MicroRNA in central nervous system trauma and degenerative disorders

Nai-Kui Liu and Xiao-Ming Xu

Spinal Cord and Brain Injury Research Group, Stark Neurosciences Research Institute and Department of Neurological Surgery, Indiana University School of Medicine, Indianapolis, Indiana

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MicroRNAs (miRNAs) are a novel class of small noncoding RNAs that negatively regulate gene expression at the posttranscriptional level by binding to the 3′-untranslated region (UTR) of target mRNAs leading to their translational inhibition or sometimes degradation. miRNAs are predicted to control the activity of at least 20–30% of human protein-coding genes. Recent studies have demonstrated that miRNAs are highly expressed in the central nervous system (CNS) including the brain and spinal cord. Although we are currently in the initial stages of understanding how this novel class of gene regulators is involved in neurological biological functions, a growing body of exciting evidence suggests that miRNAs are important regulators of diverse biological processes such as cell differentiation, growth, proliferation, and apoptosis. Moreover, miRNAs are key modulators of both CNS development and plasticity. Some miRNAs have been implicated in several neurological disorders such as traumatic CNS injuries and neurodegenerative diseases. Recently, several studies suggested the possibility of miRNA involvement in neurodegeneration. Identifying the roles of miRNAs and their target genes and signaling pathways in neurological disorders will be critical for future research. miRNAs may represent a new layer of regulators for neurobiology and a novel class of therapeutic targets for neurological diseases.

microRNAs (miRNAs) are a novel class of small noncoding RNAs that negatively regulate gene expression at the posttranscriptional level by binding to the 3′-untranslated region (UTR) of target mRNAs leading to their translational inhibition or degradation of the target. Recent evidence suggests that expression of at least 20–30% of human protein-coding genes is modulated by miRNAs (48). A number of miRNAs were found in the mammalian central nervous system (CNS), including the brain and spinal cord, where they play key roles in neurodevelopment and are likely to be important mediators of plasticity (1, 46, 48, 73). Some miRNAs have been implicated in several neurological diseases (46). Recently, several studies suggested the possibility of miRNA involvement in neurodegeneration. Although studies on the role of miRNAs in neurobiology have just begun to emerge, their advance is rapid in this field. This review summarizes the research progress regarding miRNAs in the CNS with an emphasis placed on the possible roles of miRNAs in CNS disorders particularly in traumatic injuries and neurodegenerative diseases. We also discuss the clinical and therapeutic applications of modulating miRNAs as a novel class of therapeutic targets for neurological disorders.

miRNA HISTORY

The first miRNA, lin-4, was identified by two research groups in 1993 (58, 99). Lin-4 is a small 21-nucleotide RNA molecule that controls developmental timing in Caenorhabditis elegans by the posttranscriptional regulation of the heterochronic gene lin-14 (58, 99). Lin-4 did not code protein and contained antisense sequences complementary to a repeated sequence element in the 3′-UTR of the lin-14 mRNA (58, 99). Although the finding was published in high-profile journals, it wasn’t until the discovery of the second miRNA (let-7) in 2000 that this field began to attract lots of attention from scientists. In the following year, three papers published in the same issue of Science showed that an extensive number of small RNAs similar to lin-4 and let-7 were identified in invertebrates and vertebrates as well as in C. elegans (53, 55, 57). It was also the first time this abundant class of newly identified small RNAs were defined as microRNAs, abbreviated miRNA (55). The individual miRNAs and their genes were designated miR-# and mir-#, respectively (55). Since then, an increasing number of miRNAs have been identified in mammals. More than 800 miRNAs have been cloned and sequenced in humans. In the
past 10 years, there has been an explosive increase in the number of papers related to miRNA research, and miRNA-related research has become one of the hottest research fields in biology. Currently, 15,632 miRNAs have been identified in a variety of animals, plants, and viruses, and have been deposited in publicly available databases, such as miRBase (release 15 of the database, http://www.mirbase.org/).

miRNA BIOGENESIS

MiRNA is a single-stranded RNA of ~22 nucleotides that derives from hairpin precursor (pre-miRNA). It is a multi-step biological process to generate mature miRNAs from miRNA genes (Fig. 1) (5, 23, 24, 43, 46, 80). miRNA genes are generally transcribed by RNA polymerase II or III (Pol II or Pol III) in the nucleus to yield large primary transcripts (pri-miRNAs). The pri-miRNA is cleaved by the nuclear RNase III enzyme Drosha/DGCR8 to release approximately ~70 nucleotide pre-miRNA. The pre-miRNA is then translocated to the cytoplasm by exportin 5-RanGTP. Once in the cytoplasm, the pre-miRNA is subsequently processed into ~22-nucleotide miRNA duplexes by the cytoplasmic RNase III enzymes named Dicer and loquacious. Generally, the strand named the guide strand is selected to function as a mature miRNA, and the other strand, known as the passenger strand, is degraded. The guide strand is loaded into an RNA-induced silencing complex (RISC), a complex of proteins that includes the Argonaute proteins, where it binds to a target mRNA. Recently, it was found that some miRNAs are from another biogenesis pathway: the mirtron pathway (5, 80). Short introns with hairpin potential, termed mirtrons, can be spliced and debranched into pre-miRNAs and bypass Drosha cleavage. The pre-miRNA is then translocated to the cytoplasm by exportin 5/RanGTP and is subsequently processed by Dicer to generate miRNAs.

miRNA ACTION AND MECHANISMS

The primary action of miRNAs is to downregulate gene expression by binding to the 3' UTR of target mRNAs (4, 46). The 2–8 nt of the 5’ end of the miRNA, called the seed region, is crucial for the recognition of target mRNAs (4, 46). Therefore, most computational algorithms designed for target predictions use a miRNA seed region to search for complementarity in the 3’-UTR of mRNAs. Two common mechanisms have been described for miRNA mediated gene regulation: translational repression and mRNA degradation (4, 46). In most cases, it is dependent on the degree of complementarity between the miRNA and targeted mRNAs, and on other criteria that have yet to be defined. When a miRNA imperfectly pairs to its targeted mRNAs, translational repression is thought to be the primary mechanism for miRNA mediated gene regulation. In contrast, if a miRNA perfectly or near-perfectly pairs to the targeted mRNAs, it is thought that miRNAs regulate genes by target mRNA cleavage (4, 46). In animals, miRNAs downregulate gene expression mostly, but not always, through the translational repression (48). A recent study showed that decreasing miRNA levels by destabilization of mammalian target mRNAs is a major mechanism in miRNA gene repression (30). Since the requirement for target complementarity is only partial in animals, this means that one miRNA can potentially have hundreds of targets. In fact, several studies have shown that each miRNA can regulate hundreds or more mRNA targets (48, 60, 62) and each mRNA can be regulated by many miRNA (47, 48).

Fig. 1. MicroRNA (miRNA) biogenesis pathway. miRNA genes are transcribed by RNA Pol II or Pol III to generate pri-miRNAs whose hairpin structures are cleaved by Drosha/DGCR8 to release pre-miRNAs. Mirtrons are short hairpin introns and are spliced and debranched into pre-miRNAs. Pre-miRNAs are exported from the nucleus by Exportin-RanGTP into cytoplasm. Once in the cytoplasm, pre-miRNAs are further processed by Dicer/TRBP to form an ~22 nt duplex. One strand is then selected to function as a mature miRNA, and the other strand is degraded.
miRNA DISTRIBUTION IN THE MAMMALIAN CNS

A large number of miRNAs are found in the CNS including the brain and spinal cord (1, 46, 48, 49, 64, 73). Several miRNAs have been shown to be specifically expressed in the brain (86). Using microarray, real-time RT-PCR, and in situ hybridization, Bak et al. (1) found that 44 miRNAs displayed more than threefold enrichment in the CNS including the brain and spinal cord, suggesting that a large number of CNS-expressed miRNAs may be associated with specific functions within these regions. During development, many miRNAs show distinct expression patterns within the developing CNS, implying their importance in brain development and function (49, 73, 86, 90). Recently, we examined expression of 350 Rattus norvegicus miRNAs based on version 11.0 of the Sanger miRBase (Sanger Institute, Cambridge, UK; http://microrna.sanger.ac.uk/sequences) in the rat spinal cords by microarray analysis. The results showed that 269 of 350 miRNAs were detected in the adult rat spinal cord. More than half of 269 miRNAs were highly expressed in the spinal cord (64). miRNAs are also expressed in neurons (42, 86), astrocytes (89), and oligodendrocytes (56). The experimental data reveal that some miRNAs, such as miR-124 and miR-128, are preferentially expressed in neurons, whereas others, such as miR-23, are restricted to astrocytes; some miRNAs, such as miR-26 and miR-29, are more strongly expressed in astrocytes than in neurons (89).

miRNAs IN NEUROBIOLOGY

MiRNAs are predicted to control the activity of 20–30% of all protein coding genes. Increasing evidence shows that miRNAs play an important role in diverse neurobiological processes such as cell differentiation, growth, proliferation, apoptosis, and neural activity (27, 61, 91). For example, expression of miR-124 and miR-9 increases during differentiation of mouse embryonic stem (ES) cell-derived neural progenitors, and experimental overexpression or inhibition of miR-124 and miR-9 affects neural lineage differentiation in the ES cell-derived cultures (50). Other group reported that miR-7 and miR-214 are specifically expressed during neuroblastoma differentiation, cortical development, ES cell differentiation, and control neurite outgrowth in vitro (11). Recently, let-7b has been shown to regulate neural stem cell proliferation and differentiation by targeting the stem cell regulator TLX and the cell cycle regulator cyclin D1 (101). Overexpression of let-7b led to reduced neural stem cell proliferation and increased neural differentiation, whereas antisense knockdown of let-7b resulted in enhanced proliferation of neural stem cells (101).

miR-124 is one of the well-known miRNAs that are involved in neuronal differentiation. It has been shown to be expressed in neurons, but not astrocytes, and the levels of miR-124 increase over time in the developing nervous system (49, 73, 89). Inhibition of miR-124 with an antisense 2′-OMeRNA increases levels of nonneuronal transcripts in primary cortical neurons (15). Three recent studies show that miR-124 downregulates a subset of predicted mRNA targets in vivo (9, 69, 94). One of these studies showed that miR-124 had no effect on neuronal differentiation in the developing chick spinal cord (9). The other two studies revealed that miR-124 mediated neuronal differentiation (69, 94). This is achieved in part by targeting poly(pyrimidine tract binding protein 1 (PTBP1), a repressor of neuron-specific splicing (69), and small COOH-terminal domain phosphatase 1 (CTDSP1, also known as SCP1), a component of the repressor element 1-silencing transcription factor (REST) transcription repressor complex (94). More recently, Cheng et al. (12) found that knockdown of endogenous miR-124 maintained purified subventricular zone stem cells as dividing precursors, whereas ectopic expression led to precocious and increased neuron formation, and blocking miR-124 function during regeneration induced hyperplasias, followed by a delayed burst of neurogenesis. Furthermore, the SRY-box transcription factor Sox9 was identified as being a physiological target of miR-124 at the transition from the transit amplifying cell to the neuroblast stage (12).

miRNAs are also involved in the specification of glia. The phosphorylation of signal transducer and activator of transcription 3 (STAT3) can inhibit the differentiation of neural progenitor cells into neurons and promote differentiation into glia. Inhibition of miR-124a and miR-9 increased the level of phosphorylated STAT3, whereas overexpression of miR-124a and miR-9 decreased STAT3 expression, suggesting that these miRNAs could have a role in modulating glial cell fate determination by regulating the levels of STAT3 (50). The dynamic changes in the expression of 43 miRNAs were found during the transition from A2B5-positive oligodendrocyte precursor cells (OPCs) to premyelinating oligodendrocytes. Combined expression profiling of miRNA and mRNA revealed that miR-9 was downregulated during oligodendrocyte differentiation, which inversely correlated with the expression of its predicted targets, particularly peripheral myelin protein 22 (56). Recently, miRNAs have also been shown to be critical regulators of oligodendrocyte differentiation and myelination in the vertebrate CNS. For example, inhibition of miRNA maturation by selectively deleting the miRNA processing enzyme Dicer1 in oligodendrocyte lineage cells results in severe myelination deficits in the CNS (102). In addition, Dicer1 deletion induced proliferation of OPCs and inhibited the differentiation of OPCs into mature myelinating oligodendrocytes (102), suggesting that miRNAs normally inhibit OPC proliferation, while promoting their differentiation. Furthermore, miR-219 and miR-338 were identified as oligodendrocyte-specific miRNAs in the spinal cord. Overexpression of these miRNAs is sufficient to promote oligodendrocyte differentiation. Additionally, blockage of these miRNA activities in oligodendrocyte precursor culture and knockdown of miR-219 in zebrafish inhibit oligodendrocyte maturation (102). Finally, this study revealed that miR-219 and miR-338 control oligodendrogenesis by directly targeting negative regulators of oligodendrocyte differentiation such as transcription factors Sox6 and Hes5 (102). Another study also showed that Dicer1 and miR-219 were required for normal oligodendrocyte differentiation and myelination (19). For example, deletion of Dicer1 resulted in abnormal CNS myelination and miR-219 was showed to directly repress the expression of PDGFRA, Sox6, FoxJ3, and ZFP238 proteins, all of which normally help to promote OPC proliferation (19).

MiRNAs have also been shown to be involved in apoptosis. For example, some miRNAs (miR-15, miR-16, miR-29b, miR-127) can induce apoptosis by targeting BCL2 and certain oncoproteins (MCL1, CCND1, and WNT3A) (8, 14, 97). Some miRNAs (miR-21, miR-210, miR-155) are known inhibitors of apoptosis.
miR-21 can reduce apoptosis by targeting PTEN and PDCD4, while miR-210 and miR-155 can inhibit apoptosis by separately targeting caspase-8-associated protein-2 and p53 inducible nuclear protein-1 (40, 97). In addition, knockdown of Dicer has been shown to result in increased cortical apoptosis (16).

miRNAs IN THE CNS DEVELOPMENT

Many miRNAs have been shown to be enriched or specifically expressed in the CNS, and their expression is precisely regulated during brain development, implying their importance in brain development and function (49, 73, 86, 90). Dicer is one of the key enzymes in miRNA biogenesis (24, 43, 46). Several studies inactivating Dicer show that miRNAs are essential for brain morphogenesis in zebrafish (29) and mice (16). Recent studies show that specifically deleting Dicer expression in the CNS and in the cerebral cortex using two Cre lines results in reduced progenitor numbers, abnormal neuronal differentiation, and a thinner cortical wall (39). Incomplete Dicer deletion during early embryonic stages contributes to normal development of early-born neurons in the cortex and motor neurons in the spinal cord (39). However, at late embryonic stages when Dicer is completely ablated in the CNS, the migration of late-born neurons in the cortex and oligodendrocyte precursor expansion and differentiation in the spinal cord are greatly affected (39). Recent studies on the role of individual miRNAs in CNS development also showed that miRNA were involved in multiple steps of CNS development, from early neurogenesis to synaptogenesis (Table 1). Some miRNAs (let-7b, miR-9, and miR-124) were involved in neuronal differentiation and neurogenesis, whereas some miRNAs (miR-132, miR-134, and miR138) were involved in synapse formation plasticity.

### miRNA IN SYNAPTIC PLASTICITY

The proper formation and function of synapses is a prerequisite for the proper function of the CNS, and several neurological disorders are characterized by synaptic dysfunction. Many excellent reviews focusing on miRNAs and synapse plasticity (13, 25, 26, 45, 83, 84) show the involvement of a large number of miRNAs in synapse formation and plasticity. It is believed that local translation plays a key role in synaptic plasticity (52, 92). Both miRNAs and pre-miRNAs have been found in dendrites and synapses (52, 65, 66, 88). miRNA pathways such as Dicer, Argonaute, FMR1, and various P-body components are also found in dendrites of mature neurons (2, 65). These findings suggest that miRNAs may regulate local protein synthesis. Recent studies on local translation regulatory mechanisms of miRNAs provide a model for the role of miRNAs in local protein synthesis and dendritic spine morphogenesis (13, 83, 84). This model shows that miRNAs control dendritic spine growth by two antagonistic pathways: a RhoA-ROCK cascade leading to actomyosin contraction and spine shrinkage, and a Rac-LIMK1 signaling module promoting actin polymerization and spine growth (13, 83, 84). For example, miR-138 induces spine shrinkage by the RhoA-Rock pathway. This is achieved by miR-138-dependent downregulation of the deamidation enzyme APT1 and the resulting membrane localization and activation of the RhoA stimulatory G protein Go12/13. In contrast, miR-132 and miR-134 promote spine growth by inhibiting separately the synthesis of the Rac-inactivating protein p250RhoGAP and LIMK1 of Rac-LIMK1 pathway.

### miRNAs IN DISEASES OF THE CNS

Although studies on the role of miRNAs in the disorders of the CNS have just begun to emerge, increasing evidence

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**Table 1. List of individual miRNAs involved in different steps of neural development**

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Species</th>
<th>Target</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-7b</td>
<td>rodent</td>
<td>TLX cyclin D1</td>
<td>regulation of neural differentiation</td>
<td>(104)</td>
</tr>
<tr>
<td>miR-9</td>
<td>human</td>
<td>REST</td>
<td>regulation of neurogenesis</td>
<td>(76)</td>
</tr>
<tr>
<td>miR-9</td>
<td>rodent</td>
<td>Foxg1</td>
<td>regulation of neural differentiation</td>
<td>(87)</td>
</tr>
<tr>
<td>miR-124</td>
<td>rodents</td>
<td>SC1F1</td>
<td>regulation of neural differentiation and neurogenesis in the developing spinal cord</td>
<td>(94)</td>
</tr>
<tr>
<td>miR-124</td>
<td>rodents</td>
<td>SOX9</td>
<td>regulation of the neurogenesis in the SVZ stem cell niche and neurite outgrowth in neuronal differentiation</td>
<td>(15)</td>
</tr>
<tr>
<td>miR-124</td>
<td>rodent</td>
<td>PTBP1</td>
<td>regulation of neural differentiation</td>
<td>(70)</td>
</tr>
<tr>
<td>miR-124</td>
<td>rodent</td>
<td>LAMC1 ITGB1</td>
<td>regulation of neural differentiation</td>
<td>(10)</td>
</tr>
<tr>
<td>miR-125b</td>
<td>rodent</td>
<td>NR2A</td>
<td>dendrite spine development</td>
<td>(23)</td>
</tr>
<tr>
<td>miR-132</td>
<td>rodents</td>
<td>MeCP2</td>
<td>neuronal homeostasis</td>
<td>(47)</td>
</tr>
<tr>
<td>miR-132</td>
<td>rodents</td>
<td>RFX4</td>
<td>regulation of the circadian clock</td>
<td>(14)</td>
</tr>
<tr>
<td>miR-132</td>
<td>rodent</td>
<td>P250GAP</td>
<td>regulation of neuronal morphogenesis and dendrite development</td>
<td>(95, 99)</td>
</tr>
<tr>
<td>miR-133b</td>
<td>rodents</td>
<td>Ptx3</td>
<td>regulation of the maturation of midbrain dopaminergic neurons</td>
<td>(44)</td>
</tr>
<tr>
<td>miR-134</td>
<td>rodents</td>
<td>LimK1</td>
<td>regulation of the dendritic spine development</td>
<td>(84)</td>
</tr>
<tr>
<td>miR-134</td>
<td>rodent</td>
<td>Pum2</td>
<td>dendrite development</td>
<td>(27)</td>
</tr>
<tr>
<td>miR-138</td>
<td>rodent</td>
<td>APT1</td>
<td>spine development</td>
<td>(88)</td>
</tr>
<tr>
<td>miR-196</td>
<td>rodents</td>
<td>HOXB8</td>
<td>downregulation of HOXB8, HOXD8 and HOXA7</td>
<td>(102)</td>
</tr>
<tr>
<td>miR-219</td>
<td>rodent</td>
<td>SCOP</td>
<td>regulation of the circadian clock</td>
<td>(14)</td>
</tr>
<tr>
<td>miR-219</td>
<td>rodent</td>
<td>Sox6, Hes5</td>
<td>regulation of oligodendrocyte differentiation and myelination</td>
<td>(105)</td>
</tr>
<tr>
<td>miR-219</td>
<td>rodent</td>
<td>Sox6, Fox3, ZFP238, PDGFe</td>
<td>regulation of oligodendrocyte differentiation and myelination</td>
<td>(22)</td>
</tr>
<tr>
<td>miR-338</td>
<td>rodent</td>
<td>Sox6, Hes5</td>
<td>regulation of oligodendrocyte differentiation and myelination</td>
<td>(105)</td>
</tr>
</tbody>
</table>

MiRNA, microRNA.
indicates that miRNAs are involved in a number of neurological disorders including traumatic CNS injuries and neurodegenerative diseases (Table 2).

### Traumatic Spinal Cord Injury

Acute spinal cord injury (SCI) triggers a secondary injury by multiple injury processes including extensive temporal changes in gene expression (3, 18, 75). Alteration in expression of many genes has been shown to play important roles in the pathogenesis of secondary SCI (3, 18, 75). miRNAs are attractive candidates as upstream regulators of the secondary SCI progression because miRNAs can posttranscriptionally regulate the entire set of genes (10, 62).

Recently, we found that a large set of miRNAs (97 of 269) were significantly deregulated after SCI in adult rats (64). Of those, 60 miRNAs were expressed above the low level (intensity >500). These miRNAs can be classified into three categories: 30 were upregulated, 16 downregulated, and 14 upregulated at 4 h post-SCI and then subsequently downregulated at 1 and 7 days (64). The bioinformatics analysis indicates that the potential targets for miRNAs altered after SCI include genes encoding components that are involved in the inflammation, oxidation, and apoptosis that are known to underlie the pathogenesis of SCI (64), suggesting that abnormal expression of miRNAs may contribute to the pathogenesis of SCI. More recently, 10 miRNAs (5 decreased and 5 increased) were also identified to be deregulated after SCI in mice by miRNA-based array screening (74). Quantitative PCR further revealed two peaks of miR-223 expression at 12 h and 3 days after SCI, while miR-124a expression was significantly decreased at 1 day through 7 days after SCI (74). In situ hybridization showed that miR-223 was located around the injury. In contrast, miR-124a, which was detected in the normal spinal cord, was not observed at the injury site (74).

Astrogliosis following SCI involves an early hypertrophic response that is beneficial and the subsequent formation of a dense scar. Increasing evidence suggests that bone morphogenetic protein (BMP) signaling plays a fundamental role in

### Table 2. Changes in miRNA profiles in CNS disorders

<table>
<thead>
<tr>
<th>Disorders</th>
<th>Implicated miRNAs</th>
<th>Potential Targets and/or Signaling Pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI</td>
<td>97 miRNAs were deregulated; 60 of the 97 expressed above the moderate level; among the 60 miRNAs, 30 ↑, 16 ↓, 14 ↑ at 4 h and then ↓ at 1 &amp; 7 days</td>
<td>target proteins involved in inflammation, oxidation, and apoptosis that are known to underlie pathological progression of SCI</td>
<td>(65)</td>
</tr>
<tr>
<td>SCI</td>
<td>miR-1, miR-133a, miR-133b, miR-223, and miR-451 ↑; miR-124a, miR-129-3p, miR-342, miR-495, and miR-541 ↓; miR-223 ↑: 2 peaks at 12 h and 3 days after SCI</td>
<td>N/A</td>
<td>(74)</td>
</tr>
<tr>
<td>SCI</td>
<td>miR-124 ↓: at 1 day through 7 days after SCI</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>TBI</td>
<td>expression changes &gt;2-fold: 13 miRNAs ↑ and 14 miRNAs ↓ at 6 h after TBI; 4 miRNAs ↑ and 23 miRNAs ↓ at 1 day after TBI; 16 miRNAs ↑ and 11 miRNAs ↓ at 2 days after TBI; 19 miRNAs ↑ and 5 miRNAs ↓ at 3 days after TBI</td>
<td>target proteins involved in signal transduction, transcriptional regulation, proliferation, and differentiation that are known to be initiated after TBI</td>
<td>(60)</td>
</tr>
<tr>
<td>TBI</td>
<td>50 miRNAs ↑, 35 miRNAs ↓</td>
<td>N/A</td>
<td>(78)</td>
</tr>
<tr>
<td>Brain ischemia</td>
<td>a number of miRNAs were deregulated</td>
<td>4 target genes known to be important in the progression of cerebral ischemia.</td>
<td>(38)</td>
</tr>
<tr>
<td>Brain ischemia</td>
<td>8 miRNAs ↑ and 12 miRNAs ↓ at 3 h and 3 days; decreased miR-145 resulted in increased translation of its miRNA target, superoxide dismutase-2; in silico analysis showed sequence complementarity of 8 miRNAs induced after focal ischemia to 877 promoters</td>
<td>several target proteins known to mediate inflammation, transcription, neuroprotection, receptors function, and tonic homeostasis</td>
<td>(20)</td>
</tr>
<tr>
<td>Brain ischemia</td>
<td>miR-497 ↑ in brain after transient ischemia, and in N2A neuroblastoma cells after oxygen-glucose deprivation; miR-497 promoted ischemic neuronal death</td>
<td>bcl-2 and bcl-w</td>
<td>(103)</td>
</tr>
<tr>
<td>Brain ischemia</td>
<td>a number of miRNAs were deregulated in the brain; many miRNAs changed &gt;1.5-fold</td>
<td>N/A</td>
<td>(64)</td>
</tr>
<tr>
<td>AD</td>
<td>miR-29a, miR-29b-1 ↓</td>
<td>BACE1</td>
<td>(36)</td>
</tr>
<tr>
<td>AD</td>
<td>miR-107 ↓</td>
<td>BACE1</td>
<td>(97)</td>
</tr>
<tr>
<td>AD</td>
<td>miR-9, miR-125b, miR-128 ↑</td>
<td>N/A</td>
<td>(68)</td>
</tr>
<tr>
<td>AD</td>
<td>miR-146a ↑</td>
<td>complement factor H</td>
<td>(69)</td>
</tr>
<tr>
<td>AD</td>
<td>miR-298, miR-328 ↓</td>
<td>BACE1</td>
<td>(8)</td>
</tr>
<tr>
<td>AD</td>
<td>miR-20a, miR-17-5p and miR-100b (only miR-100b decreased in AD)</td>
<td>APP</td>
<td>(35)</td>
</tr>
<tr>
<td>AD</td>
<td>miR-101</td>
<td>APP</td>
<td>(93)</td>
</tr>
<tr>
<td>AD</td>
<td>miR-15a, miR-195 and miR-497 (only miR-15a decreased in AD)</td>
<td>ERK1</td>
<td>(37)</td>
</tr>
<tr>
<td>PD</td>
<td>miR-133b ↓</td>
<td>PTPX3</td>
<td>(44)</td>
</tr>
<tr>
<td>PD</td>
<td>miR-433</td>
<td>FGF20</td>
<td>(96)</td>
</tr>
<tr>
<td>PD</td>
<td>miR-7 ↓</td>
<td>gSYN</td>
<td>(41)</td>
</tr>
<tr>
<td>Huntington’s disease</td>
<td>miR-9, miR-9* ↓</td>
<td>REST, CoREST (RCOR1)</td>
<td>(76)</td>
</tr>
<tr>
<td>Huntingtons disease</td>
<td>miR-124a, miR-132 ↓</td>
<td>N/A</td>
<td>(39)</td>
</tr>
<tr>
<td>Rettsyndrome</td>
<td>miR-132</td>
<td>MeCP2</td>
<td>(47)</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>miR-125b, miR-132</td>
<td>NR2A</td>
<td>(23)</td>
</tr>
<tr>
<td>Tourette’s syndrome</td>
<td>miR-189</td>
<td>SLITRK1</td>
<td>(1)</td>
</tr>
</tbody>
</table>

CNS, central nervous system; SCI, spinal cord injury; TBI, traumatic brain injury; AD, Alzheimer’s disease; PD, Parkinson’s disease; N/A, nonapplicable.
generation of astrocytes. Recent studies showed that miRNA may mediate effects of BMP signaling on astrogliosis. It has been shown that BMPR1a and BMPR1b signaling exerts opposing effects on hypertrophy (81). For example, BMPR1a signaling promotes reactive gliosis and wound closure, whereas BMPR1b signaling inhibits these beneficial processes and increases formation of the glial scar. The two type I receptors have also been shown to exert opposing effects on levels of astrocytic miR-21(81). BMPR1a decreases levels of miR-21 and BMPR1b opposes this effect. miR-21 overexpression in astrocytes caused a reduction in glial fibrillary acidic protein (GFAP) levels as well as in astrocytic size (81). These results indicate that BMPR1a signaling decreases levels of miR-21 and that miR-21, in turn, negatively regulates GFAP levels in astrocytes.

**Traumatic Brain Injury**

Two recent studies on expression profiling of miRNA after traumatic brain injury (TBI) suggest a role of miRNAs in TBI (59, 79). In the rat cortex, TBI induced deregulation of a number of miRNAs (59). At 6 h postinjury 136 miRNAs were expressing, in which 13 miRNAs were more than twofold upregulated, and 14 miRNAs were more than twofold downregulated; 118 miRNAs were expressing at 24 h postinjury, in which four miRNAs were more than twofold upregulated, and 23 miRNAs were more than twofold downregulated; 149 miRNAs were expressing at 48 h post injury, in which 16 miRNAs were more than twofold upregulated, and 11 miRNAs were more than twofold downregulated; and 203 miRNAs were expressing at 72 h post injury, in which 19 miRNAs were more than twofold upregulated, and five miRNAs were more than twofold downregulated. Furthermore, only miR-21 was globally upregulated within all the four time points postinjury. Similarly, in the mouse hippocampus, 50 miRNAs were decreased, whereas 35 miRNAs were increased after TBI (79). Bioinformatic analysis of the predicted targets for validated miRNAs (miR-107, -130a, -223, -292-5p, -433-3p, -451, -541, and -711) regulated by TBI revealed an overrepresentation of proteins involved in several biological processes and functions known to be initiated after injury, including signal transduction, transcriptional regulation, proliferation, and differentiation (79).

Posttraumatic ischemia is a key mechanism of secondary injury after acute TBI (28). Several studies revealed that brain ischemia also induced alteration of a large set of miRNA expression (17, 36, 63, 100). Bioinformatic analysis indicated a correlation between miRNAs altered by ischemia to several miRNAs known to mediate inflammation, transcription, neuroprotection, receptors function, and ionic homeostasis (17). It is interesting to note that in silico analysis revealed eight miRNAs induced by transient ischemia with complementarity to 877 gene promoters, suggesting that miRNAs also regulate gene expression (17). In addition, miR-497 has been shown to be induced in the mouse brain after transient ischemia, and in mouse N2A neuroblastoma (N2A) cells after oxygen-glucose deprivation (100). Loss-of-function and gain-of-function studies revealed that miR-497 promoted neuronal death. Moreover, miR-497 directly bound to the predicted 3'-UTR target sites of bcl-2/-w genes (100). Furthermore, knockdown of cerebral miR-497 in mice enhanced Bcl-2/-w protein levels in the ischemic region, attenuated brain infarction, and improved neurological outcome after focal ischemia (100). A deregulated expression of miRNAs was also detected in the brain in rat models of ischemia, brain hemorrhage, and kainate-induced seizures, many of which changed >1.5-fold (63).

**Alzheimer’s Disease**

Alzheimer’s disease (AD) is a progressive degenerative neurological disorder and is the most common form of dementia. The majority of AD cases are sporadic. This progressive disease is characterized by the accumulation of plaques formed of short β-amyloid (Aβ) peptides (78). These peptides are from proteolytic cleavage of the β-amyloid precursor protein (APP), a type 1 transmembrane protein, by a β-secretase known as the β-site APP-cleaving enzyme (BACE), and γ-secretase (78). BACE1/β-secretase cleavage of APP is the rate-limiting step for Aβ peptide production in the brain, and increased BACE1 expression is thought to be an important risk factor for sporadic AD (31). In AD, a number of miRNAs exhibit abnormal expression levels, and many of them have been shown to regulate BACE1 (7, 33, 67, 68, 96). Hebert et al. (33) have recently found that miR-29a and miR-29b-1 can regulate BACE1 expression in vitro, which is consistent with significantly decreased expression of miR-29a and miR-29b-1 in AD patients with abnormally high levels of BACE1. Additionally, miR-107 predicted to target the BACE1 3'-UTR at multiple sites was significantly decreased in patients with the earliest stages of pathology (96). The further finding that BACE1 mRNA levels increase as miR-107 levels decrease in the progression of AD suggests that miR-107 may be involved in accelerated disease progression through the regulation of BACE1 (96). Increased expression of BACE protein and decreased expression of miR-298 and miR-328 were also found in a mouse model of AD, and in vitro studies showed that both miR-298 and miR-328 bind to the 3'-UTR of BACE1 mRNA-regulated BACE1 (7). These findings suggest that loss of specific microRNAs contributes to increased BACE1 and Aβ levels in sporadic AD.

In vitro studies showed that miR-20a, miR-17-5p, miR-106, and miR-106b could regulate APP expression (32, 93). Interestingly, miR-106 was found to be decreased in the sporadic AD brain, suggesting that miR-106 was involved in an elevated production of Aβ (32). Recent studies also showed that the absence of Dicer in the adult forebrain was accompanied by a mixed neurodegenerative phenotype (34). Further studies revealed that miR-15 family members (miR-15a, miR-195, and miR-497), whose levels were reduced in the Dicer-deficient animals, could regulate ERK1 expression and phosphorylation as well as tau phosphorylation in cultured mouse neurons. Finally, miR-15a displayed reduced expression in the AD brain. These results suggest that changes in the miRNA network may contribute to a neurodegenerative phenotype by affecting tau phosphorylation.

In contrast to above, miR-9, miR-125b, and miR-128 were found to be elevated in the AD brain (67). Other studies on the AD brain revealed that increased expression of miR-146a correlates with decreased expression of the predicted mRNA target, complement factor H, an important repressor of brain inflammatory responses (68), suggesting that miRNAs may be involved in the neuroinflammatory process associated with Alzheimer’s disease.
deposition of the Aβ peptide. Increased expression of miR-9, miR-125b, and miR-146a in the AD brain has been shown to be correlated with neuropathological changes (87). Taken together, these studies suggest that the deregulated microRNAs may contribute to AD pathogenesis.

**Parkinson’s Disease**

Parkinson’s disease (PD) is characterized by the progressive neurodegeneration of dopaminergic neurons in the substantia nigra, which is manifested in such symptoms as tremors, rigidity, and bradykinesia. Recent studies showed that miR-133b was specifically expressed in midbrain dopaminergic neurons (DNs) and was deficient in midbrain tissue from patients with PD (41). Further studies revealed that miR-133b regulated the maturation and function of midbrain DNs within a negative feedback circuit that includes the paired-like homeodomain transcription factor Pitx3 (41). A disruption of this feedback mechanism may promote the multitude of symptoms associated with PD (41). Moreover, the disruption of the binding site for miR-433 resulted in increased expression of fibroblast growth factor 20 (FGF20) in the PD brain and in vitro cell models. FGF20 was previously identified as a risk factor for PD corrected with increased α-synuclein expression that can cause PD (95). Lastly, miR-7 expressed mainly in neurons was found to reduce α-synuclein protein levels through binding the 3′-UTR of α-synuclein mRNA and protect cells against oxidative stress (38). In the MPTP-induced neurotoxin model of PD in cultured cells and in mice, miR-7 expression was decreased, suggesting that this reduction may contribute to increased α-synuclein expression (38).

**Huntington’s Disease**

Huntington disease (HD) is a dominantly inherited neurodegenerative disorder. HD is primarily caused by a trinucleotide repeat expansion of the gene encoding Huntingtin (Htt). In healthy individuals, the transcriptional repressor protein REST is primarily found in the cytoplasm through interaction with Htt. In HD patients, REST is incapable of binding Htt and accumulates in the nucleus. In the nucleus, REST recruits corepressors, including the REST corepressor 1 (CoREST), to inactivate neuron-specific genes (13). Recent studies revealed the dysregulated expression of many neuronal-specific microRNAs including miR-124a and miR-132 in mouse models of HD and in human HD, probably as a result from REST repression (37). Moreover, the reduction in expression of miR-124a and miR-132 have been shown to lead to increased levels of their target mRNAs, supporting the idea that HD reflects a loss of neuronal identity caused in part by dysregulation of both transcriptional and posttranscriptional gene expression (37). Further in vitro studies showed that miR-9/miR-9* targeted REST and CoREST, respectively, and in vivo studies showed that miR-9/miR-9* were decreased early in HD disease progression, suggesting that bifunctional microRNAs serve as effectors of a double negative feedback loop between the REST-silencing complex and the REST-regulated microRNAs (76).

**miRNAs as Therapeutic Targets**

Although the roles of microRNAs in human neurological diseases including traumatic CNS injuries and neurodegenerative diseases are only beginning to be elucidated, increasing evidence suggests that microRNAs represent a new class of drug target (22, 72, 98). MicroRNAs reduce steady-state protein levels for the targeted gene(s) by posttranscriptional regulation (46, 48). Thus, inhibition of a particular microRNA linked to disease can remove the block against expression of a therapeutic protein, and conversely, administration of a microRNA mimic can boost the endogenous microRNA population repressing a detrimental gene. If we take advantage of their small size and the current knowledge of microRNA biogenesis, modified RNAs can be transiently delivered as a synthetic, preprocessed microRNA or anti-microRNA oligonucleotides (70).

The anti-microRNA oligonucleotides are single-stranded reverse complement oligonucleotides. The stability, binding affinity, and specificity of these oligonucleotides have been improved through chemical modification. The most common oligonucleotide modifications are locked nucleic acids (LNA), 2′-O-methyl-modification, and phosphorothioate backbones. The 2′-O-methyl modified oligonucleotides have been proved to be effective inhibitors in several cell lines and cultured primary neurons (35, 44, 71, 77, 85). The first mammalian in vivo study using anti-miRNA showed that 2′-O-methyl modified cholesterol-conjugated single-stranded oligonucleotides, named “antagomirs,” inhibited miR122, an abundant liver-specific microRNA (51). The silencing of endogenous microRNAs by the antagomirs was specific, efficient, and long-lasting (51). Recently, administration of LNA and phosphorothioate modified oligonucleotides targeting miR-122 intravenously in mice and nonhuman primates have also been shown to result in an effective and long-lasting decrease in total plasma cholesterol without any apparent toxicity (20, 21). An elegant study further demonstrated the therapeutic feasibility and safety of microRNA inhibition of this approach in a primate disease model (54). The LNA-modified oligonucleotides targeting miR-122 (SPC3649) is currently in phase I clinical trials for hepatitis C virus infection and will become the first microRNA therapeutic target in humans (http://santaris.com/).

The microRNA mimics are small, usually double-stranded, and chemically modified oligonucleotides that can be used to downregulate a specific target proteins. The double-stranded structure is required for the efficient recognition and loading into the RISC. One strand in the mimic is the mature microRNA, and the complementary strand is complexed with the mature microRNA sequence. Although synthetic microRNA mimics are frequently used in culture research (7, 33, 102), there is no in vivo data demonstrating efficacy of microRNA mimic.

Although there are many challenges for microRNAs as therapeutic targets such as delivery, potential off-target effects, and safety, the strategy of microRNA manipulation in vivo to regulate disease-related processes is already becoming a feasible future therapeutic approach. In future, a better understanding of microRNA biogenesis and function will undoubtedly promote the development of microRNA-based therapies.

**Conclusion**

Investigating the role of microRNAs in neurological disorders is a new frontier for neurological research. Although the studies on the role of microRNAs in neurological disorders have just begun to emerge, a growing body of exciting evidence suggests that microRNAs are important regulators of diverse neurobiological processes such as neurodegeneration, neuroregeneration, neuroprotection, and neuroplasticity. The emerging therapeutic potential of microRNAs as drug targets for neurological diseases highlights the promise of microRNA-based treatments and opens new avenues for the development of novel therapeutic strategies for neurological disorders.
as neurogenesis, neurodifferentiation, growth, proliferation, and apoptosis. Moreover, miRNAs have been implicated in a number of CNS disorders. More importantly, identifying the gene targets and signaling pathways responsible for their neurological effects is critical for future studies. The ultimate goal is the development of new therapeutic and diagnostic strategies for neurological disorders.

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