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REVIEW

microRNAs as neuroregulators, biomarkers and therapeutic agents in neurodegenerative diseases

Indranil Basak[1] · Ketan S. Patil[1] · Guido Alves[2] · Jan Petter Larsen[2] · Simon Geir Møller[1,2]

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Abstract The last decade has experienced the emergence of microRNAs as a key molecular tool for the diagnosis and prognosis of human diseases. Although the focus has mostly been on cancer, neurodegenerative diseases present an exciting, yet less explored, platform for microRNA research. Several studies have highlighted the significance of microRNAs in neurogenesis and neurodegeneration, and pre-clinical studies have shown the potential of microRNAs as biomarkers. Despite this, no bona fide microRNAs have been identified as true diagnostic or prognostic biomarkers for neurodegenerative disease. This is mainly due to the lack of precisely defined patient cohorts and the variability within and between individual cohorts. However, the discovery that microRNAs exist as stable molecules at detectable levels in body fluids has opened up new avenues for microRNAs as potential biomarker candidates. Furthermore, technological developments in microRNA biology have contributed to the possible design of microRNA-mediated disease intervention strategies. The combination of these advancements, with the availability of well-defined longitudinal patient cohort, promises to not only assist in developing invaluable diagnostic tools for clinicians, but also to increase our overall understanding of the underlying heterogeneity of neurodegenerative diseases. In this review, we present a comprehensive overview of the existing knowledge of microRNAs in neurodegeneration and provide a perspective of the applicability of microRNAs as a basis for future therapeutic intervention strategies.

Keywords microRNA · Neurodegenerative diseases · Tissue-enriched · Body fluid · Biomarker · Therapeutic agent · microRNA technological advancements

Introduction

Although it was generally assumed that the human genome would mainly contain protein-coding sequences, the human genome project revealed that protein-coding sequences only constitute ~1.5 % of the entire genome. The remaining ~98.5 % of the genome contains introns, regulatory DNA sequences, interspersed elements and non-coding RNA (ncRNA) molecules [1]. Indeed, the majority of mammalian genomes are transcribed into ncRNAs, many of which are alternatively spliced or processed into smaller products. To date, two types of non-coding RNAs have been identified—short non-coding RNA and long non-coding RNA molecules [2]. The short non-coding RNA molecules can further be subdivided into microRNA (miRNA), small interfering RNA (siRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), piwi-interacting RNA (pi-RNA), transfer RNA (tRNA), ribosomal RNA (rRNA) and other uncharacterized small molecules (Fig. 1a) [2, 3]. Among all the regulatory molecules, miRNAs are the most studied, particularly as regulators in human diseases. One of the main drivers for this is that miRNA expression

I. Basak and K. S. Patil contributed equally.

✉ Simon Geir Møller
mollers@stjohns.edu

[1] Department of Biological Sciences, St. John's University, 8000 Utopia Parkway, New York, NY 11439, USA

[2] Norwegian Center for Movement Disorders, Stavanger University Hospital, 4068 Stavanger, Norway

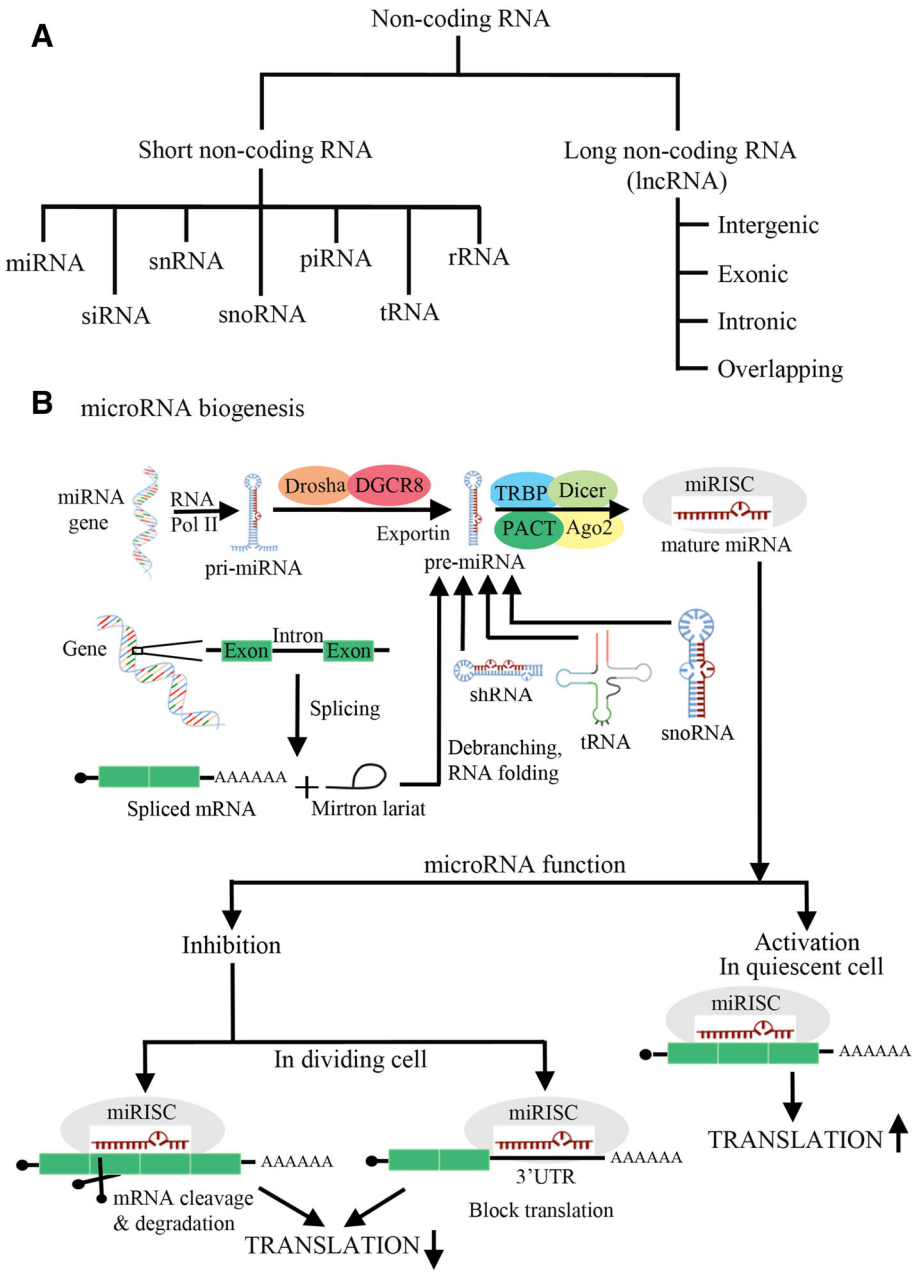

Fig. 1 Schematic overview of non-coding RNA types, microRNA biogenesis and their functions. **a** Two types of non-coding RNAs, short non-coding RNA and long non-coding RNA molecules, have been identified. The short non-coding RNA molecules can further be subdivided into microRNA (miRNA), small interfering RNA (siRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), piwi-interacting RNA (pi-RNA), transfer RNA (tRNA), ribosomal RNA (rRNA) and other uncharacterized small molecules. **b** Schematic overview of microRNA biogenesis and their function. In addition to the canonical pathway, involving the microprocessor complex, nuclear, and cytosolic processing, other sources such as introns, shRNAs, tRNAs and snoRNAs contribute to the miRNA biogenesis pathway. miRNAs exert their function predominantly by inhibiting translation in dividing cells, but they can also activate translation in quiescent cells

profiles very often show significant modifications in response to a disease state suggesting that miRNAs represent key regulators of disease-associated pathways [4, 5]. Recent developments in miRNA research, in relation to human disease, have revealed that miRNAs represent valuable tools as biomarkers and as potential disease-modifying agents [6–8]. Despite these advances, miRNA biology in neurodegeneration is poorly understood. In this review, we comprehensively discuss miRNA biology in the context of neurodegeneration and highlight technological developments that ultimately may lead to new diagnostic and intervention strategies.

Expression and biogenesis of miRNAs

miRNAs are evolutionarily conserved regulatory molecules that are synthesized after processing by both nuclear and cytosolic proteins. In the nucleus, non-coding RNAs (can be more than 1 kb), called the primary miRNAs (pri-miRNA), are transcribed from miRNA-encoding genomic sequences by RNA polymerase II [9–12]. Following this initial transcription event a protein complex comprised of the RNase III enzyme (RNASEN), Drosha and DGCR8 (in addition to several cofactors) recognizes the pri-miRNA and cleaves the 5′ and 3′ arms of the pri-miRNA hairpin to

form the premature miRNA (pre-miRNA—70–110 nts, Fig. 1b) [13, 14]. Exportin-5 then recognizes and transports the pre-miRNA to the cytosol for further processing via a RAN-GTP-dependent mechanism [10]. In the cytosol, further processing of pre-miRNAs into 22 nucleotide mature miRNA duplexes takes place by a protein complex consisting of Dicer (RNase) and the double-stranded RNA-binding domain proteins TRBP, PACT and Ago2 [15, 16]. This is followed by separation of the miRNA guide strand and association with Ago2 within the RNA induced silencing complex (RISC) (Fig. 1b) [16]. Alternatively, and without the RISC involvement, miRNAs can be generated from short hairpin introns, snoRNAs, tRNAs and endogenous shRNAs as a result of splicing, debranching and complex processing mechanisms (Fig. 1b) [17–20].

miRNA biogenesis is a complex process and it is clear that miRNAs are not only generated through the classical canonical pathway, but also from a number of other miRNA sources. As the field progresses and miRNA biogenesis in its entirety becomes fully understood, we will be better equipped to comprehensively understand miRNA biology and its impact on cellular functions.

Brief overview of miRNA functions

Canonical functions

miRNAs are proposed to be downregulators of gene expression via two principal mechanisms: (i) mRNA cleavage and (ii) translational repression. On one hand, miRNAs have been shown to bind to complementary regions of protein-coding mRNA sequences resulting in RISC-mediated cleavage. Alternatively, and in the absence of appropriate complementarity, miRNAs also have the ability to bind to 3′UTRs and block translation (Fig. 1c) [12].

Non-canonical functions

A relatively recent and significant discovery changed the way miRNAs were perceived as bona fide translational repressors. Vasudevan and colleagues convincingly demonstrated that miRNAs can also act as translational upregulators in non-dividing cells (Fig. 1c) [21]. Indeed, miRNAs, along with microRNPs (micro ribonucleoproteins), attain a dual ‘switch’ role as both upregulator and downregulator under quiescent and proliferating conditions, respectively. Furthermore, indirect upregulation of translation may also occur via specific miRNA-mediated downregulation of a repressor protein(s).

The discovery that miRNAs can have dual roles suggests that one miRNA can regulate several mRNAs, and that several miRNAs can share a common target [22].

Regulation of biogenesis, expression, function and decay of miRNAs

In addition to understanding the biogenesis and functions of miRNAs, recent technological advancements have shed considerable light on the biology associated with the regulation of expression and function of miRNAs. Several biological pathways have been identified, involving numerous protein–protein and protein–RNA interaction, that contribute significantly to the cell- or tissue-specific functions of miRNAs [23]. For example, a battery of cofactors and accessory proteins such as DGCR8/Pasha, p68, p72, EWSR1, Fus, Argonaute 2, assist Drosha, Dicer and miRISC to execute their functions, which indirectly control miRNA expression [15, 24–27].

Similar to protein-encoding genes, miRNA genes transcription is regulated by transcription factors [11]. Furthermore, several miRNAs work in tandem with transcription factors within autoregulatory feedback loops to drive or repress the expression of miRNAs [23]. Moreover, several activators and repressors have been identified that regulate miRNA biogenesis, either via protein–protein or protein–RNA interactions [23]. Interestingly, at different processing stages pri-miRNAs and pre-miRNAs may undergo adenosine deaminase-mediated catalysis altering base-pairing and structural properties resulting in abnormal processing of miRNAs [28]. At the functional level, miRNAs incorporated into the miRISC, can be further regulated by proteins targeting the RISC-associated proteins [23].

In contrast to our knowledge on miRNA biogenesis, expression and function, miRNAs turnover mechanisms remain less explored. miRNAs are considered to be highly stable molecules with slow turnover rates, often at very high cellular copy numbers. It is, however, known that miRNA turnover is nucleotide sequence-dependent, and is further influenced by target-mediated degradation, uridylation status and viral infections [29].

Initially miRNA regulation was not generally viewed as being cell specific, but neuronal miRNA regulation indicates that some regulatory aspects may exhibit quasi cell-specificity [30]. For example, Krol and colleagues suggest that the miRNA-mediated regulation of synaptic stimulation is due to miRNAs being expressed at distal sites in dendrites [23]. Furthermore, it has been suggested that miRISC-mediated repression can be relieved by neuronal stimulation-facilitated proteolysis of miRISC assembly

factors resulting in normalized synaptic plasticity or memory formation [23].

Combined, these findings imply that miRNAs play significant regulatory roles during normal neuronal functioning, thereby suggesting that possible dysregulation may have an impact on neurodegeneration.

Tissue-enriched expression of miRNAs

Although all tissues harbor miRNAs, the levels of individual miRNAs differ substantially between different tissue types. In addition, a significant number of miRNAs are enriched in a cell compartment-specific manner. For example, microRNA miR-1 is enriched in expression in muscle and heart tissue, whilst miR-124 expression is augmented in brain tissues [31]. Other examples of tissue enrichment of a miRNAs include miR-122 in hepatocytes, miR-142 is lymphoid tissue-specific, miR-375 is expressed in pancreatic cells and miR-223 is found in myeloid tissue [31–34]. microRNA expression in a particular tissue or cell type can also be enhanced as a result of endogenous and exogenous stimuli. For example, Let-7d, Let-7e, miR-768-3p and miR-768-5p are significantly upregulated in human fibroblasts under oxidative stress caused by ionizing radiation, H_2O_2 and etoposide [35].

There are many examples of miRNAs that are specifically expressed in brain tissue which play a critical role in regulating neuronal activity [36]. As an example, miR-9, miR-124 and miR-128 represent brain-specific miRNAs, and interestingly, their expression patterns change in disease states [37]. The tissue specificity of miRNAs is especially evident in neurons, as they regulate and influence key neuronal features such as neurogenesis, synaptic plasticity, neuronal differentiation, neuronal proliferation and the self-rejuvenation of neuronal stem cells [38].

The well-documented tissue enrichment of miRNA suggests not only that many miRNAs influence cell specific processes, but also it highlights the potential of miRNAs as diagnostic biomarkers and as possible targets in future disease intervention strategies.

miRNAs in brain

Many miRNAs have been shown to be involved in neuronal differentiation and overall brain development. This is evident from studies showing that inactivation of miRNA biogenesis via Dicer removal, results in inappropriate neurulation and neuronal differentiation, ultimately culminating in brain morphogenesis defects [39]. Similarly, Dicer dysfunction in post-mitotic motor progenitor cells results in lack of neuronal generation [40]. miRNA biogenesis is also vital for the appropriate development of dopaminergic neurons. For example, abnormal miRNA biogenesis abolishes differentiation of dopaminergic neurons derived from mouse embryonic stem cells [41]. Furthermore, Dicer inactivation in mouse post-mitotic midbrain results not only in loss of dopaminergic neurons, but also reduced axonal projections [42]. It is also interesting to note that loss of Dicer in spinal motor neurons causes muscular atrophy and a decline in motor function coupled with reactive astrocytosis, a marker for neurotoxicity [43, 44].

miRNAs, such as miR-124a and miR-9, have been shown to determine the fate of neuronal precursors derived from embryonic stem cells [45]. Interestingly, the brain-specific miR-124a regulates neuronal differentiation and maintenance by decreasing the levels of many hundred non-neuronal gene transcripts [46]. Indeed, the expression of miR-124a in non-neuronal cell lines results in neuronal-like mRNA profiles, suggesting a pivotal role for this miRNA in neuronal differentiation [46]. miR-124 has also been shown to have a functional interaction with the alternative pre-mRNA splicing pathway where miR-124 inhibits Polypyrimidine tract-binding protein-1 (PTBP1). PTBP1 encodes a global repressor of alternative pre-mRNA splicing and its inhibition leads to PTBP2 exon skipping and PTBP2 mRNA decay, ultimately resulting in a transition from non-neuronal to neuronal-specific alternative splicing patterns [47].

In the nervous system, the regulation of mRNA translation is important for synaptic development and plasticity [38]. Interestingly, the first study to demonstrate the presence of synapse function-specific miRNAs revealed that the brain-specific miR-134 is expressed in the synaptodendritic compartment of hippocampal neurons, and that it negatively regulates the size of dendritic spines through the inhibition of translation of the Limk1 kinase [48].

This study and others demonstrate the critical role of miRNAs in brain developmental processes and highlights that subtle change or defects in miRNA profiles may result in inappropriate neuronal development, including premature neuronal cell death, a hallmark of neurodegenerative disorders.

miRNAs and neurodegeneration

Neurodegeneration, in diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's diseases (HD), and amyotrophic lateral sclerosis (ALS), is a complex process often influenced by a combination of genetic, molecular and environmental perturbations [49]. Although neurodegeneration is still poorly understood, recent advances in the field have started to unravel numerous

pathways that impact neuronal cell death. Indeed, the emergence of miRNAs as key regulators in neuronal stem cell differentiation, neurogenesis, neuronal survival, neurite outgrowth, synapse formation, and synaptic plasticity has not only revealed their involvement in neuronal development and differentiation, but also neurodegeneration, opening up new avenues for biomarker discovery and potential therapeutic intervention strategies [42, 50–54].

Several studies have demonstrated that miRNAs are not only neuroregulators but also neuroprotectors. For example, a number of findings clearly suggest that the miR-29 family impacts neuronal protection [55–58] and in line with these findings, miR-29 levels are reduced in individuals with AD or HD and in mouse models [59, 60]. Of particular interest are reports documenting that miR-29 family members regulate the expression of serine palmitoyl transferase-1 (SPTLC1), the first rate-limiting enzyme in de novo ceramide synthesis, and beta-secretase-1 (BACE1) [61]. Disruptions of both these proteins play key roles in amyloid beta (Ab) formation, contributing to AD pathology [56, 58]. In AD, the neuroprotective miR-29a-1 and miR-29b-1 are downregulated, promoting disease progression by inversely regulating BACE1 causing increased Ab plaque formation [58].

Age has a tremendous impact on most neurodegenerative diseases [62] and miRNA profiles show dramatic changes as the brain ages [63]. Genome-wide expression analysis of miRNAs and ncRNAs in brain tissue has demonstrated differential regulation of small RNAs in chimpanzees and humans in an age-dependent manner [64]. For example, miR-144 is upregulated in aging human cerebellum and cortex, as well as in aging chimpanzee brains [64]. It is interesting to note that miR-144 can bind to the 3′ UTR of programmed cell death protein 4 (PDCD4) suggesting a possible role for miR-144 in apoptosis in aging brains [64]. Further evidence revealing a link between miRNA expression and apoptosis comes from studies showing that miR-16, miR-128, miR-15 and miR-497 can regulate B cell lymphoma 2 protein (BCL2) to induce programmed cell death [65, 66]. Another example showing the influence of miRNAs on the aging process is the finding that the anti-aging factor Sirtuin-1 (SIRT1), promoting neuronal survival and suppression of neurodegeneration in AD and ALS models, is also regulated miR-34a [67–69]. Indeed, SIRT1 levels are inversely correlated with miR-34a expression in aging brains.

Although the full extent of miRNAs directly affecting neurodegenerative pathways is still not fully understood, a large number of studies have demonstrated that the expression of miRNAs change in neurodegeneration. It is interesting to note that there are numerous miRNAs that are specifically deregulated in a disease-specific manner. For example, miR-548d, miR-224, miR-373, miR-198 show altered expression in PD patients [70, 71, 73]. Multiple studies demonstrate miR-148a, miR-17-5p, miR-137, miR-181c, miR-101, miR-184, miR-15a, miR-185 and miR-210 are only some of the miRNAs that can be AD specific [58, 60, 61, 72, 74]. There are some miRNAs that show a Prion specific expression pattern, miR-342-3p, let-7b, miR-342-3p, miR-490 and miR-188-5p [75–77]. In addition there are many miRNAs such as miR-26b, miR-106, miR-21, miR-128, miR-125b, miR-124a, miR-132, miR-9, miR-29a, miR-338-3p, miR-27b, miR-151, miR-219, miR-145, miR-16, miR-30a, miR-320 and the miR-34 subfamily that are indicative of neurodegeneration in general, evident from the expression changes in case of multiple neurodegenerative disorders (Table 1). A comprehensive list of miRNA expression profile changes in brains of patients with PD, AD, HD, MSA (multiple system atrophy) and ALS are shown in Table 1.

Trinucleotide repeat disorders (TRD) are characterized by trinucleotide repeat expansions either within or outside the coding region of a gene [78]. TRDs with repeats within a gene's coding region include Huntington's disease (HD), Kennedy disease, Haw-River syndrome and five types of spinocerebellar ataxia (SCA), whilst TRDs with repeats outside a gene's coding region includes Fragile X syndrome, Fragile XE syndrome, Friedreich's ataxia, myotonic dystrophy, spinocerebellar ataxia type 8 and spinocerebellar ataxia type 12 [79]. Several mRNAs have been shown to have altered expression in neurodegenerative TRDs. For example, miR-144 is upregulated in the cortex of SCA type 1 patients [55]. Furthermore, miR-34b is upregulated and miR-25, miR-29a and miR-125b are downregulated in SCA type 3 patients [80]. In addition, miR-25 and miR-125b are associated with progression of the disease [80]. HD is one of the most studied TRDs and it has been shown that miR-100, miR-139-3p, miR-196a, miR-133a and miR-330 are indicative of the disease [81–83].

In addition to the above-mentioned types of neurodegenerative diseases, several studies have also linked miRNAs to prion diseases, a transmissible and invariably fatal class of neurodegenerative disease including Kuru and Creutzfeldt–Jakob disease (CJD) in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cattle [84]. In 2008, Saba and colleagues reported aberrant expression of 15 miRNAs in prion disease-affected mouse brains (Table 1) [75]. They further demonstrated that two downregulated miRNAs, miR-203 and miR-191, targets EGR1 that shares a functional relationship with CREB1, a protein downregulated in prion disease [75]. Montag and colleagues further reported on a number of miRNAs that showed differentially expressed in BSE-infected macaque brain samples (Table 1). In addition, they also highlighted the involvement of miR-342-3p in different prion

Table 1 microRNA expression profile changes in neurodegeneration

Disease	Source	Differentially expressed microRNAs	Method	References
Parkinson's disease	Blood	miR-126-3p, miR-126-5p, miR-147, miR151-5p, miR-151-3p, miR-199a-3p, miR-199a-5p, miR-19b, miR-26a, miR-28-5p, miR-29b, miR29c, miR-301a, miR-30b, miR-30c, miR-335, miR-374a, miR-374b	Microarray, ChIP-seq	[97]
	Peripheral blood	miR-1, miR-16-2-3p, miR-22-5p, mir-26a-2-3p, miR-29a, miR-30a	qRT-PCR	[55]
	Plasma	miR-1826, miR-450b-3p, miR-626, miR-505	Microarray, qRT-PCR	[98]
	Plasma	miR-181c, miR-331-5p, miR-193a-p, miR-196b, miR-454, miR-125a-3p, miR-137	TaqMan low-density arrays, TaqMan assay	[144]
	Serum	miR-339-5p, miR-223-5p, miR-324-3p, miR-24, miR-30c, miR-148b	TaqMan low-density arrays, TaqMan assay	[57]
	Serum	miR-338-3p, miR-30e-3p, miR-30a-3p, miR-16-2-3p, miR-1294	TruSeq small RNA sequencing,	[74]
	White blood cells	miR-320a/b/c, miR-769, miR-92b, miR-16, miR-199b, miR-1274b, miR-21, miR-150, miR-671, miR-1249, miR-20a, miR-18b*, miR-378c, miR-4293	Small RNA sequencing (ABI SOLiD)	[136]
	CSF	miR-132-5p, miR-19a-3p, miR-19b-3p, miR-127-3p, miR-409-3p, miR-370, miR431-3p, miR-873-3p, miR-136-3p, miR-10a-5p, miR-1224-5, miR-4448, let-7 g-3p, miR-128, miR-433, miR-485-5p, miR-212-3p	TruSeq small RNA sequencing,	[74]
	Brain	miR-34b, miR-34c	Microarray, qRT-PCR	[108]
	Brain	miR-133b	qRT-PCR, northern blot analysis, luciferase assay	[42]
	Substantia nigra pars compacta	miR-198, miR-135b, miR-485-5p, miR-548d	TaqMan low-density arrays, TaqMan assay	[71]
	Substantia nigra pars compacta	miR-26b, miR-106a*, miR-301b, miR-21, miR-224, miR-373*	qRT-PCR	[70]
	Amygdala	miR-224, miR-373*	qRT-PCR	[70]
Alzheimer's disease	Blood	miR-34a, miR-181b	Microarray, qRT-PCR	[99]
	Blood	miR-137, miR-181c, miR-9, miR-29a, miR29b,	qRT-PCR	[56]
	Peripheral blood	miR-112, miR-161, let-7d-3p, miR-5010-3p, miR-26a-5p, miR-1285-5p, miR-151a-3p, miR-103a-3p, miR-107, miR-532-5p, miR-26b-5p, let-7f-5p	Next generation sequencing, qRT-PCR	[100]
	Plasma	let-7d-5p, let-7 g-5p, miR-15b-5p, miR-142-3p, miR-191-5p, miR-301a-3p, miR-545-3p	Nanostring, qRT-PCR	[101]
	Serum	miR-137, miR-181c, miR-9, miR-29a, miR29b	qRT-PCR	[56]
	Serum	miR-125a-3p, miR-125b-1-3p, miR-127-3p, miR-1285, miR-135a-5p, miR-30c-2-3p, miR- 21-5p, miR-219-2-3p, miR-34c-5p, miR-34b-3p, miR-34b-5p, miR-22-5p, miR-375, miR-873, miR-1307-5p, miR-887, miR-182-5p, miR-184, miR-671, miR-3176	TruSeq small RNA sequencing,	[74]
	CSF	miR-105, miR-10a, miR-10b, miR-143, miR-142-5p, miR-146b, miR-151, miR-125a, miR-126*, miR-126, miR-127, miR-135a, miR-138, miR141, miR-181a, miR-181c, miR-15b, miR-154, miR-186, miR-191, miR-194, miR-195, miR-197, miR-199a*, miR-204, miR-205, miR-214, miR-216, miR-221, miR-302b, miR-30a-3p, miR-30a-5p, miR-30b/c/d, miR-32, miR-99a, miR-501, miR-517a/b, miR-518b/f, miR-520a*, miR-526a, miR-338, miR345, miR-362, miR-371, miR-374, miR-375, miR-380-3p, miR-422b, miR-429, miR-448, miR449, miR-451, miR-455, miR-494, miR-497, miR-7f	qRT-PCR	[72]
	CSF	miR-9, miR-125b, miR-146a, miR-155	Microarray	[145]

Table 1 continued

Disease	Source	Differentially expressed microRNAs	Method	References
	CSF	miR-10a-5p, miR-33b-5pmiR-101-5p, miR-124-3p, miR-127-3p, miR-127-5p, miR-132-3p, miR-129-5p, miR-134, miR-136-3p, miR-136-5p, miR-138-5p, miR-139-5p, miR-181a-5p, miR-181a-3p, miR-181b-5p, miR-181d, miR-184, miR-218-5p, miR-323a-3p, miR-326, miR-329, miR-377-5p, miR-381, miR-410, miR-431-3p, miR-433, miR-488-3p, miR-495, miR-708-5p, miR- 769-5p, miR-874, miR-9-3p, miR-9-5p, miR-95, miR-598, miR-760, miR-708-3p, miR-873-5p, miR-3200-3p	TruSeq small RNA sequencing,	[74]
	Brain	miR-125b, miR-106b, miR-107, miR-124, miR-132, miR-145, miR-146b, miR-148a, miR-17-5p	qRT-PCR	[72]
	Cortex	miR-137, miR-181c, miR-9, miR-29a, miR29b	qRT-PCR	[61]
	Cortex	miR-212, miR-424, miR-29a, miR-29b-1, miR-107, miR-15a	LNA-microarrays, northern blot analysis	[60]
	Cortex	miR-210, miR-320, miR-29a, miR-29b-1, miR-106b, miR-15a, miR-181c, miR-9, miR-22, miR-101, miR-197, miR-511, miR-19b, miR-26b, miR-363, miR-93, let-7i	Microarry, qRT-PCR, northern blot analysis	[58]
	Cortex	miR-29a, miR29b, miR-338-3p	Microarray, qRT-PCR	[146]
	Cortex	miR-101, miR-106b, miR-107, miR-125b, miR-137, miR-142-3p, miR-142-5p, miR-145, miR-151-5p, miR-15a, miR-181c, miR-184, miR-185, miR-194, miR-197, miR-19b, miR-210, miR-212, miR-214, miR-219-2-3p, miR-22, miR-223, miR-26b, miR-27b, miR-298, miR-29a, miR-29a/b-1, miR-29b-1, miR-300, miR-301a, miR-320, miR-326, miR-330-5p, miR-338-3p, miR-338-5p, miR-361-3p, miR-363, miR-382, miR-		
	Hippocampus	miR-9, miR-124a, miR-125b, miR-128	Microarray	[73]
Huntington's disease	Plasma	miR-34b		[147]
	Brain	miR-16, miR-100, miR-151-3p, miR-219-2-3p, miR-27b, miR-451, miR-92a, miR-128, miR-139-3p, miR-222, miR-382, miR-433, miR-485-3p	RNAseq, microarrays, qRT-PCR	[81]
	Cortex	miR-9, miR-9*, miR-29b, miR-124a, miR-132, miR-196a, miR-486	Microarray, qRT-PCR	[83]
	Cortex	miR-1-1, miR-124a, miR-29a, miR-132, miR-133a, miR-203, miR-204, miR-21, miR-330	qRT-PCR	[82]
Multiple system atrophy	Serum	miR-339-5p, miR-223*, miR-324-3p, miR-24, miR-29c, miR-148b, miR-483-5p, miR-652, miR-744, miR-1274A, miR-1274B, miR-1291	TaqMan low-density arrays, TaqMan assay	[57]
	Cerebellum	miR-202, miR-129-3p, miR-129-5p, miR-337-3p, miR-380, miR-433, miR-132, miR-410, miR-206, miR-409-5p, miR-199a-5p	Microarray	[148]
Amyotrophic lateral sclerosis	Blood	miR-338-3p, miR-451, miR-1275, miR-328, miR-638, miR-149, miR-665, miR-583	Microarray, qRT-PCR	[102]
	$CD14^{+}CD16^{-}$ monocytes	miR-27a, miR-155, miR-146a, miR-32-3p	TaqMan low-density arrays, TaqMan assay	[103]
	Spinal cord	miR-146*, miR524-5p, miR-582-3p	qRT-PCR	[149]
	Spinal cord	miR-24-2*, miR-142-3p, miR-142-5p, miR-1461, miR-146b, miR-155	Microarray, qRT-PCR, Luciferase assay	[136]

Table 1 continued

Disease	Source	Differentially expressed microRNAs	Method	References
Prion diseases	Brain	miR-342-3p, miR-320, let-7b, miR-328, miR-191, let-7d, miR-370, miR-128, miR-139-5p, miR-146a, miR-339-5p, miR-203, miR-181a-1*, miR-338-3p, miR-337-3p, miR-200a, miR-200b, miR-26a, miR-186, miR-331-3p, miR-152, miR-221	Microarray, qRT-PCR	[75]
	Brain	miR-26a, miR-30a-5p, miR-30d, miR-103, miR-106b, miR-107, miR-124a, miR-125a, miR-128a, miR-132, miR-143, miR-145, miR-181a, miR-191, miR-195, miR-219, miR-320, miR-342-3p, miR-361, miR-490, miR-494	TaqMan low-density arrays, TaqMan assay	[76]
	Exosomes	miR-126-3p, miR-134, miR-146a, miR-182, miR-186, miR-188-5p, miR-193b, miR-222, miR-296-3p, miR-29b, miR-380-5p, miR-424	qRT-PCR (TLDA cards)	[77]
	Exosomes	Let-7b, let-7i, miR-103, miR-125a-5p, miR-125b, miR-130a, miR-130b, miR-16, miR-21, miR-23a, miR-23b, miR-24, miR-296-6p, miR-29a, miR-29b, miR-29c, miR-301a, miR-30b, miR-30c, miR-342-3p, miR-344-4p, miR-378, miR-93	RNA sequencing	[77]

disorders, suggesting a potential biomarker in prion diseases in animals and humans [76]. In 2012, deep sequencing revealed that an array of miRNAs exist in exosomes released from prion-infected neuronal cells, which initiated the notion that miRNA exosome signatures may represent a novel avenue in terms of new diagnostic intervention in prion disorders [77].

Combined, these studies demonstrate the aberrant nature of miRNA profiles within different classes of neurodegenerative diseases suggesting that miRNAs hold promise as not only diagnostic tools but as vehicles to further our understanding of neurodegenerative disease mechanisms.

miRNAs in autophagy

The misfolding and aggregation of proteins, as well as inappropriate functioning of organelles, represent hallmarks of neurodegeneration [85]. Although the proteasome is responsible for protein clearance, the proteasome is often dysfunctional in neurodegeneration, often due to the vast amount of aggregated proteins [85]. Because of this, the autophagy plays a pivotal role in terms of clearing dysfunctional proteins and organelles in neurodegeneration [86].

Autophagy has clearly been linked to PD and several key PD proteins, such as LRRK2, Pink1, and Parkin [87]. Autophagy does not directly degrade miRNAs, but influences miRNA processing proteins thereby preventing miRNA mediated repression of appropriate target genes. Indeed, Gibbings and colleagues have found the autophagy selectively degrading Dicer1 and Ago2 ultimately regulating miRNA biogenesis [88].

miR-106a and miR-224, which are both upregulated in the substantia nigra pars compacta and amygdala of PD brains, cause a dose-dependent decrease in heat shock 70 kDa protein (hsc70) and lysosome-associated membrane protein 2 (LAMP-2A), respectively [70]. Both hsc70 and LAMP-2A are mediators of autophagy, namely chaperone-mediated autophagy (CMA) [89]. Interestingly, miR-34 has been shown to affect lifespan by regulating autophagy [90]. Further evidence supporting the regulation of autophagy by miRNAs comes from studies showing that the integral autophagy-promoting protein Beclin 1 (BECN1) is directly regulated by miR-30a, and that miR-206 regulates HDAC4 which is associated with autophagy [91, 92]. Noteworthy, is also the fact that reduced expression of miR-9, which is reduced in brains of HD and AD patients, causes deregulation of histone acetylation, ultimately resulting in autophagy-dependent cell toxicity [58, 82, 93].

Autophagy represents an integral part of neurodegeneration and although autophagy does not directly control miRNA turnover, it regulates the protein targets of miRNAs. Indeed, several studies suggest that miRNAs may play an important role in autophagy as they can regulate proteins promoting or blocking autophagy.

miRNAs as biomarkers of neurodegeneration

Neurodegenerative diseases are often highly heterogeneous in nature and, although this necessitates the need for accurate diagnostic and prognostic biomarkers, the heterogeneity in itself has hampered the successful development of robust markers for disease onset and progression. However, the finding that miRNAs are present in biofluids as stable molecules has led to recent studies suggesting that miRNA may represent candidates as biomarkers for neurodegenerative disease [8].

Biofluids as a source of miRNAs

miRNAs have been detected in many clinically relevant body fluids including cerebrospinal fluid (CSF), serum/plasma, saliva, and urine [94]. Of these, CSF clearly

represents the most relevant biofluid for biomarker discovery in neurodegenerative disorders because of its proximity to the brain. However, the use of CSF has some disadvantages related to risks associated with CSF collection, as the procedure can be painful which may limit patient participation in cohort studies. Serum/plasma represents a more convenient source for biomarker discovery as sample collection is simple, and moreover, it is thought that miRNAs are released by apoptotic cells, possibly mirroring miRNA profiles as neurons degenerate in the brain [6]. In addition, several studies have found that extracellular serum/plasma miRNAs are exceptionally stable [95].

Circulating biomarkers in neurodegeneration

Because of the heterogeneous nature of neurodegenerative diseases, misdiagnosis is rather common. Moreover, the characteristic motor symptoms of PD normally do not become clinically overt before more than 60–80 % of the dopaminergic neurons are already irreversibly lost [96]. Because of this, accurate and sensitive diagnostic tools need to be developed, not only to better diagnose neurodegenerative diseases at early stages of progression, but also to help pave the way for future therapeutic intervention strategy design.

As described earlier, changes in miRNA profiles in brain tissue from individuals with neurodegenerative disease has been documented. Recently, there has also been a significant increase in studies showing that miRNA profiles change in biofluids of individuals with established neurodegenerative diseases (Table 1). In terms of PD, potential diagnostic miRNA biomarkers include, but are not limited to, miR-125-3p, miR-16, miR-19a/b, let-7, and miR-29/miR-30 family members. These miRNAs were detected in varied biofluids ranging from whole blood, plasma, serum, and CSF using a variety of technologies including microarray, qRT-PCR, and RNA-Seq techniques (Table 1) [55, 57, 73, 97, 98]. Similarly, many differentially expressed miRNAs have been identified from biofluids of AD patients including, but not limited, to miR-34a/b, miR-30b/c/d, miR-9, miR-29a/b, miR-125a/b and let-7 family members (Table 1) [72, 73, 99–101]. Although miRNA biomarker discovery studies in biofluids is not as developed for MSA (atypical parkinsonian disorder) and ALS, potential MSA biomarkers include miR-223*, miR-324-3p, miR-24, miR-29c, miR-148b, miR-miR-1274a/b (Table 1) [57], and for ALS patients miR-338-3p, miR-451, miR-328, miR-149, miR-27a, miR-155, miR-146a, and miR-32-3p (Table 1) [102, 103].

The emerging view that circulating miRNAs have the potential to represent robust biomarkers has not only opened up new horizons in terms of disease diagnosis and prognosis, but also fueled interest into exploring miRNA targets as potential therapeutic interventions.

miRNA-mediated protein deregulation in neurodegeneration

Many miRNAs have been shown to regulate key proteins involved in neurodegenerative disease (Table 2). For example, miR-7 and miR-153 inhibits SNCA expression at both the transcriptional and translational levels in a synergistic manner (Fig. 2) [104, 105]. Another key PD protein, LRRK2, is regulated in a reverse manner by miR-205, and pathogenic mutations in LRRK2 disrupts let-7 and miR-184* expression causing deregulation of E2F1/DP implicated in cell cycle and survival control (Fig. 2) [106, 107]. Minones-Moyano and colleagues have further demonstrated the miR-34b/c regulates DJ-1/PARK7 and Parkin in SHSY-5Y neuroblastoma cells and in brains of PD patients (Fig. 2) [108].

Tauopathies, such as AD, have many key proteins regulated by miRNAs. Strikingly, Tau aggregation in AD is directly regulated by miR-34a [109]. The amyloid precursor protein (APP) is also regulated by numerous miRNAs, including miR-101, miR-16, miR-106a and miR-644 (Table 2; Fig. 2) [110–113]. Further, the amyloid protein fragment Ab, is also regulated by miR-24, miR-186, miR-455, miR-146a, and miR-98 (Fig. 2) [113–115]. It has also been speculated that miR-137, miR-146a, and miR-181c may regulate the levels of Ab in AD patients [61, 113–115]. Interestingly, the APP-processing enzyme b-secretase enzyme I (BACE1) is also regulated by miR-107, miR-29a-1/b-1, miR-9 and miR-124 (Fig. 2) [49, 116, 117].

Further, several proteins known to be involved in HD and ALS, are regulated by miRNAs (Table 2). miR-196a overexpression has been shown to alleviate HD phenotype in both in vitro and in vivo by indirectly targeting mutant huntingtin (htt), an integral protein in HD etiology (Fig. 2) [118]. The miR-9 family of microRNAs, which are differentially expressed in the cortex of patients suffering from Huntington's disease, regulate the expression of the transcription repressor-co-repressor REST-coREST (RE1-silencing transcription factor) which interacts with htt [81]. In addition, miR-9, miR-9* regulates neurofilament expression by positively regulating neurofilament heavy subunit (NEFH) in mouse models of ALS (Fig. 2) [43].

Table 2 Regulation of key proteins in neurodegeneration by microRNA

Disease	microRNA	Gene regulated	Experimental design	References
Parkinson's disease	miR-7	SNCA	Regulation in cells, luciferase reporter assay	[105]
	miR-153	SNCA	Mouse model, regulation in cells	[104]
	miR-34b, miR-34c	SNCA	Regulation in cells, 3′UTR binding/ inhibition	[150]
	Let-7, miR-184*	Lrrk2, E2F1, DP		[106]
	miR-34b/c	DJ-1, Parkin	SHSY-5Y cells, human brains	[108]
	miR-433	FGF20	Patients, regulation in cells	[151]
	miR-26b, miR-106a*, miR-301b	Hsc-70	Patients, regulation in cells, luciferase reporter assay	[70]
	miR-21, miR-224, miR-373*, miR-379	LAMP-2A	Patients, regulation in cells, luciferase reporter assay	[70]
	miR-133b	Pitx3	Patients, regulation in cells	[42]
	miR-64/65	mdl-1, ptc-1	Mouse model, *C. elegans*	[152]
Alzheimer's disease	miR-34a	TAU	Patients, in vitro reporter assay	[109]
	miR-101	APP	In vitro reporter assay, regulation cells	[110]
	miR-16	APP	Mouse model	[111]
	miR-106a, miR-520c	APP	In-vitro reporter assay, overexpression in cells	[112]
	miR-147, miR-655, miR-323-3p, miR-644, miR-153	APP	Regulation in cells, luciferase reporter assay, ELISA	[113]
	miR-24, miR-186, miR-455	Ab	Regulation in cells, luciferase reporter assay, ELISA	[153]
	miR-137	Ab	Patients, in vitro reporter assay	[61]
	miR-146a	Ab	Patients, regulation in cells, mouse model	[127]
	miR-181c	Ab	Patients, in vitro reporter assay	[61]
	miR-98	Ab	Regulation in cells	[115]
	miR-107	BACE1	Patients, in situ hybridization	[116]
	miR-29a-1, miR-29b-1, miR-9	BACE1	Patients, in vitro reporter assay	[58]
	miR-124	BACE1	Overexpression in cells	[117]
	miR-181c, miR-137	SPTLC1	Patients, in vitro reporter assay	[61]
	miR-29a, miR-29b1, miR-9	SPTLC2	Patients, in vitro reporter assay	[61]
	miR-107	Cofilin	Mouse model, In-vitro reporter assay	[154]
	miR-106b	TGF-b	Mouse model	[116]
	miR-98	IGF1	Mouse model, regulation in cells	[115]
	miR-17, miR-20a, miR-106a, miR-106b	APP	Patients, in vitro reporter assay	[155]
Huntington's disease	miR-9, miR-9*	REST-coREST	In-vitro reporter assay	[81]
	miR-196a	mut-Htt	Mouse model, Overexpression in Stem cells	[118]
Amyotrophic lateral sclerosis	miR-9, miR-9*	Neurofilament expression	Mouse model	[43]
	miR-659	Progranulin	Polymorphism	[119]
	miR-206	HDAC4	Mouse model	[92]
	miR-9	NEFH	Mouse model	[43]

miR-659 can regulate progranulin (GRN), by binding more efficiently to the T-allele or rs5848 variant, which in turn leads to a 3.2 fold higher risk of developing frontotemporal dementia (FTLD-U) [119].

miRNAs can clearly regulate both the activation and inhibition of translation of genes associated with neurodegeneration, and Table 2 summarizes these miRNAs and their protein targets.

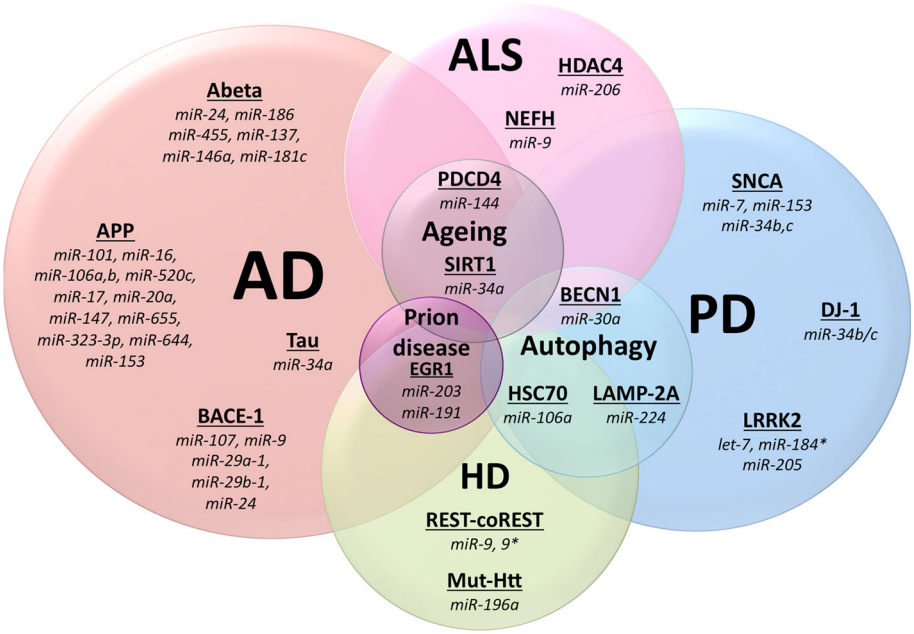

Fig. 2 Schematic diagram highlighting microRNAs that have been identified to show differential expression in various neurodegenerative diseases. The miRNAs shown have been identified using a variety of different techniques, as described throughout the text. Although several miRNAs are specific to a given class of neurodegenerative disease many also overlap suggesting their involvement in more common pathways, such as neuronal cell death. As both aging and autophagy are associated with neurodegeneration there are also shared miRNAs between these two processes and the four main neurodegenerative disease states

Technological advancements in search for miRNA biomarkers in neurodegenerative diseases

miRNA microarray coupled to quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) have been extensively used for miRNA biomarker discovery. SYBR Green-based qRT-PCR has been most widely used, representing a fluorescent dye-based technology that detects and enables quantification of double-stranded DNA amplification [120]. Recently, TaqMan-based qRT-PCR applications have become more common practice, which are based on the use of fluorescent probes, specific to target genes, allowing increased specificity [121, 122]. Several commercial companies have marketed TaqMan probe-based qRT-PCR assays, which offer robustness and flexibility in terms of miRNA analysis. For example, TaqMan low density array (TLDA) cards are arrays pre-filled with TaqMan assays for qRT-PCR analysis, which can be ordered as customized cards, thereby providing flexibility to choose disease-specific miRNAs.

To date, only a small number of potential neurodegenerative disease-specific miRNA biomarkers have been identified using microarray and qRT-PCR (Table 1). However, as more are discovered, the future development of neurodegenerative diseases-specific microarray or TLDA arrays will enhance the diagnostic capability and accuracy for neurodegenerative diseases.

As mentioned earlier, the discovery of circulating miRNAs has presented an opportunity to mine for biomarkers in neurodegenerative diseases. With respect to this, Soreq and colleagues used Next Generation Sequencing (NGS) to provide a comprehensive list of 254 miRNAs in PD patients before and after deep brain stimulation treatment [123]. Similarly, Kumar and colleagues reported 140 unique mature miRNAs from blood samples of AD patients using NGS [101]. In addition, Burgos and colleagues combined NGS with CSF and serum samples from AD and PD patients investigating extracellular miRNAs which could possibly be used as prognostic markers for disease [73].

The application of NGS, microarrays and qRT-PCR has led to the discovery of hundreds of miRNAs associated with neurodegeneration (Table 1); however, target validation is still lacking.

Therapeutic intervention strategies involving miRNAs in neurodegeneration

Although we are far from understanding the complete mechanistic picture of neuronal cell death, studies on animal models, cell models, biofluids, and post-mortem brain tissue have provided insight into perturbations of miRNA expression profiles associated with neurodegenerative diseases. Combined with genetic and biochemical studies, progress has been made in developing miRNA-based therapeutic intervention strategies.

miRNA mimics

In 2007, Wang and colleagues developed double-stranded small RNA molecules that would mimic miRNAs and serve as gain-of-function tools for specific miRNAs [124]. These miRNA mimics specifically target mRNAs through

miRNA-like actions in mammalian cells. Their 5′ end possesses a motif that is partially complementary to the 3′UTR of a specific target gene. Once inside the cells, these double-stranded miRNA-like RNA fragments inhibit the translation of the specific target genes, thereby producing a gene-specific effect. This technology has been further developed by the industry sector and a battery of miRNA mimics is available for the majority of human miRNAs, including miRNAs in neurodegeneration. For example, miR-153 and miR-205 target α-synuclein and LRRK2, respectively (Table 2) [105, 107], and it is, therefore, possible to use miR-153 and miR-205 mimics to unravel protein targets associated with PD, allowing the discovery of new therapeutic targets.

miRNA anti-miRs and antagomirs

Similar to miRNA mimics, blocking a miRNA also shows potential in terms of disease intervention. Initial studies focused on anti-miRs or antisense oligonucleotides that block endogenous miRNA. The antisense oligonucleotide would bind to the mature guide strand of the miRNA and induce degradation or stoichiometric duplex formation [125]. Hutvagner and colleagues then showed that a modification on the antisense oligonucleotide, which gave rise to the now known antagomiR, contributed to nuclease resistance and increased binding affinities to miRNA targets [126]. For example, in Locked Nucleic Acid (LNA) antimiRs, the ribose rings are 'locked' by a methylene bridge connecting the 2′-O atom and the 4′-C atom. This locking mechanism not only increases its stability, but also increases the hybridization efficiency to the target single stranded RNA molecule [127]. This technology has been adopted by the industry sector that has developed a library of custom oligos mimicking human miRNAs. Using this technology, Koval and colleagues inhibited miR-155, which is elevated twofold in the spinal cord of ALS patients, in ALS-model mice through delivery of a miR-155 anti-miR into the central nervous system. This inhibition caused global repression of targets in peritoneal macrophages and increased the survival rate [128].

miRNA sponges

miRNA sponges, first recognized by Ebert and colleagues [129], are able to inhibit multiple miRNAs simultaneously. For example, miRNA sponges with a heptameric seed sequence are able to target an entire miRNA family sharing the same seed sequence [130], thereby enabling widespread miRNA repression. Similar to anti-miRs or antagomiRs, these sponges can also be used to target multiple mRNAs [10]. Recently, Tan and colleagues used a miR-277 sponge to block rCGG repeat-mediated neurodegeneration in Fragile X-associated tremor/ataxia syndrome (FXATS), a late-onset neurodegenerative disorder [131].

miRNA masks

Another decoy-based mechanism for controlling miRNA regulation is through the use of miR-Masks. miR-Masks are single stranded 2′-*O*-methyl-modified antisense oligonucleotide that bind to the 3′UTR of a miRNA's target mRNA, thereby masking the miRNA binding site and derepressing the target gene. This approach, has to date, not been used in neurodegeneration. However, the masking of the 3′UTR region of mRNAs that prevent neurodegeneration represents exciting prospects [132].

Small drug molecule inhibitors

A study in 2008 by Gumireddy and colleagues identified small drug molecules, which indirectly altered the onco-miR miR-21 at the transcription level [133]. Although this study was related to oncogenesis, their observations may have implications in pre-clinical neurodegeneration studies by screening compounds for a target miRNA, which is involved in neuronal cell death. For example, studies have shown that L-dopa treatment results in altered miRNA profiles in PD patients [134] suggesting that perhaps small molecules may alter miRNA profiles in neurodegeneration that ultimately modifies the disease course.

Challenges of miRNA treatment

The stability and the immunogenicity of miRNAs has always been a concern when it comes to miRNA-based therapies. The overall benefits and limitations of different miRNA-based therapies have been elegantly reviewed by Garzon and colleagues where they clearly underline the need for increased understanding of miRNA biology in general [135]. Neurodegeneration is a process that develops over several years, often decades, and hence miRNA stability becomes the biggest concern in this context. However, the high turnover rates of miRNAs and their higher half lives as compared to mRNAs may provide ample time to track onset and progression of neurodegeneration, thereby paving the way for the development of potential biomarkers and therapeutic interventions.

Another potential obstacle in developing molecules to treat neurodegeneration is the inherent capability of molecules to cross the blood brain barrier. However, a couple of recent studies in ALS have shown that oligonucleotides and miRNAs can cross the blood brain barrier and also cross into the central nervous system by using intrathecal infusion and a ventricular osmotic pump, respectively [128, 136].

miRNAs very often target and regulate the expression of several mRNAs which may pose potential challenges in terms of off target effects in the context of miRNA-based therapies. This is probably the reason why only a handful of miRNAs have reached clinical trials. Complicating matters further for neurodegeneration-driven applications is the highly complex nature of neurodegenerative diseases in terms of progression rates, in addition to the age and sex dependency.

Despite the current limitations of miRNAs as therapeutic agents, recent advances in the field have paved the way for the future design of novel intervention strategies.

Technological advancements in miRNA target discovery in neurodegenerative diseases

Microarrays have been extensively used for expression analysis. However, the emergence of NGS has enabled the use of RNA-seq as an alternative to microarrays for the identification of miRNA targets [137]. Despite its extensive application to identify neurodegenerative disease-specific miRNAs, the approach has limitations as it will identify miRNA targets irrespective of a direct or indirect association with any given miRNA. Therefore, other alternative techniques, which can differentiate between direct and indirect targets of miRNAs, have been developed. For example, immunoprecipitation of RISC components followed by a microarray analysis provides direct miRNA:mRNA target pair identification [138]. Furthermore, high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP), has provided promising data on miRNA binding sites and generated genome-wide interacting maps for endogenous and exogenously expressed miRNAs [139]. A similar advancement called photoactivable-ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP), with a better RNA recovery rate than HITS-CLIP, utilizes photoactivable nucleosides to isolate proteins associated with RNA [140]. Recently, yet another immunoprecipitation-based technique was developed, known as ChIP-Seq, which has the ability to analyze the transcriptional regulation of miRNAs [141].

In addition to the above techniques, comparative two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), followed by mass spectrometry (MS), or liquid chromatography MS (LC/MS) can be employed to identify miRNA:mRNA target pair combinations in cells or tissues where specific miRNAs are artificially overexpressed or inhibited [142, 143].

The combination of the abovementioned techniques will allow further deciphering of miRNA:mRNA associations as well as perturbations in the microRNAome and proteome in neurodegeneration.

Future prospects

miRNAs hold promise to be the next generation biomarkers and therapeutic candidates in neurodegenerative diseases. Cell-based, as well as clinical sample-based, studies involving miRNA have shed much light on understanding the etiology of neurodegenerative diseases. Although no candidate miRNA are currently undergoing clinical trials, as is the case for cancer, recent advancements suggest that we are not far from reaching this milestone. Clearly the technological advancements in modifying the structural properties of miRNAs, in terms of bioavailability and stability, have paved way for a new dimension in miRNA research. Likewise, the emergence of larger, well-defined patient cohorts, coupled to the availability and stability of miRNAs in biofluids, has opened up the possibility of discovering robust diagnostic and prognostic miRNA biomarkers for neurodegenerative disease. Although the tissue specificity and off-target effects still remain a concern, conjugating neural stem cell research with miRNA biology might provide a better platform to develop potential therapeutic candidates. Our understanding of the etiology of neurodegeneration is still very much in its infancy, but miRNAs, their biology and potential applications, hold tremendous promise for individuals suffering from this class of devastating diseases.

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