high ability to target mutant genes. This technique includes a variety of methods, such as antisense oligonucleotides (ASO), RNAi technology (siRNA, miRNA, shRNA), ribozyme, DNAzyme and aptamer. Many studies based on the antisense technology in pre-clinical and clinical phases are currently underway to find a suitable solution for the challenge in managing ND. For example, RO7234292 or Tominersen, an investigational drug from ASO class, is undergoing clinical trials at phase 3 (NCT03761849) to treat patients with Huntington's disease. WVE-120102 is another ASO which is currently under investigation at Phase 1b/2a clinical study (NCT03225846) for the same disease [7-8]. Some of them have even received FDA approval. Spinraza™ (Nusinersen) is the first FDA-approved antisense drug for the management of a CNS disease, spinal muscular atrophy (SMA), that recovers the expression of survival motor neuron protein through splicing correction [9-10].

One of the gene targeting procedures is RNAi technology that also has been used for the treatment of ND in recent years. siRNA, a promising class of RNAi, has been a significant achievement in the world of biology in the last two decades.[11]. Theoretically, this can focus on any mRNA target that is translated into protein [12]. Hence, siRNA is a powerful means for drug discovery in medical research [13]. siRNA has some advantages over other common

therapeutic approaches such as antibodies, small molecules, and proteins. siRNA does not require a particular target on the cell membrane surface or a druggable target [14]. Compared to other typical drugs, siRNA can be designed easily since it has only a fewer number of nucleotides (21-23) and follows the Watson–Crick base pairing rules [15]. The siRNA can work in lower concentrations suggesting siRNA has high fidelity and efficacy [12]. siRNA also has some advantages over ASO. For *in vitro* experiments, siRNAs are preferred. Unmodified RNAs have a great potency, so finding a potent siRNA is comparably easier than ASO since ASOs must have chemical modifications to function appropriately [16].

Considering the highlighted benefits of siRNA technology, it is not surprising many investigators used this method to find a solution for the treatment of ND. This review focuses on the application of the therapeutic potential of siRNA in the treatment of ND based on the existing evidence.

2. siRNA-mediated RNAi pathway

The RNAi process is started when a double-strand (ds) RNA is introduced into the cell [17]. It comprises of an initiation stage followed by and effector stage. In the initiation stage, an endoribonuclease enzyme, Dicer, cleaves the dsRNA and produces a shorter fragment (21-23 base pair), called siRNA. Dicer belongs to the RNase III family and is described as the "molecular ruler" (**Figure 1**). The 3' end of new siRNA has two nucleotides overhangs, which are necessary for its specific function, whereas the 5' end consists of a monophosphate group [18-22]. In the second (effector) stage, the siRNA molecule is loaded into a multiprotein complex called the RISC (RNA induced silencing complex). The rest of the steps such as completion of the siRNA processing, target recognition and the digestion are facilitated with the help of this complex [23]. After the siRNA-RISC formation, one of two-strand with the more stable 5' end, namely the guide or antisense strand, remains in connection with the RISC. While the other strand, the so-called passenger or sense strand, is digested and is discharged from the complex by the argonaute protein 2 (AGO2), which is an important part of the RISC [24-28]. AGO is the major player and the critical effector molecule in the RNAi associated silencing. It is a family with 4 members (AGO 1-4) in which only the AGO2 has the catalytic function in the mammalian cells [26, 29] It is thought that following the release of the passenger strand, the RISC is activated and then the guide strand can bind to the target mRNA. An impressive gene silencing will be accessible only if the guide strand of siRNA and mRNA transcript are paired completely (unlike miRNA) which leads to the cleavage of

the target mRNA by AGO2 part of RISC [26, 30]. Subsequently, the cleaved mRNA is degraded by the cellular nuclease [31].

3. siRNA delivery systems

The development of an effective and safe approach for the siRNA delivery to the target cells is the major impediment for the clinical use of siRNA [32-33]. There are various reasons for these challenges with siRNA including the large size of siRNA (13 kD), its polyanionic nature and its inability to pass from the cell membrane because of the negative charge [13, 34]. A suitable delivery approach should have some features such as no or low toxicity, improve the cellular uptake of the siRNA, siRNA protection from the serum nuclease attack, lowering the rate of siRNA renal filtration, ability to extravasate from the blood to the target site (after intravenous injection administration) and preservation of siRNA from the phagocytosis [33, 35-38].

There are two major types of delivery systems for the siRNA transfer which are viral vectors and non-viral vectors. The hallmark of viral vectors is their high efficiency but some safety issues limit their clinical application. The most common viral vectors consist of adenoviruses (AVs), adeno-associated viruses (AAVs) and lentiviruses (LVs) [39]. The non-viral vectors are more preferred rather than the viral vectors due to their safety profile, although their efficiency is not very high. They can be divided into different types [40] including lipid base (e.g. liposome)[38, 41], non-lipid inorganic-based (e.g. golden nanoparticles [42] and superparamagnetic iron oxide nanoparticles (SPIONs)) [43] and non-lipid organic-based (e.g. chitosan, PEI, polyplexes) [38, 41]. Moreover, siRNA can be modified to increase its stability [44-46].

4. siRNA and Neurodegenerative disorders

4.1. Alzheimer's disease

Alzheimer's disease (AD) is a progressive, devastating and the most prevalent neurodegenerative disorder [47]. The clinical symptoms of the disease include ongoing deterioration of memory, learning, cognition and consequently personality and behavioral changes [47-48]. AD is an age-dependent disease and the most common reason for dementia (>80%) in the aging population. It is predicted that by 2060, the number of peoples who lives with AD in the U.S. will increase to 9.3 million [49].

AD is highlighted by two major forms of pathological protein aggregates namely extracellular amyloid plaques which are an accumulation of β-amyloid (Aβ) and intracellular neurofibrillary tangles (NFTs) which are an aggregation of abnormally phosphorylated tau (**Figure 2**) [50]. The precise process of AD is not elucidated yet. Many factors could promote the development of AD but it is not easy to ascertain the exact role of each in the development of AD [51].

There is no effective treatment for AD yet [52], however, a few drugs are prescribed to alleviate some of the symptoms of patients suffering from AD. As mentioned above, the siRNA is a powerful technique to suppress the expression of specific genes [53]. Inhibition of AD-related genes by the siRNA approach could be a good therapeutic option for AD's treatment (Table 1).

One of the most recognized hypotheses regarding the development of AD is the amyloid cascade theory. This hypothesis suggests that the aggregations of Aβ activate a harmful cascade in the brain, which leads to the degeneration of the neurons, progressive deterioration of cognition and development of dementia [54-56]. Aβ is a peptide that is normally produced from the amyloid precursor protein (APP) cleavage. The APP is a membrane protein that takes part in cell signaling. Alternative splicing can produce different isoforms of the APP [50, 58]. In normal cell processing, the APP first is cut by the α-secretase(s) and then is cleaved by the γ-secretase. The result of this enzymatic process is a very soluble and non-pathological product, a p3/p3-like portion [54, 59-60]. In opposition to this, the APP could be cut first by the β-secretase, then the different left-over membrane linked fragments are cleaved by the γ-secretase [61-62]. The resultant fragment consists of 99 residues from the C-terminal of APP. Hereafter a distinctive γ-secretase cleaves this fragment at position 40 (Aβ 1-40) or 42 of the Aβ region (Aβ 1-42). Notably, these forms of Aβ could pass from presynaptic end to the ECM (extracellular matrix) and consequently, the insoluble fibrillary Aβ plagues are formed in the outer space of the neurons [63-66].

Besides Aβ, tau is another major player in the AD pathology. In AD, hyperphosphorylated tau protein can aggregate and form intracellular bodies known as NFT. Tau is a microtubule-related protein with an important role in both axonal and dendritic function. It has been revealed that tau protein could mediate Aβ toxicity through the regulation of dendritic function [67].

APP and tau could be a favorable target for RNAi therapy, because of their critical role in both familial and sporadic forms of AD [54, 68]. Accordingly, in an *in vitro* study using SHSY5Y cells (human neuroblastoma cell line) the expression of three AD-related genes, APP, tau and VDAC1, were silenced by a specific siRNA. Consequently, the level of their mRNA and protein is reduced in the cells. The results of this study demonstrated that the transfected SHSY5Y cells by specific siRNA against APP, tau and VDAC1 showed an improved synaptic activity and also a better mitochondrial function. Based on these findings, the reduction of expression of these three genes could have a protective role in AD [69].

Not only the APP gene but also the APP-related pathways could be targeted by the siRNA to decrease the Aβ plaque formation. For example, BACE1 is a β-secretase that is involved in the cleavage processing of APP. This step is the limiting rate of the Aβ formation [70-71]. Hence, it is not surprising that this gene quickly became an attractive therapeutic target for the researchers. For instance, in 2005, a group of investigators used a transgenic mouse model of AD to assess the effect of reducing the BACE1 level on the improvement of Alzheimer-like symptoms in AD's models. They utilized a lentiviral vector which expressed siRNA against the BACE1. Their experiment showed that the BACE1 suppression specifically diminished amyloid plaque rate *in vivo* and the neuropathological, as well as behavioral signs of mouse models, got better [72].

Interestingly, BACE1 has a positive regulator known as BACE1-antisense transcript (BACE1-AS). It is a long noncoding RNA (lncRNA) which is transcribed from the reverse strand of the BACE1 gene. The BACE1-AS enhances the stability of BACE1 through forming the RNA duplex. It has been demonstrated that the concentrations of BACE1-AS are increased in patients with AD and also in the transgenic model mouse of AD. Also, changes in the BACE1-AS level could alter the amount of Aβ1–40 and Aβ 1–42 product [73]. Consistently, in a recent study, BACE1-AS expression was inhibited through the administration of siRNA lentivirus to bilateral hippocampi of SAMP8 mice (an AD mouse model). The main result of BACE1-AS knockdown was the amelioration of learning problem and memory loss in mice models, probably because of the improvement in neuronal growth in the hippocampus, BACE1 suppression, blocking of Aβ accumulation and decreasing of the phosphorylated tau protein [52].

As mentioned earlier, γ-secretase has an indispensable function in the cleavage of APP and producing the Aβ peptide. Additionally, it has been proven that presenilins (PS1 and PS2) are a critical unite of the γ-secretase complex and are

needed for the γ-secretase cleavage action. On the other hand, some mutations in the PS1 gene is seen in a large group of inherited AD [75-76]. Hence, some researchers studied the siRNA technology in the IMR-32 neuronal cell line to find out the role of the PS1 in Aβ42 formation. Their results showed that the transfected IMR-32 cells with anti-PS1 siRNA reduced the level of Aβ42. Therefore, PS1 also could be a potential therapeutic target for gene therapy of AD [78].

4.2. *Parkinson's disease*

Parkinson's disease (PD) is the most common movement-related disorder and also the second most prevalent neurodegenerative disease following AD [79]. PD is an extremely disabling, finally fatal, and until now an incurable disease [80]. The frequency of PD has grown up during the past two decades [79]. PD has some common symptoms including rigidity, resting tremor, bradykinesia and posture instability [81]. The psychological problems may also appear in later stages. Two main processes lead to the progression of PD including the formation of intracellular bodies, Lewy bodies (LB), which consist of filamentous α-synuclein aggregations in the brain of patients and the destruction of the dopaminergic neurons [82].

The current accessible therapeutic approach for PD is limited to some medications, none of them can cure the symptoms of disease entirely. They only can decelerate the progression of the disease and also have unfavorable side effects [83]. The PD is a multifactorial disorder with a combination of both genetics and environmental factors [84]. Based on this rationale, the siRNA dependent approach suggests a novel treatment strategy for the management of PD.

A large number of the studies which used the siRNA technology for the treatment of PD focused on the α-Synuclein (α-syn) gene, because of its critical role in the pathology of PD. α-syn is a small peripheral membrane protein that is expressed in the axonal end of the neurons [85]. The main role of this protein is to process the neurotransmitters in the presynaptic region. In this region, the α-syn interacts with the pre-synaptic membrane proteins and synapsis derived vesicles. The other functions of α-syn include proteasome processing and mitochondrial function [87-91]. The first evidence demonstrating the important role of α-syn in the PD pathology was obtained from the identification of a missense mutation (A53T) in the α-syn in four unrelated families with inherited PD. The high susceptibility of people with duplicated α-syn to the PD is another confirmation for the critical role of α-syn [85, 93-94].

Several lines of experiments using different methods to target the α-syn by siRNA were used to assess the potential of this approach in the treatment of the PD (Table 2). For instance, the effect of the naked siRNA against the *SNCA* (the gene of α-syn) was evaluated, both *in vivo* and *in vitro*, and the ability of this siRNA to decrease the expression of *SNCA* was demonstrated [95]. In another study for the first time, the anti-*SNCA* siRNA was administrated to the brain (substantia nigra) of a monkey model. There was a reduction in the level of α-syn mRNA and protein. Also, no tissue-specific or systematic toxicity was reported in these monkeys. These results showed the feasibility and safety of using siRNA in the primates [96]. The efficacy of naked siRNA is very low, for the reasons mentioned before. Hence, a research group used a viral vector (AAV vector) containing α-syn siRNA in a mice model. This vector was tolerated well in the mouse models of PD and successfully reduced the amount of α-syn mRNA and protein [97]. In another study, an anomalous RNAi by siRNA, namely "expression-control RNAi" (ExCont-RNAi) was developed. This method was designed to regulate the level of overexpressed *SNCA.* In this study, the PD model flies were exposed to the ExCont-RNAi. They showed motor function recovery following the reduced level of the *SNCA*. There was a positive association between the grade of motor dysfunction and the level of SNCA in the PD flies [98].

4.3. Huntington's disease

Huntington's disease (HD) is an inherited disorder with an autosomal dominant pattern. The genetic cause of HD is trinucleotide expansion (CAG: glutamine codon) in the exon 1 of the *Htt* gene [99-100]. The product of this gene is the huntingtin protein, a 348-kDa protein which is present in various cells especially in the neurons of the brain [102]. This protein plays a crucial role in a wide range of functions including endocytosis, regulation of transcription, transport in synapsis and axonal transport [104]. The normal alleles of the Htt gene have <36 repeats of the CAG. But if these repeats increase to 36 and more, the mutant alleles are formed at the HD locus [105]. Cognitive impairment, motor dysfunction, dementia and neuronal death are the results of this gain of function mutation in patients with HD [107].

Among all the neurodegenerative diseases, HD is one of the best one to be targeted by siRNA since this treatment is a suitable therapy for the autosomal dominant disorders [108-112]. The effect of the *Htt* gene silencing by siRNA method was assessed through different *in vitro* and *in vivo* experiments (Table 3). As a first step towards developing

an effective siRNAs as a therapeutic tool for HD, three different siRNA against Htt was tested in the cell culture. The results showed that one of them, which was specific for an upstream region of CAG repeated, successfully suppressed the expression of the *Htt* [113].

In a study of the anti-Htt siRNA in HD, R6/2, a transgenic mouse model of HD was used. These animals expressed the mutant alleles of Huntingtin and have unusual behavior. They also formed the aggregations of polyglutamine in their neurons, namely neuronal intranuclear inclusions (NIIs). Intraventricular injection of anti-Htt siRNA showed promising results including inhibition of the Htt in transgenic mouse and reduction in the size and number of NIIs [109]. In a modified study, a "cholesterol-conjugated (cc) siRNA" was used to target the Htt gene. This was used since it has been demonstrated that *in vitro* conjugation of cholesterol and bioactive molecules could improve the uptake process [114]. This conjugation could also increase siRNA uptake [115]. In addition, the LDL receptors are present in the brain cells [116]. Their results also showed the knockdown of the Htt gene, extended survival of neurons, diminished NIIs and improvement of movement with the cholesterol-conjugated (cc) siRNA [112].

4.4. Spinal cord injury

Spinal cord injury (SCI) is a serious clinical issue all over the world, because of the irreversible impairment of the neurons and the secondary problems [117]. SCI has a heavy economic and social burden on the affected people, their family and the health services [118]. SCI results in transitional or constant damage in the sensory, motor and autonomous function of the spinal cord [119]. It is regarded as a permanent disability since the CNS is not able to regenerate its neuronal axons [120]. So far, significant progress has been made in the diagnosis, recovery and has increased the survival rate of SCI, although there is a long way to develop an effective treatment.

Since some genetic aspect of SCI was established in the last years, using the siRNA technology to silence the involving genes have been considered as an alternative therapeutic approach (Table 4). For example, ephrinB3 (ephB3) is a useful target since it has been proven that this gene is involved in the inhibition of axonal growth and also decreasing the rate of recovery after the CNS injury [121]. Accordingly, the effects of a lentiviral vector expressing the anti-ephB3 siRNA were tested in a rat model. The results of this experiment revealed that the spinal cord administration of anti-ephB3 siRNA and consequently reducing the expression of ephB3 gene lead to the recovery of the axonal regeneration and the motor function after SCI. It could also enhance the Basso-Beattie-Bresnahan (BBB) score [122].

One of the pathological features of SCI is the accumulation of reactive astrocytes in the damaged region. The regeneration process of the neurons is disrupted and the permanent disability is the inescapable result of such events. Reactive astrocytes are characterized by up-regulation of the intermediate filament (IF) proteins such as glial fibrillary acidic protein (GFAP) and vimentin [123]. In a study using siRNA technology, the expression of GFAP and vimentin were down-regulated in a rat model. For the assessment of its efficacy, the improvement of the bladder function was tested. There was an improvement of bladder function demonstrating the efficacy of siRNA [120].

Another pathological condition in the SCI is neuroinflammation where M1 macrophages have a critical role [124-126]. M1 macrophages produce a large number of inducible nitric oxide synthase (iNOS) and its product, nitric oxide (NO) which following SCI can lead to axon degeneration and demyelination [127-128]. Hence, in the acute stage of the SCI, iNOS can be a suitable target. Recently a siRNA-chitosan-antibody nanoparticle complex was used to suppress the iNOS expression *in vitro* and *in vivo*. This antibody complex helped the M1 macrophages to phagocytosis

the nanoparticle by the Fc-receptor. There was a successful reduction of the iNOS expression by this complex. The results demonstrate promising evidence for improving the secondary damage following the SCI [129].

Recently a newly discovered protein with specific expression in neurons, Nischarin (Nis), was used as a target for siRNA therapy in the SCI. Nischarin can suppress neurite outgrowth as well as neurons regeneration [130-131]. For silencing of the Nis a nano complex consisting of Nis-siRNA and PEI-ALG was developed and then administrated to a rat model with SCI. The improvement of motor function in the rat models confirmed the therapeutic potency of this method [132].

4.5. Multiple sclerosis

Multiple sclerosis (MS) is the most common non-traumatic debilitating disorder that affects a young person [133]. It is a chronic, demyelinating, neurodegenerative and inflammatory disorder of the CNS [134]. Although the precise etiology of this disease is not clear, it is obvious that MS is a heterogeneous, multifactorial complex disease that is developed by both the genetic susceptibility and the environmental factors [134-135].

The focal plaques made of demyelinating lesions are the generic hallmark of all MS subtypes. They appear over the post-capillary venules in the grey and white matter of the spinal cord as well as the brain of the patients [134, 136-137]. MS is also defined as an autoimmune disorder in which both autoantibody and autoreactive T cells can destroy the myelin sheath [138]. It has an early inflammation stage and a delayed neurodegeneration stage which are related to, respectively, relapsing-remitting form, and non-relapsing forms such as the primary and secondary progressive MS [139-140].

The existing treatments for MS are limited to the immunomodulatory or immunosuppressant agents meaning that the patients have to take treatment continuously. Moreover, these medications do not improve the patient's quality of life [141-142]. It can be said that MS is a more convenient target for the treatment by siRNA than other neurodegenerative diseases. Firstly, MS has an immunological basis so the target cells can be triggered easily through systemic administration. Secondly, usually in MS, BBB has been broken, hence getting the siRNA to the target lesion is simpler [143].

Different genes and molecular pathways can be triggered by this method (Table 5). It has been revealed that T-bet is an important regulator of the IFN-γ gene in Th1 (major T cell in MS pathogenesis), but not TH2. IFN-y is also a major mediator in the signaling pathway that leads to the naive T cell differentiation into the T helper cells [144-146]. The investigation through siRNA against T-bet had interesting results, in both prevention and treatment. Normally, the transfection of myelin derived antigen into the mice could induce MS, namely the EAE model. But if treated T cells with both specific myelin antigen and anti-T-bet siRNA transfer to the naïve mice, the EAE induction process would fail [147]. However, if anti-T-bet siRNA was injected intravenously during the EAE induction, will block the development of disease [147].

There is a close association between the potency of remyelination and the level of oligodendrocyte progenitors in MS [148]. It has been revealed that in the animal models the noch1 signaling pathway plays a role in the inadequate and impaired remyelination process [149]. More confirmation was obtained by a study in which the Notch1 specific siRNA was injected into the MS mice models. Improvement in the potency of oligodendrocyte differentiation and promotion of remyelination were the major results of this study [150]. It has also been demonstrated that LINGO-1 protein could suppress the myelination and oligodendrocyte differentiation. Accordingly, in a recent study, a chitosan-based nanoparticle was loaded with siRNA against LINGO-1, and was administrated intranasally to the rat model of demyelination. The results in the treatment group were promising. In the molecular sight, the downregulation of LINGO-1 leads to higher level of myelin basic protein (MBP) and lower level of caspase-3. The motor function in the remyelination treated group was also improved, indicating the neuroprotective effect of LINGO-1 silencing via siRNA [151].

5. Conclusions

Finding an optimal treatment for ND is still a tremendous medical challenge, maybe due to the specific conditions of these diseases such as their complex nature, incompletely understood etiology or the physiological barrier such as BBB (blood-brain barrier) which make them difficult for drug delivery. siRNA as an alternative strategy, with its features to specific gene silencing, is a potential therapeutic option for the treatment of ND. Although more than two decades have passed since the discovery of siRNA, there are only two siRNA drugs that have been approved for clinical use yet (Onpattro and Givlaari) [152-153]. There are some hurdles which slow the progression of siRNA

technique including immunological adverse effects [154-155], off-target effects [156-157], instability of naked siRNA and nuclease susceptibility [158] and most importantly the development of an optimal *in vivo* delivery system [159].

As reviewed in this paper, many siRNAs were used in different experiments for various ND. Nevertheless, they have hardly entered the clinical phase, indicating that some issues with siRNAs must be clarified before their translation into clinic applications. This suggests that more studies, especially clinical studies, should be performed in this field. Our increasing understanding of the different aspects of siRNA and also the growing advancement in the development of novel delivery systems will pave the way for the next generation of research studies.

Conflict of interest

The authors declare no conflict of interest.

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None

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Abbreviations

AAVs; Adeno-associated viruses, Aβ; β-amyloid, ACAT-1; Acetyl-CoA acetyltransferase 1, AD; Alzheimer's disease, AGO;Argonaute protein, APP;Amyloid precursor protein, AVs; Adenoviruses, BACE; Beta-Secretase, BACE1-AS; BACE1-antisense transcript, BBB; Basso-Beattie-Bresnahan, CaMKII; Calcium/calmodulin dependent protein kinase II, CC; cholesterol-conjugated, CNS; Central nervous system, EAE; Experimental autoimmune encephalomyelitis, ECM; Extracellular matrix, EphB3; EphrinB3, ExCont-RNAi; Expression-control RNAi, HD; GFAP; Glial fibrillary acidic protein, Huntington's disease, HVJ-E; Hemagglutinating virus of japan envelope, I2 PP-2A; Inhibitor 2 of protein phosphatase 2A, IFN-γ; Interferon gamma, iNOS; Inducible nitric oxide synthase, IL-17; Interleukin 17, IF; Intermediate filament, LDL; Low-density lipoprotein, LVs; Lentiviruses, LBs; Lewy bodies, lncRNA; Long noncoding RNA, MS; Multiple sclerosis, ND; Neurodegenerative diseases, NFTs; Neurofibrillary tangles, NIIs; Neuronal intranuclear inclusions, Nis; Nischarin, NO; nitric oxide, Notch1; Notch homolog 1, translocation-associated (Drosophila), NR4A2; Nuclear receptor subfamily 4 group A member 2, ON; Optic neuritis, OLs; Oligodendrocytes, PD; Parkinson's disease, PEI; Polyethylenimine, PEI-ALG; Polyethyleneimine-alginate, PS; presenilin, RGC; Retinal ganglion cells, RhoA; Ras homolog family member A, RISC; RNA induced silencing complex, RNAi; RNA interference, RNFL; Retinal nerve fibre layer, ROCK; Rho-associated protein kinase, TRIF; TIR-domain-containing adapter-inducing interferon-β, SAMP8; Senescence accelerated mouse-prone 8, SCI; Spinal cord injury, siRNA; Small Interference RNA, SPIONs; Superparamagnetic iron oxide nanoparticles, T-bet; T-box transcription factor, VDAC1; Voltage-dependent anion-selective channel 1, WHO; World Health Organization

Figure Legends

Figure 1. Mechanism of RNAi by dicer.

Figure 2. Mechanism of Tau formation and aggregation in Alzheimer.

408 **Table 1.** siRNA therapeutic applications in Alzheimer's disease.

Target gene(s)	Delivery approach	Model(s)	Effect(s)	Reference
BACE1‑AS	lentiviral vector	*In vivo*: SAMP8 mice	-Improvement of memory and learning behaviors	[52]*
APP Tau VDAC1	Naked siRNA	*In vitro*: human neuroblastoma cell line (SH-SY5Y)	-Improvement in synaptic activity and mitochondrial function	[69]*
BACE1	Lentiviral vectors	*In vivo*: mouse model of Alzheimer disease	-Decreasing amyloid plaque rate -improvement in neuropathological and behavioral signs	[72]*
presenilin1 (PS1)	Naked siRNA	*In vitro*: IMR-32 (human neuroblastoma cells)	-Reducing the level of Aβ 42	[78]*
ROCK-II	PEG–PEI *co*-polymer	*In vitro*: C17.2 (neural stem cells)	-Promoting axonal regeneration	[160]
mutant presenilin1 (L392V PS-1) BACE1	Lentiviral vector and synthetic chemically modified siRNA	*In vivo*: rat model *In vitro*: dividing and neural stem cells	-Decreasing the level of amyloid plaque	[161]
I2 PP-2A	lentiviral vector	*In vivo*: TG2576 mice	-Decreasing the level of Aβ and APP and phosphorylated tau -Improvement of memory and learning ability	[162]
ACAT-1	chemically synthesized siRNA	*In vitro*: human APP751 (H4APP751)	-Reducing the enzymatic process of APP -Enhancing the level of free cholesterol	[163]
BACE1	PEGylated magnetite nanoparticles	*In vitro:* HFF-1 cells	-Significant suppression of BACE1 expression	[164]

BACE1	Fusion protein TARBP-BTP	*In vivo:* AbPP-PS1 mice	-Reduction of plaque load in the cerebral cortex and hippocampus	[165]
Nogo receptor	poly - lysine starch nanoparticle	*In vivo:* Male SD mice	Promoting the regeneration and repair of cholinergic neurons	[166]
BACE1	PEG-PDMAEMA nanocomplex	*In vivo:* APP/PS1 transgenic mice *In vitro:* bEnd.3	- Increasing the level of synaptophysin - Rescued memory loss	[167]

*Explained in the text

431 **Table 2.** siRNA therapeutic applications in Parkinson's disease.

Target gene	Delivery system	Model(s)	Effect(s)	Reference
α-synuclein (SNCA)	Anionic liposomes decorated with a rabies virus glycoprotein -derived peptide	*In vitro*: neuronal cell from P0 newborn C57BL/6J mice	-Reducing the level of SNCA	[80]
	Naked siRNA	*In vitro*: human neuroblastoma cells (BE(2)-M17) *In vivo*: wild-type C57BL6 female mice	-Reducing the level of SNCA	[95]*
	Naked siRNA	*In vivo*: Primate Substantia Nigra	Reducing the level of SNCA and the first evidence of successful anti-α-synuclein intervention in the primate	[96]*
	Viral vector (AAV vectors))	*In vivo*: Thy1-hSNCA mice	-Decreased hSNCA expression -Rescue of hSNCA-mediated behavioral deficits	[97]*
	ExCont-RNAi	*In vitro*: Drosophila S2 cells and human fibroblasts *In vivo*: flies model of PD	-Reducing the level of SNCA - Improvement in motor dysfunction	[98]*
	Nanoparticle (LDH)	*In vitro*: human neuroblastoma cell line (SH-SY5Y)	-Reducing the level of SNCA	[168]
	PEG-PEI	*In vitro*: PC12 cells	-Protect cells from death via apoptosis	[169]

	PEI F25- LMW	*In vitro*: human neuroblastoma cell line (SH-SY5Y) *In vivo*: Thy1-aSyn mice	-Reducing the level of SNCA	[170]
	Peptide mediated delivery	*In vivo:* transgenic mouse model of PD	-Reducing the accumulation of α-syn -Amelioration of inflammatory pathology	[171]

*Explained in the text

454 **Table 3.** siRNA therapeutic applications in Huntington's disease.

Target gene(s)	Delivery approach	Model(s)	Effect(s)	Reference
Htt	Naked siRNA	*In vivo*: HD transgenic mouse model, R6/2	-Inhibition of the Htt expression - reduction of size and number of NIIs	[109]*
	cholesterol-conjugated (cc) siRNA	*In vivo*: viral transgenic mouse model of HD	-Inhibition of the Htt expression - Improvement of some movement problem -Survival of neurons	[112]*
	Naked siRNA	*In vitro* : -COS-7 (African green monkey fibroblasts); -SH-SY5Y (human neuroblastoma); -Neuro-2A (mouse neuroblastoma).	- Inhibition of the Htt expression	[113]*
	Chitosan-based nanoparticle	*In vivo:* transgenic YAC128 mouse	- Decreasing the level of mutant htt protein	[172]

455 *Explained in the text

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Table 4. siRNA therapeutic applications in spinal cord injury.

Gene target(s)	Delivery system	Mode(s)	Effect(s)	Reference
GFAP Vimentin	adenovirus vectors	*In vitro*: C6 glioma cells *In vivo*: SCI model rat	-Improvement of urinary function	[120]*
EphB3	Lentiviral vector	*In vivo*: female Wistar rats	-Improvement in axonal regeneration and the motor function	[122]*
iNOS	chitosan	*In vivo*: Female BALB/c mice1	-Improvement of the secondary damage following SCI	[129]*
Nischarin	PEI-ALG	*In vivo*: SCI model rat	-Improvement of motor function	[132]*
RhoA	2'O-methylated siRNA	*In vivo*: female Sprague-Dawley rats	-Improvement in walking -declining of allodynia	[173]

*Explained in the text

484 **Table 5.** siRNA therapeutic applications in multiple sclerosis.

Target	Delivery system	Model(s)	Effect(s)	Reference
T-bet	Naked siRNA	*In vivo*: EAE mice (mouse model of MS)	- Specifically regulate IFN - Prevented the onset of disease	[147]*
Notch1	pIRES2‐EGFP vector	*In vivo*: Mouse model of acute demyelination	- Promotion of the remyelination - Improve OL differentiation -Increase mature OL	[150]*
LINGO-1	Chitosan nanoparticles	*In vivo:* Male Wistar rats	- Better motor function -Repair in histopathological sections	[151]*
NR4A2	hemagglutinating Virus of Japan envelope (HVJ-E) vector kit	*In vivo*: EAE mice (mouse model of MS)	-Inhibiting the pathogenic potentials of IFN and IL-17	[174]
TRIF	Liposome	*In vivo*: EAE mice (mouse model of MS)	-Alleviating the severity of EAE via the inhibition of interleukin and cytokine release	[175]
caspase-2	Naked siRNA	*In vivo*: EAE mice (mouse model of MS)	-Significant inhibition of nerve cell loss -Decreasing in RNFL thickness - Increased survival of RGC after ON	[176]
CaMKII	Naked siRNA	*In vivo*: EAE mice (mouse model of MS)	-Reduced mechanical and thermal hypersensitivity - Essential role of CaMKII_ in inducing and maintaining the evoked and non-evoked pain in EAE.	[177]

485 *Explained in the text

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