Chapter 6
microRNAs and Neurodegenerative Diseases

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Abstract microRNAs (miRNAs) are small, noncoding RNA molecules that through imperfect base-pairing with complementary sequences of target mRNA molecules, typically cleave target mRNA, causing subsequent degradation or translation inhibition. Although an increasing number of studies have identified misregulated miRNAs in the neurodegenerative diseases (NDDs) Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, which suggests that alterations in the miRNA regulatory pathway could contribute to disease pathogenesis, the molecular mechanisms underlying the pathological implications of misregulated miRNA expression and the regulation of the key genes involved in NDDs remain largely unknown. In this chapter, we provide evidence of the function and regulation of miRNAs and their association with the neurological events in NDDs. This will help improve our understanding of how miRNAs govern the biological functions of key pathogenic genes in these diseases, which potentially regulate several pathways involved in the progression of neurodegeneration. Additionally, given the growing interest in the therapeutic

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potential of miRNAs, we discuss current clinical challenges to developing miRNA-based therapeutics for NDDs.

Keywords microRNAs • Neurodegenerative diseases • Alzheimer's disease • NDD • Parkinson's disease • Huntington's disease • Gehrig • ALS • Amyotrophic lateral sclerosis

Introduction

Neurodegenerative diseases (NDDs) are characterized by progressive neuronal degeneration in the central nervous system (CNS). The most prevalent NDDs include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). These diseases are usually incurable but have many similarities, such as protein aggregation, mitochondria dysfunction, and axonal transport defects. Except HD, the majority of cases of the other three NDDs are sporadic, meaning no significant associations with certain genes are detected in these cases. Thus, searching for more general pathological mechanisms is required.

microRNAs (miRNAs) have emerged as an attractive candidate mechanism because they are regulatory molecules that are widely involved in many biological processes. miRNAs are 21- to 24-nucleotide (nt)-long, noncoding RNAs that are transcribed by RNA polymerase II or III as pri-miRNA. Pri-miRNAs are cleaved by Drosha to yield approximately 70- to 100-nt pre-miRNAs [1]. The pre-miRNAs are exported out of the nucleus and are cleaved again by Dicer to generate a 21- to 24-nt mature miRNA. The mature miRNAs form mismatching-permitted complementary binding with the 3′ untranslated region (3′UTR) of the target mRNA through the RNA-induced silencing complex (RISC) [2] (see Chap. 2 of the volume "microRNA: Basic Science" for a detailed discussion of miRNA machinery). Upon binding with their target mRNA, the miRNA's main function is to induce the degradation or translational inhibition of their target mRNAs [2, 3]. Based on computational predictions, one miRNA can downregulate the expression of hundreds of proteins, and this has been experimentally demonstrated [4, 5]. Therefore, the role of miRNA must be considered a crucial aspect in almost every biological process, including the pathogenesis of NDDs.

Brain-enriched miRNAs account for less than one-thirtieth of the total number of identified miRNAs [6, 7], which indicates that the temporal and spatial expression of miRNAs is strictly regulated. miRNAs have been shown to play an important role in neuronal development [8–12]. For example, deletion of Dicer at a stage when ES cells enter the postmitotic state to initiate dopaminergic (DA) neuronal differentiation can completely eliminate DA neuron differentiation [13]. This phenotype can be rescued by introducing small RNA species, including miRNA, into the ES cells, which means that these small RNA species are crucial for DA neuron generation [13]. In addition to neurodevelopment, accumulating evidence has gradually revealed the essential role of miRNA in nervous system morphogenesis, synaptic plasticity, and neurodegeneration [14, 15]. For instance, conditional deletion of Dicer in the adult brain results in obvious neuronal degeneration in the mouse cortex

[15]. All of these findings indicate that miRNA research is becoming increasingly crucial and complicated in neuroscience, including with respect to NDDs.

Here, we summarize the involvement of miRNAs in NDD. We will discuss the dysregulation of miRNAs in each NDD and the roles of specific miRNAs that show promising involvement in these NDDs. Moreover, given that an abnormal increase or decrease in the expression of various miRNAs may contribute to the pathophysiology of NDDs, the use of miRNAs as a therapeutic method to modulate and correct aberrant activity of the nervous system may therefore provide potential clinical treatment for these disorders, which will also be discussed.

miRNA in Neurodegenerative Diseases

miRNAs and AD

Dysregulation of miRNA in AD

Alzheimer's disease (AD) is a neuronal degeneration disease that shows cognitive impairment and dementia clinically [16]. The pathological hallmark of AD is a progressive spread of extracellular Aβ accumulation and intracellular tangles of hyperphosphorylated tau [16]. These pathological proteins in turn cause neuronal loss, starting from the hippocampus and progressing to the entire cortex [16].

miRNA expression profiling has been extensively studied in AD patients, and a number of miRNAs have been shown to be dysregulated in AD patients [17–23]. In human brain, Lukiw found that miR-9 and miR-128 were increased in the hippocampus of AD patients [21]. Cogswell and colleagues identified 8 miRNAs, including miR-9, -26a, -132, and -146b, that were downregulated and 15 miRNAs, including miR-27, -29, -30, -34, and -125b, that were upregulated in the frontal gyrus of AD patients [17]. Hebert and colleagues also detected 13 downregulated miRNAs, including miR-181c, -15a, -9, -101, -29b, -19b, -106b, and -26b, and 3 upregulated miRNAs in the cortex of sporadic AD patients [19]. In human CSF, miR-146b and miR-27a-3p were identified as downregulated [17, 23], and miR-30 family members were upregulated in patients compared to controls [17]. In the cerebral cortex of AD mice (APPswe/PSΔE9 mice), miR-20a, -29a, -125b, -128a, and -106b were downregulated compared to their expression in age-matched controls [18, 22].

miR-9 in AD

Several independent studies have found altered expression of miR-9 in AD brains. However, both upregulation and downregulation have been reported [17, 19, 21, 24–28]. For example, Lukiw and colleagues reported an upregulation of miR-9 in the temporal cortex and hippocampus of AD patients compared to age-matched controls [21, 25]. However, Hebert and colleagues found a decrease in miR-9 in the cortex of sporadic AD patients [19]. In an animal model of AD, miR-9 shows a

significant decrease in the hippocampus of 6-month-old, but not 3-month-old, APPswe/PSΔE9 mice [22].

Because Aβ plaques form in 6-month-old APPswe/PSΔE9 mice, this decreased expression of miR-9 might indicate that miR-9 decreases in response to Aβ accumulation. This hypothesis is consistent with what Schonrock and colleagues found in a cell culture model. They detected miRNA expression changes in response to Aβ treatment in primary neurons [29]. Interestingly, most of the miRNAs that show significant expression changes compared with untreated cells are downregulated in response to Aβ treatment. The proportion of upregulated miRNAs is much smaller. miR-9 is one of the miRNAs that are rapidly downregulated in response to Aβ treatment in primary neurons [29]. miR-9 targets BACE1, which cleaves APP and is a rate-limiting enzyme in Aβ generation [30].

miR-29 in AD

miR-29 was found to be decreased in the brains of sporadic AD patients [19]. Consistently, a decrease in miR-29 has also been observed in the cerebral cortex of APPSwe/PS1ΔE9 mice [22]. miR-29 is an astrocyte-enriched miRNA that is also expressed, to a lesser extent, in mature primary neurons [31, 32]. Its expression dramatically increases with aging [33–35]. The emerging role of miR-29 is to protect cells from apoptosis by targeting and repressing a family of pro-apoptotic proteins, including Bim, Bmf, Hrk, and Puma [31]. Overexpression of miR-29 protects cells from various stimuli-induced cell death. These stimuli include growth factor deprivation, ER stress, and DNA damage [31]. In addition to pro-apoptotic proteins, BACE1 is another confirmed target of miR-29, and the decrease in miR-29 in AD patients is correlated with the increase of BACE1 in these patients [19]. Therefore, decreased expression of miR-29 in AD patients might accelerate Aβ generation by de-repressing BACE1. In summary, miR-29 exhibits a protective role in neuronal survival via both the apoptotic and APP pathways.

miRNA let-7b in AD

miRNA let-7b is increased in the CSF of AD patients compared with controls [36]. This miRNA is involved in the immune response in AD. The increased level of let-7b in the CSF of AD patients might contribute to neurodegeneration by binding to RNA-sensing toll-like receptor (TLR) 7, which is an innate immune receptor [36].

miR-106b in AD

miR-106b, which directly targets APP [33], shows downregulation in both the human AD brain [19, 33] and in the brains of APPSwe/PS1ΔE9 mice [22]. Although it is unclear whether the downregulation of miR-106b is triggered by Aβ

accumulation, decreased expression of miR-106b can possibly enhance the expression of APP, which may accelerate Aβ accumulation.

However, we need to bear in mind that miR-106b might contribute to AD pathology in other ways. One possibility is that miR106b might regulate cell cycle reentry by targeting retinoblastoma protein 1 [37–39] and p21 [37]. Both retinoblastoma protein 1 and p21 are involved in cell cycle regulation. Additionally, miR-106 also targets ITCH, which is an E3 ubiquitin ligase that is involved in the p73 apoptotic signaling pathway [40] and the Wnt signaling pathway [41]. Furthermore, miR-106b can also be involved in autophagy regulation, which is closely related to Aβ accumulation [42], by targeting SQSTM1/p62 [43]. Therefore, miR-106b might participate in AD pathology by regulating APP expression, cell cycle reentry, apoptosis, or even neuronal differentiation.

miRNAs and PD

Dysregulation of miRNA in PD

PD is a neural degenerative disease with symptoms that include tremors,) rigidity, and bradykinesia [44]. The most prominent pathological features are neuronal loss in the substantia nigra [44] and the cellular accumulation of α-synuclein in Lewy bodies [45, 46].

miRNA microarray analyses revealed that miR-34c-5p and miR-637 were significantly downregulated in the amygdala of PD patients compared with controls [47]. Another miRNA, miR-133b, was found to be downregulated in the midbrain of PD patients compared with controls [13]. In peripheral blood mononuclear cells (PBMCs), miR-335, -374a/b, -199, -126, -151-5p, -29b/c, -147, -28-5p, -30b/c, -301a, and -26a were found to be decreased in PD patients compared with controls [48]. In PD *C. elegans* models, miR-64/65 and let-7 were shown to be downregulated compared with controls [49].

miR-133b in PD

In sporadic PD patients, miR-133b is significantly downregulated [13]. miR-133b is a midbrain-enriched miRNA. Overexpression of miR133b moderately promotes the initial stage of DA neuronal differentiation, while inhibiting terminal differentiation, as indicated by the reduced expression of DAT and TH [13]. Dopamine release is also reduced in miR-133b-overexpressing cells. Conversely, inhibition of miR133b in ES cells potentiates DA neuron terminal differentiation and enhances dopamine release [13].

Pituitary homeobox 3 transcription factor (Pitx3) is one of miR-133b's targets [13]. Pitx3 is important not only for DA neuron differentiation but also for the long-term survival of these neurons [50]. Thus, it seems that the decreased expression of

miR-133b in PD patients might participate in PD pathogenesis by affecting DA neuron differentiation. miR-133b and Pitx3 also form a feedback loop in the regulation of DA neuron differentiation. Bioinformatics searches predicted that Pitx3 is one of miR-133b's targets. This was confirmed by a luciferase assay.

However, overexpression of Pitx3 in differentiating ES cells causes an increase in the expression of miR-133b. The regulation of Pitx3 by miR-133b seems direct because the binding between Pitx3 and the promoter of miR-133b was also confirmed by a luciferase assay [13, 51].

miR-107 in PD

Kim and his colleagues reported downregulation of miR-107 in the midbrain of PD patients [13]. One of miR-107's targets is progranulin. Progranulin is a secreted growth factor and also a major genetic cause of frontal temporal dementia [52, 53]. miR-107 is also involved in cell cycle regulation through targeting CDK6, which is important for entry into the G1 phase [54, 55]. As cell cycle reentry commonly leads to cell death in postmeiotic neurons, decreased miR-107 may lead to increased expression of CDK6 and then the promotion of cell cycle reentry, which finally causes cell death. The expression of miR-107 is directly upregulated by p53 [56]. Using a luciferase assay and a ChIP assay, Yamakuchi and colleagues identified a p53 binding site in the 5′UTR of the miR-107's parent gene, pantothenate kinase enzyme 1 (PANK1). Genotoxic stress induces increased expression of miR-107 in a p53-dependent manner [56].

miR-34 in PD

Decreased expression of miR-34 is found in various brain regions of PD patients compared with age-matched controls [22]. The decreased expression of miR-34 in PD patients is an early-stage event, indicating that it is not a secondary response to disease development or drug treatment. It has been shown that the reduction of miR-34 in differentiated SH-SY5Y cells can lead to disruption of the mitochondrial membrane and increased oxidative stress, which in turn leads to decreased cell viability [22].

Bioinformatic studies indicated that Parkin is one of the targets of miR-34; however, this relationship cannot be proven by Western blot analysis. Targets of miR-34 also include SIRT1 [57, 58], Bcl-2 [22], Cdk4, and cyclin D2 [59]. Bcl-2, which is an anti-apoptotic protein, showed an increase in response to miR-34 inhibition and might protect cells from apoptosis. Cdk4 and cyclin D2 are cell cycle regulators that are involved in cell cycle reentry, which triggers cell death in mature neurons [59]. By using a luciferase assay, it was found that p53 can directly bind to the promoter of miR-34a [60]. Conversely, miR-34a can indirectly regulate the expression of p53 by repressing the expression of HDM4, which can

inhibit the expression of p53 by its RING domain [61]. Thus, the double-positive feedback between p53 and miR-34a forms a locked checkpoint to execute the pro-apoptotic process.

miR-205 in PD

In sporadic PD patients, Lrrk2 is considered an important pathogenic factor; Lrrk2 is significantly increased in patients compared with controls [46]. Interestingly, miR-205, which targets the 3′UTR of Lrrk2 and can regulate Lrrk2 expression, was significantly downregulated in the frontal cortex and striatum of sporadic PD patients compared with controls [62].

In contrast, miR-181, -19, and -410, which also contain the 3′UTR binding site of Lrrk2, lacked a significant change in expression in sporadic PD patients. Overexpression of miR-205 in Lrrk2 R1441G mutant primary neurons rescued the shortened neurite phenotype [62]. Interestingly, overexpression of miR-29, which functions in the apoptotic pathway, cannot rescue the phenotype [62], which means that miR-205 works through Lrrk2 in regulating neuronal survival and that the downregulation of miR-205 contributes, at least in part, to the PD pathology induced by elevated Lrrk2 activity [62].

miRNAs and HD

Dysregulated miRNA in HD

HD is a NDD characterized by cognitive and motor defects. It is caused by CAG repeat expansion ,)in the huntingtin (HTT) gene. Pathologically,,) progressive neuronal death is found in the cortex and striatum of HD patients.

Mutant HTT protein, which harbors an expanded polyglutamine tract, has been shown to be associated with Ago2 and P-bodies [63], cytoplasmic sites of RNA metabolism, and RNAi and miRNA activity [64, 65]. Thus, a change in miRNA activity has been proposed to be responsible for the defects in HD. By using RNA sequencing, microarray, and qRT-PCR techniques, Marti et al. found a set of miRNAs that showed dysregulation in the frontal cortex and striatum of HD patients. These miRNAs include miR-100, -151-3p, -16, -219-2-3p, -27b, -451, and -92a, which showed increased expression in the diseased tissue, and miR-128, -139-3p, -222, -382, -433, and -483-3p, which showed decreased expression in the HD patients [66]. Moreover, HTT also interacts with repressor element-1 silencing transcription factor (REST) [67].

Johnson et al. selectively detected changes in the expression of REST-targeting miRNAs, including miR-9, -29a, -29b, -124a, -132, -135b, -139, -203, -204, -212, -330, and -346, in the cortex of R6/2 mice, which are an animal model of HD [68].

They found that four miRNAs, including miR-29a, -124a, -132, and -330, were decreased, while their mRNA targets were correspondingly increased. However, only the change in miR-132 has been further confirmed in the parietal cortical tissue of human patients [68]. Packer et al. also detected the expression change of REST-targeting miRNAs in HD patients, but using Brodmann's areas [69]. They found that the expression of five miRNAs, including miR-9, miR-29b, miR-124a, and miR-132, were significantly changed in HD patients [69].

miR-9 in HD

miR-9-5p and miR-9-3p are both decreased in early HD. As mentioned above, REST, which is a transcription factor that inhibits genes important for neuronal differentiation, is one of the downstream effectors of HTT in HD pathology, and miR-9 is one of the miRNA targets of REST [67, 69]. Aberrant subcellular distribution of REST has been observed in HD [67, 69]. On the other hand, REST and CoREST are also the targets predicted for miR-9-5p and miR-9-3p (TargetScan). Thus, the feedback regulation loop between miR-9 and REST plays an important role in neurogenesis.

Overexpression of miR-9 inhibits the proliferation of neural progenitor cells (NPCs) and promotes neuronal morphogenesis. The pro-neuronal differentiation role of miR-9 is not only mediated by its repression of REST expression but also by its repression of the expression of BAF53a (ACTL6A), which inhibits neurogenesis by regulating chromatin remodeling [70]. Some researchers also claimed that the pro-neurogenesis effect of miR-9 is mediated by the phosphorylation of STAT3 [71]. It is not surprising that miR-9 promotes neurogenesis by regulating various protein targets with regard to the divergent effect of miRNAs on the proteome [5]. The genes that are important for neuronal differentiation are also important for neuronal survival. For example, one of the targets of REST is BDNF, which is essential for neuronal survival. Thus, the decreased expression of miR-9 in HD might contribute to the development of the disease by impairing neurogenesis and neuronal survival.

miR-22 in HD

miR-22 is decreased in HD [69]. Bioinformatic studies indicated that miR-22 targets histone deacetylase 4 (HDAC4), REST co-repressor 1 (Rcor1), and regulator of G-protein signaling 2 (Rgs2) [72]. All three of these genes are important for neurogenesis and neuronal survival and have been implicated in HD pathogenesis. Importantly, the targeting of these three genes by miR-22 has been demonstrated by luciferase assay [72]. Moreover, overexpression of miR-22 in vitro protects neurons against various stresses [72]. miR-22 overexpression has been shown to reduce the activity of caspases, and miR-22 can also directly inhibit the expression of pro-apoptotic proteins, such as mitogen-activated protein kinase 14/p38

(MAPK14/p38) and tumor protein p53-inducible nuclear protein 1 (Tp53inp1). Furthermore, miR-22 can also decrease the accumulation of mutant HTT via an unknown mechanism. Taken together, these results indicate that miR-22 is strongly associated with HD through the neurogenesis and neuronal survival pathway [72].

miR-128 in HD

miR-128 is a neuronally enriched miRNA that plays an important role in neuronal differentiation and survival [73]. It is decreased in the brains of HD mice, monkeys, and humans [66, 69, 74, 75]. In addition to its effect on neurogenesis and neuronal survival, miR-128 is also directly involved in the HTT signaling pathway by targeting HTT, HTT interacting protein 1 (HIP1), SP-1, and GRM5. The targeting of these three genes by miR-128 has been demonstrated by luciferase assay [74]. Thus, miR-128 might be involved in the pathogenesis of HD either by regulating neuronal differentiation and survival or by regulating the HTT signaling pathway.

miR-132 in HD

miR-132 is also downregulated in the cortex of HD patients [75]. miR-132 is another neuronally enriched miRNA. The transcription of miR-132 is directly regulated by CREB and the BDNF pathway, which is known to be crucial in neuronal differentiation and survival [76]. miR-132 can target p250GAP [77], which is important in neurogenesis, and MeCP2 [78], which regulates BDNF expression in the brain. Thus, dysregulation of miR-132 in HD might contribute to disease development by affecting BDNF balance and impairing neurogenesis in the diseased brain.

miRNAs and ALS

Dysregulated miRNA in ALS

ALS is a progressive NDD that affects nerve cells in the brain and spinal cord. Motor neuron,) loss gives rise to malfunctions in muscle,) tissues, causing weakness, atrophy, and ultimately paralysis and death.

Although 90 % of ALS cases are sporadic, a subset of ALS patients is found to carry a TDP-43 mutation [79]. TDP-43 is an RNA binding protein and interacts with Drosha, which is a key miRNA processing enzyme [80, 81]. These findings raise the possibility that TDP-43 may play a role in miRNA processing. In fact, the expression levels of some miRNAs are affected in TDP-43 mutant flies [82]. Furthermore, P-bodies, where RNA metabolism happens, form in response to cellular stress and are central to ALS pathogenesis [83, 84]. Taken together, these findings indicate that miRNAs might be dysregulated in ALS and that the misregulated miRNAs might also be involved in the pathogenesis of ALS.

Using RT-qPCR, Campos-Melo et al. have demonstrated that miR-146, miR-524-5p, and miR-582-3p are dysregulated in the spinal cords of ALS patients. miRNA target analysis has shown that these miRNAs target the 3′UTR of low molecular weight neurofilament (NFL) [85]. Meantime, Koval et al. also identified expression changes of miR-24, miR-142-3p, miR-142-5p, miR-1461, miR-146b, and miR-155 in the spinal cords of both ALS animal models and human patients [86]. Besides the CNS, miRNA dysregulation was also found in the immune system in HD. miRNAs, such as let-7a, let-7b, miR-27a, -146a, -451, -223, -142-5p, and -155, were significantly upregulated in spleen-derived Ly6Chi monocytes both prior to and during the onset of ALS in SOD1 transgenic mice (an ALS mouse model) [87].

miR-206 in ALS

miR-206 is a skeletal muscle-specific miRNA. It is observed to be dramatically increased during the nerve reinnervation process in SOD1 transgenic mice. Consistently, miR-206 knockout worsens the disease and shortens the lives of SOD1 mice [88]. miR-206 was found to induce the secretion of fibroblast growth factor binding protein 1 (FGFBP1) from muscle by inhibiting HDAC4 translation [88]. FGFBP1 can promote presynaptic differentiation at the neuromuscular junction by binding to FGF [89]. Thus, miR-206 is beneficial to heal ALS due to its promotion of synaptogenesis through FGFBP1 signaling.

miR-155 in ALS

miR-155 is increased in both sporadic and familial ALS patients. This increase is harmful because when the increased expression of miR-155 is inhibited in the brains of SOD1G93A rats, the survival of the rats increases [86]. miR-155's function is mostly known in the immune system, where miR-155 was shown to promote tissue inflammation and macrophage inflammatory responses [90]. It has been shown that miR-155 can target SOCS1 mRNA and in turn increase pro-inflammatory cytokine secretion [91]. Furthermore, miR-155 can also downregulate the action of TGF-β, an important cytokine involved in immunosuppression by suppressing two of its targets, SMAD2 [92] and SMAD5 [93].

Therapeutic Potential of miRNAs in Neurodegenerative Diseases

Abnormal increases or decreases in the expression of various miRNAs may contribute to the pathophysiology of NDDs (Table 6.1). The replacement or inhibition of downregulated or upregulated miRNAs may therefore be clinically beneficial in the treatment of these disorders [94–96].

Table 6.1 Summary of miRNAs involved in NDDs

NDDs	miRNAs	Target genes	Biological process involved	References
AD	miR-9	BACE1	APP cleavage	[30]
	miR-29	Bim; Bmf, Hrk; Puma	Neuronal survival	[31]
	let-7b	TLR7(binding)	Immune response	[36]
	miR-106b	APP	APP signaling	[33]
PD	miR-133b	Pitx3	DA neuron differentiation and survival	[13]
	miR-107	Progranulin; CDK6	Neuronal survival	[53–56]
	miR-34	SIRT1; Bcl-2; CDK4	Neuronal survival	[22, 57–59]
	miR-205	Lrrk2	Neuritis outgrowth	[62]
HD	miR-9	REST/CoREST	Neurogenesis	[67, 69]
	miR-22	HDAC4; Rcor1; Rgs2; p38; Tp53inp1	Neurogenesis and neuronal survival	[72]
	miR-128	HTT; HIP1	Neuronal differentiation	[74]
	miR-132	P250GAP; MeCP2	Neurogenesis	[77, 78]
ALS	miR-206	HDAC4	Nerve reinnervation	[88]
	miR-155	SOCS1; TGF-β	Immune response	[91–93]

Most of the progress made in the development of RNA-based therapies has been associated with hereditary NDDs, such as HD [97]. Delivery of a U6 promoter-driven shRNA effector using an adeno-associated virus serotype 1 (AAV1) vector has been shown to target human huntingtin protein, and this treatment could yield improvements in HD-associated neuropathology and behaviors [97]. Although it is difficult to define the appropriate therapeutic targets in sporadic NDDs, recent progress has been made in RNA-based silencing of targets linked to common sporadic forms of PD and AD. For instance, Sapru et al. have successfully targeted the alpha synuclein pathway in the context of PD [98], while in the case of AD, the amyloid [99, 100] and BACE1/β-secretase [101] pathways have both been targeted, with the latter yielding a striking improvement in disease phenotypes.

Development of miRNA-Based Therapeutics

There are two main miRNA-based therapeutic strategies being developed in vivo:

- microRNA mimics
- Anti-microRNAs

miRNA mimics, which are small RNA molecules that resemble miRNA precursors, are used to downregulate the expression of specific target proteins. These proteins can be associated with disease pathogenesis, or with the disease gene itself, and have a gain-of-function pathogenic mutation. The goal of this

strategy is based on the hypothesis that decreasing the level of a specific protein is a protective therapeutic approach.

The second strategy, which has been more widely studied, involves the exploitation of anti-miRNA molecules to create a loss-of-function in the miRNA of interest [102]. In certain disease conditions in which miRNAs are over-expressed, the aim would be to block these miRNAs by introducing a complementary RNA sequence that binds to and inactivates the targeted miRNAs. A major objective in these efforts is to develop oligonucleotides that achieve high in vivo efficacy without significant toxicity.

Development of Anti-microRNA-Based Therapeutics

Antisense Oligonucleotides

Mature miRNAs can be inactivated by administering short complementary synthetic antisense oligonucleotides (ASOs) [103]. Recently, ASOs have been shown to block miRNA precursors and miRNAs without degrading the target RNA [104]. While the complete mechanism behind ASO-mediated inhibition of miRNA activity remains to be determined, anti-miRNA ASOs are likely to be featured as important future therapeutic agents.

Antagomirs

Antagomirs are usually cholesterol-conjugated ASOs that are capable of improving the degradation of specific mRNAs when administered in vivo [105]. To achieve this, Stoffel's group designed "antagomirs," which are RNA snippets conjugated to cholesterol molecules that help the RNA enter into the cells [105]. Intravenous administration of antagomirs results in an apparent decline of corresponding miRNA levels in many organs, such as liver, lung, kidney, heart, and intestine. The silencing of endogenous miRNAs by this method is specific, efficient, and long-lasting. However, antagomirs cannot cross the blood–brain barrier (BBB) but can penetrate brain cells if injected directly into the brain [106].

miRNA Sponge

Another experimental strategy developed in recent years to inhibit miRNA function is to exploit synthetic sponge mRNA, which contains complementary binding sites to a miRNA of interest [107, 108]. When the sponge is expressed from transgenes, it specifically hampers the activity of a family of miRNAs that have a common seed sequence, resulting in the sequestration and blocking of a whole family of related miRNAs [108].

Typical sponge constructs are composed of four to ten binding sites separated by a few nucleotides each. The efficiency of a miRNA sponge depends not only on its affinity for its binding sites but also on need for a high amount of sponge RNAs relative to the amount of the miRNA, which can be achieved by expressing the sponge from a strong promoter, such as the CMV promoter. The sponge method presents several advantages [108]. First, making a sponge to miRNA is easier than knocking it out. Second, many miRNAs have the same seed sequence but are encoded by multiple distant loci; therefore, each locus will have to be knocked out individually and the animals cross-bred to generate a strain with complete knockout. Further, as some miRNA precursors are transcribed in clusters, the deletion of a miRNA within a cluster will be difficult to achieve without affecting others in the same cluster. Because sponges interact with mature miRNAs, their effectiveness is unaffected by the latter two situations. Although sponge technology has advantages in experimental settings, further investigations will be required to develop it into a therapeutically viable strategy.

Locked Nucleic Acid

As the miRNA research field progressed, the first challenge was to develop and improve miRNA detection and functional analysis tools given the small size and sometimes low levels of expression of different miRNAs. An important addition to the miRNA toolbox came from locked nucleic acid (LNA) [109]. LNA-modified oligonucleotides possess high thermal stability when hybridized with their cognate mRNA target molecules and therefore appear to be ideally suited for the targeting of small RNAs, such as miRNAs [109–111]. LNA is a family of conformationally locked nucleotide analogs that are relatively resistant to nuclease activity and may prove to be a suitable platform for the development of miRNA-based therapeutics. For example, it is known that miR-122 regulates cholesterol metabolism and hepatitis C virus (HCV) replication [112]. An LNA-modified ASO blocked miR-122 activity in the liver of African green monkeys without any evidence of toxicity [112], induced long-lasting decreases in plasma cholesterol levels, and decreased HCV levels in the blood of HCV-infected chimpanzees [113].

Development of miRNA Biogenesis-Based Therapeutics

In addition to the development of mimetic and ASOs to modulate miRNA function, recent efforts have also been directed toward developing small-molecule drugs that can influence the biogenesis of miRNAs or directly influence their function [114]. The small-molecule anti-inflammatory drug enoxacin (Penetrex™) promotes the biogenesis of endogenous miRNAs [115–117]. Conversely, compounds that disrupt miRNA biogenesis have been identified [118]. It will be important to determine if similar screening approaches can be used to identify chemical probes and potentially useful drugs to selectively modulate the activity of miRNAs involved in NDDs.

Challenge of Delivering Therapeutic miRNA Targets in NDDs

The use of miRNAs as potential therapeutic targets remains controversial with regard to methods of delivery and target specificity. When considering a treatment for NDDs that is mediated by miRNA delivery, we have to assess its ability to pass through the BBB as well as its neurotoxicity and off-target effects. To overcome the problem of the BBB, several siRNA delivery systems are being developed for in vivo purposes. These include vector-based, chemically modified, and "packaged" RNA oligonucleotides [119].

Crossing the BBB to Deliver Therapeutics miRNA Targets

Exosome

A major drawback in delivering ASOs is the relative difficulty of crossing the BBB. Intriguingly, systemically injected exosomes, which are small transport vesicles secreted by many classes of mammalian cells, can be used to selectivity deliver small RNAs to the brain in mice [120]. Exosomes are cell-derived vesicles that enable cell-to-cell communication by transferring RNA molecules and proteins [120]. They have been shown to preserve mRNAs and miRNAs in the presence of RNase and to subsequently deliver them to recipient cells [121]. In particular, Alvarez-Erviti et al. isolated brain-targeting exosomes from dendritic cells bioengineered to express an exosomal membrane protein (lamp 2b) fused to a ligand of the acetylcholine receptor. Exosomes were then loaded with siRNAs targeting BACE1 mRNA by electroporation and injected intravenously, resulting in a significant knockdown of BACE1 expression [120]. Using this approach as a future therapeutic vehicle to deliver siRNA/miRNA is worthy for several reasons, including the specific targeting of exosomes to the brain following systemic delivery, the ability to load the desired siRNA/miRNA into the exosomes, and the ability to escape an immune response.

Non-viral

To avoid the action of nucleases, chemical modification or non-viral carriers can be used [122]. Conjugation with non-viral carriers might induce marked toxicity because RNA molecules will also enter the non-targeted cells due to an interaction between the negatively charged cellular membrane and cationic carriers [123]. After RNA molecules have passed these physiological barriers, they have to enter the target cells to elicit their effects. This means that they have to cross the cell membrane, escape endosomes, and localize to the nucleus. Therefore, nuclear-localization signals and cell-penetrating and endosomal-release signal peptides can influence the duration of action of injected RNA molecules [124].

Viral

Adenoviral, lentiviral, and adeno-associated virus-based local delivery has also been performed in animal models of AD and HD [97], demonstrating significant improvement. Nevertheless, the strategy to locally deliver a drug to the brain is still far from normal practice because of the complexities associated with direct injection into the human brain.

Toxicity and Off-Target Effects of Delivering Therapeutic miRNA Targets

There are considerable challenges to the clinical application of exogenous RNAi effector sequences. A number of toxicities are associated with siRNAs and exogenous RNAi precursors. High levels of expressed shRNAs in the liver are known to cause fatalities in mice due, in part, to saturation of the endogenous RNAi machinery [125], and McBride and colleagues have observed toxicity arising from shRNA-based vectors in the brain [126, 127]. Expressed shRNAs likely abrogate the function of natural miRNAs [125, 128, 129]. Therefore, careful consideration should be given to the dosage used when applying ectopically introduced RNAi effectors. Another area of concern is the possibility of off-target inhibition of unintended mRNAs through interactions between the 6- to 7-nt seed region of the exogenous siRNA guide sequences and the target mRNAs. Many off-target effects of this nature have been observed when introducing exogenous siRNAs [130–132]. Off-target effects can in some instances be mitigated by introducing specific base modifications within the siRNA duplex [133]. However, to determine the full extent of any off-target inhibition, prior screening using candidate RNA and protein expression array analyses may be required [5].

Conclusion

In summary, the current evidence clearly points to a significant role for miRNAs in NDDs. miRNAs are of particular interest in understanding these complexes of disorders because they can potentially regulate several pathways involved in the onset and progression of these diseases. The study of miRNA appears particularly promising for understanding the very prevalent but poorly understood sporadic forms of NDDs, such as AD and PD. The challenge now is to understand the role of specific miRNAs in biological models and to translate this knowledge to clinical studies. Moving forward, the development of tools for the delivery, stable expression and controlled modulation of miRNA levels and their action in vivo will be a valuable asset in enhancing the therapeutic value of miRNAs in NDDs.

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