Mol Neurobiol (2012) 45:200–212
DOI 10.1007/s12035-011-8231-z

MicroRNAs: Novel Regulators of Oligodendrocyte Differentiation and Potential Therapeutic Targets in Demyelination-Related Diseases

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Received: 31 August 2011 / Accepted: 22 December 2011 / Published online: 5 January 2012

Abstract MicroRNAs (miRNAs or miRs) are a class of endogenous small non-coding RNAs that consist of about 22 nucleotides and play critical roles in various biological processes, including cell proliferation, differentiation, apoptosis, and tumorigenesis. In recent years, some specific miRNA, such as miR-219, miR-138, miR-9, miR-23, and miR-19b were found to participate in the regulation of oligodendrocyte (OL) differentiation and myelin maintenance, as well as in the pathogenesis of demyelination-related diseases (e.g., multiple sclerosis, ischemic stroke, and leukodystrophy). These miRNAs control their target mRNA or regulate the protein levels of some signaling pathways, and participate in OL differentiation and the pathogenesis of demyelination-related diseases. During pathologic processes, the expression levels of specific miRNAs are dynamically altered. Therefore, miRNAs act as diagnostic and prognostic indicators of defects in OL differentiation and demyelination-related diseases, and they can provide potential targets for therapeutic drug development.

Keywords MicroRNAs · Oligodendrocytes · Differentiation · Demyelination-related diseases · Multiple sclerosis · Ischemic stroke · Leukodystrophy

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Introduction

Oligodendrocytes (OLs) play critical roles in brain development and neuron function by producing and maintaining the myelin sheath that surrounds vertebrate axons to deliver action potentials and they assure the survival of axons. The production of myelin-forming OLs depends on smooth terminal differentiation from oligodendrocyte progenitor cells (OPCs) [1]. A number of transcriptional and post-transcriptional factors and multiple signaling pathways contribute to the complex and paradoxical cellular processes to some degree [2, 3]. Demyelination through OL defect breaks down saltatory nerve conduction, which leads to a number of demyelination-related diseases, such as multiple sclerosis (MS), ischemic stroke, and leukodystrophy [4–8]. Remyelination has been demonstrated in animal models to be mediated by OPCs, which migrate into the lesion, proliferate, and differentiate into mature OLs and then ensheathe the demyelinated axons. Promising cell replacement therapies based on the use of stem cells or OPCs for myelin restoration have been proposed [1, 9, 10]. However, the molecular mechanisms and regulatory network of OL differentiation and myelination are poorly understood. The epigenetic controls of OL development via chromatin remodeling by histone deacetylases, DNA methylation, and gene silencing by non-coding RNAs (e.g., microRNAs) have a special influence on OL differentiation and myelination [11–13]. Recently, several studies have demonstrated that microRNAs (miRNAs or miRs) are required and sufficient in controlling OL differentiation and demyelination-related diseases [14–17]. Some progress has been achieved in this field, both in OL and in Schwann cells [18, 19].

MiRNAs, processed with the RNase III enzyme Dicer, are a class of endogenous small non-coding RNAs that consist of about 22 nucleotides. Traditionally, they bind to

the 3′-UTR of their target mRNAs by base pairing of the seed sequence in 5′-miRNAs, negatively regulating up to 30% of human genes [20], either through the inhibition of translation initiation and elongation or by degradation of target mRNAs [21–24]. They can also decrease the target mRNA levels by affecting their destabilization [25]. Hundreds of miRNAs have been identified in various multicellular organisms, and most are evolutionarily conserved. MiRNAs play important roles in various biological processes, including cell proliferation, differentiation, apoptosis, stress response, inflammation, and tumorigenesis, and they have critical functions in neurogenesis and gliogenesis [26, 27].

Currently, several pathways are known to promote OL differentiation and myelination, and rescue or repair the defect of the formation and maintenance of central nervous system (CNS) myelin. For example, one way is increasing the number of OPCs by suppressing the expression of OL or non-OL lineage (such as neurons and astrocytes) genes (Fig. 1a). Another important way is promoting OL differentiation and myelination by inhibiting the negative genes that maintain OPCs in their undifferentiated state and by regulating the transiently required high-level expression of genes from mature OLs to myelinating OLs (Fig. 2). In the present review, by collating recently available evidence, we summarized several important miRNAs and their interactions with their predicted targets in regulating OL differentiation and myelination through different mechanisms. We also reviewed their fundamental roles in some demyelination-related diseases, with emphasis on miRNA–mRNA interaction and their potential diagnostic and prognostic biomarkers for disease type and drug target values.

MiRNAs Regulate OL Differentiation via MiRNA–mRNA Interaction

Using the Cre-loxp system and two Cre lines (Emx1-Cre and Nestin-Cre) during the early and late embryonic stages to silence Dicer expression, the migration of late-born neurons in the cortex and the expansion and differentiation of OPCs in the spinal cord were greatly affected. The different timing of Dicer deletion, which blocks miRNA biogenesis, also affected distinct phases of neurogenesis and gliogenesis during CNS development in mice [28]. Moreover, postnatal Dicer ablation in mature OLs results in inflammatory neuronal degeneration, such as increased demyelination, lipid accumulation, and peroxisomal and oxidative damage. Therefore, miRNAs seem to play essential roles in the maintenance of lipid and redox homeostasis in mature OLs and are necessary for supporting axonal integrity and in the formation of compact myelin [29].

Recently, a few studies have individually identified OL lineage or stage-specific miRNAs and analyzed miRNA profiling involved in the OL differentiation process, from embryonic stem cells (ESCs) or neural progenitor cells to OPCs and myelinating OLs (see the details in Figs. 1 and 2 and ref. [14, 15, 17, 28, 30]). Briefly, miRNAs may potentially and dynamically regulate or mark key steps during OL

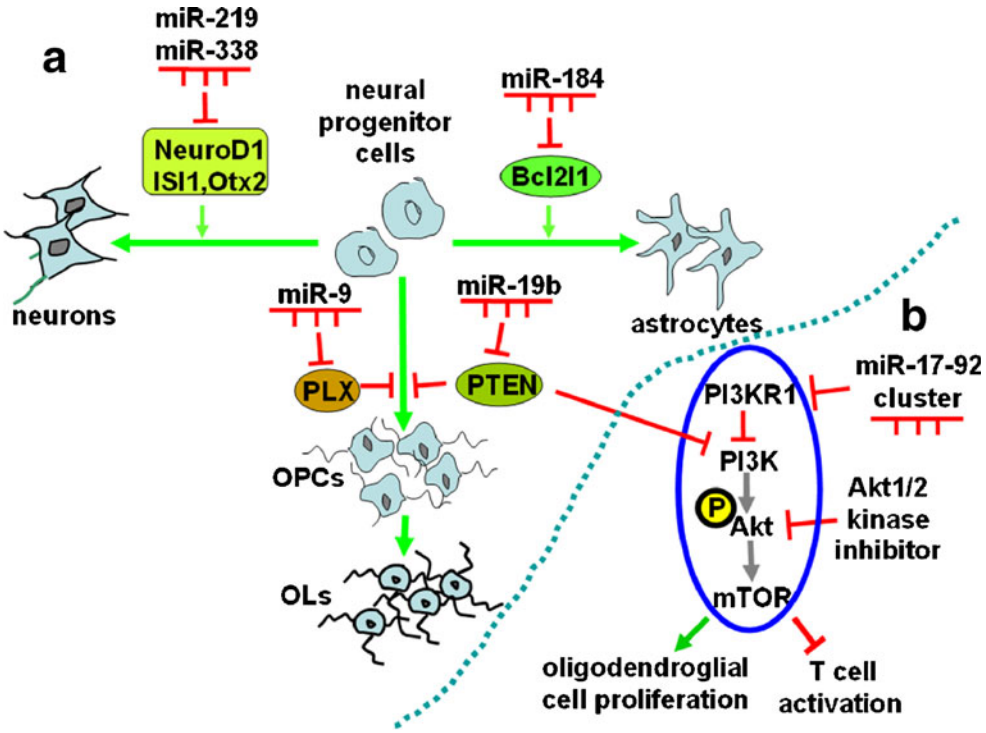

Fig. 1 **a** Specific miRNAs increase the number of OPCs and OLs by suppressing the expression of non-OL lineage (such as neurons and astrocytes) genes or the expression of genes that regulate neural progenitor cell differentiation. **b** MiR-17–92 cluster (especially miR-19b, -17, -20a) increases the number of oligodendroglial cells and inhibited MS-associated T cell activation genes by PI3K/Akt/mTOR pathway. Arrow and blocked arrow indicate activation and inhibition, respectively

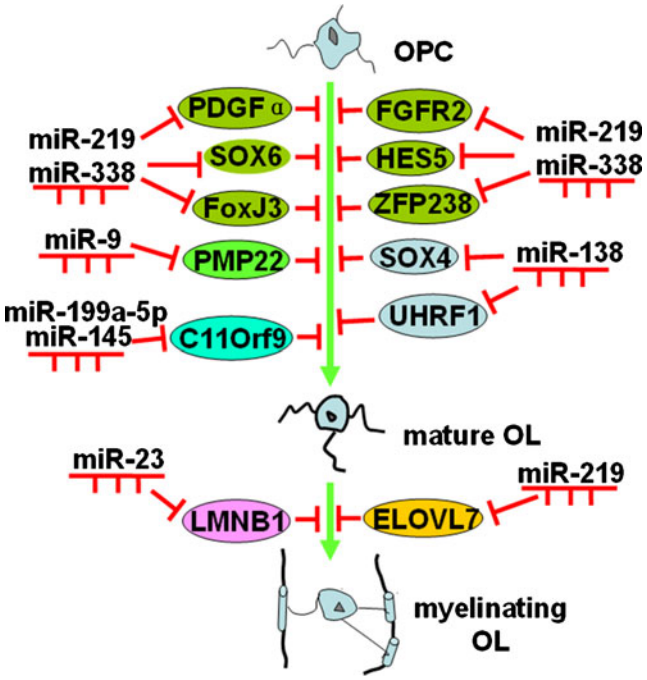

Fig. 2 Several of the most important miRNAs and the interaction with their predicted targets in regulating OL differentiation and myelination through different mechanisms, including those that inhibit the genes that maintain OPCs in their undifferentiated state and regulate other OL-related gene expression

differentiation, along with their potential gene targets. In postnatal OL lineage cells, the expression of 43 of the 98 specific miRNAs dynamically changes during the transition from $A2B5^+$ OPCs to premyelinating $GalC^+$ OLs. About 37 of the 98 miRNAs display mRNA target bias, and the expression levels of the predicted targets of 13 miRNAs are also dynamically regulated during OL differentiation [31].

In addition to studying overall patterns of miRNA expression throughout OL differentiation, several myelin-associated genes that have high target bias for the top differentially expressed miRNAs have also been identified [15]. The miRNA–mRNA interaction model reveals that the 3′-UTR of one target mRNA molecule may contain several sites where different miRNAs may bind. Their interactions may have significant roles in regulating OL differentiation and myelin production. Moreover, some miRNAs have similar temporal expression patterns, such as miR-145 and miR-199a-5p, which may co-regulate a shared set of mRNA targets; other groups, such as miR-9 and miR-223, can regulate different components (mRNAs or proteins) within common pathways [15].

Collectively, OL-specific miRNAs regulate OL differentiation and myelination at the post-transcriptional level, and they can be used to distinguish among different stages of OL differentiation. One miRNA can control many target genes, and one target gene can be regulated by multiple miRNAs. Therefore, understanding the miRNA–mRNA interaction networks is quite necessary and meaningful to reveal the molecular mechanisms that control OL differentiation and myelination and to gain fundamental knowledge for rescuing or repairing the defect of the formation and maintenance of CNS myelin as a potential therapy pathway for demyelination-related diseases.

Different Mechanisms of Specific miRNAs in Regulating OL Differentiation and Myelination

Given the importance of miRNAs in regulating vertebrate neural development [32–34], the normal differentiation and function of several different cell types, including neurons, are required. While introducing miRNAs into OLs, a number of stage-specific miRNAs that regulate OL differentiation and myelin formation have been identified, such as miR-219, miR-138, miR-9, miR-23, and miR-19b. They either increase the number of oligodendroglial cells or repress OL lineage gene expression, which maintain OPCs in their proliferative state or transiently require high-level gene expression from mature OLs into myelinating OLs to promote OL differentiation and myelination. The various miRNA molecules that have outstanding behavior in the different mechanisms are discussed below (Figs. 1 and 2).

Controlling the Number of Oligodendroglial Cells by miR-17–92 Cluster

To rescue the myelination defect, one pathway is to increase the number of OPCs and then the number of OLs. Previous studies have shown that the miR-17–92 cluster, processed from common precursor transcripts, including miR-17, miR-18a, miR-19a, miR-20a, miR-19b, miR-92a [31], and especially miR-19b, play essential roles in increasing the number of oligodendroglial cells [35].

Using the Cre-loxP recombination system and the 2′,3′-cyclic nucleotide 3′ phosphodiesterase (CNP) promoter, Dicer1 was conditionally silenced (CNP-Cre-Dicer) in E18.5 mice embryos. The number of Olig2-positive cells was reduced by ~40% in the Dicer1 knockout brains, which were observed by staining coronal P0 sections with antibodies against Olig2. This result is similar to that from RNA interference (RNAi), whereas in the spinal cord, the Olig2-positive cells were not altered. However, in the above-indexed study [28], fewer Olig2-expressing cells were detected in the E18.5 Dicer knockout spinal cord, and $PDGFR\alpha^+$ cells were greatly reduced and mainly concentrated in the ventral region of the Nestin-Cre-Dicer spinal cord [28]. Apart from the different subclasses of OLs, which are derived from multiple sources and different OPC lines that compete with each other during spinal cord and brain development, and the technological reasons [36], the results also indicate that the different mechanisms of action of

miRNA in OL development between the spinal cord and brain are still unclear.

To investigate the specific cell type further, the miRNA expression in primary cultures of OLs and astrocytes was compared by miRNA microarray profiling [35]. Among the 31 detected miRNAs, four miRNAs from the miR-17–92 cluster appear to have higher expression levels in OLs than in astrocytes. Reverse transcription polymerase chain reaction (RT-PCR) analysis confirmed that miR-19b and miR-17 are ~11-fold to ~16-fold more abundant in OLs than in astrocytes, and they are also abundant in OPCs.

Furthermore, in the brains of miR-17–92$^{flox/flox}$CNP$^{+/Cre}$ knockout mice, Olig2-positive cells had ~25% reduction compared with their control littermates. The overexpression of miR-17–19b increases the number of Oli-neu cells (a cell line of OL lineage) and those of other lines, such as A2B5-, O1-, and myelin basic protein (MBP)-positive OL lines. However, the antisense OLs reverse the effect without impairing OL differentiation. The results were further confirmed by a luciferase (target mRNA 3′-UTR) reporter assay and RT-PCR techniques. To determine the role of miR-17–19b in regulating cell proliferation and/or cell survival, an OL survival assay by TUNEL staining and BrdU assays were conducted. The expression of miR-19b significantly accelerated OPC proliferation, but did not rescue the apoptotic cells [35].

Then what is the mechanism of miR-17–92 cluster effect in OLs? In Gene Ontology (www.geneontology.org), the tumor suppressor PTEN (phosphatase and tensin homologue deleted on chromosome 10), a negative regulator of cell proliferation, may be the predicted target of miR-19b. The overexpression of miR-19b downregulates PTEN protein levels in OPCs by activating its downstream targets of the Akt signaling (PI3K/Akt/mTOR) pathway [37]. MiR-19b increases the phosphorylation of Akt, but it does not affect its overall levels. The Akt1/2 kinase inhibitor cancels miR-19b-mediated OPC proliferation [35] (Fig. 1b).

Interestingly, the miR-17–92 cluster has been found to be frequently overexpressed in lung cancers, especially small cell lung cancer [38], as well as in malignant lymphoma [39]. Thus, the miR-17–92 cluster may be the most likely candidate factors for oncogene amplification and significantly accelerated the formation of lung cancers and malignant lymphoma. The cluster is regulated by the encoding gene C13orf25 (chromosome 11 open reading frame 25), and is expressed in OPCs and OLs. The overexpression of miR-17–92 cluster is predicted to increase the number of myelinating OLs through its gene amplification effect, but simultaneously, it may also have an important role in tumorigenesis by enhancing cell proliferation and growth such as glial tumors, which indicates that controlling its appropriate expression to some degree is necessary.

Inhibiting the OL Lineage Gene Expression which Maintains OPCs in Their Undifferentiated State

Inducing stem cells or OPCs into myelinating OLs is the common and ideal pathway for replacement therapy in demyelination-related diseases. Therefore, inhibiting the expression of OL lineage genes, which maintain the OPCs in their undifferentiated state, is a vital step during the process.

Recently, miR-219, miR-138, and miR-338, especially miR-219, have been highly enriched in mature myelinating OLs and are important regulators of OL differentiation or OPC maturation, thereby providing new targets for myelin repair in demyelination-related diseases [14, 17]. Using Olig2/CNP$^{Cre/+}$Dicer1$^{flox/flox}$ and Olig1$^{Cre+/-}$Dicer1$^{lox/lox}$ (Dicer1 CKO) mice models independently, the loss of Dicer1 function in OPCs and OLs has been demonstrated to delay CNS myelination and retard OL differentiation in a cell-autonomous manner in vivo [14]. OPCs were shown to undergo extensive proliferation without further differentiation in vivo and with abnormal differentiation in vitro, which indicates that mature miRNA production is crucial to normal OL differentiation and CNS myelination [14, 17]. Furthermore, using tamoxifen-activating proteolipid protein (PLP)-CreER^{T}–dependent removal of Dicer in the OLs mice model, miR-219 was found to play an essential role in the maintenance of lipids and redox homeostasis in mature OLs by binding to its specific target ELOVL7 (elongation of very long chain fatty acids protein 7) (Fig. 2) [29]. So miR-219 is necessary to support axonal integrity and the formation and maintenance of compact myelin.

MiRNA microarray and qRT-PCR analysis revealed that several miRNAs are differentially expressed during OPC differentiation into OLs. Among the top statistically ranked candidates, miR-219, miR-138, and miR-338 are strongly induced during OL differentiation, consistent with Lau et al. report [31], via co-labeling with OL markers CC1 and Olig2, but not with the neuronal maker NeuN [17].

A sharp increase in miR-219 and miR-138 at the perinatal stage is in agreement with the onset of OL maturation and Dicer1. The expression of these miRNAs in OPCs is endogenously induced by mitogen withdrawal. Some miR-219 and miR-138 gain-of-loss function studies and mimics revealed that miR-219 is necessary and sufficient for promoting the rapid OL differentiation induced by mitogen withdrawal. MiR-219 promotes the expression of markers of both early and late OL differentiation in OPCs and partially rescues OL differentiation defects in Dicer1 knockout models. The miR-138 mimic promotes the expression of markers of the earlier stages of OL differentiation (CNP, MBP) in OPCs, but not those of the later stages [myelin oligodendroglia glycoprotein (MOG)]. MiR-138 is necessary and sufficient for delaying the later stage of OL differentiation, which plays a role in prolonging the immature

phase of OL differentiation, thereby extending the window of time in which terminally differentiating OLs could select and correctly myelinate nearby axons [14, 17]. The inhibitors of miR-219 and miR-138 abrogate this response, strongly repressing OL differentiation and blocking OL maturation in zebra fish embryos [17].

Then, what is the mechanism by which miR-219 promotes OL differentiation? Further studies revealed that miR-219 directly represses the expression of several genes that inhibit OL differentiation and maintain the undifferentiated state of OPCs, such as PDGFRα, Sox6, Hes5, ZFP238/RP58, FoxJ3, and FGFR2 [14, 17]. These predicted targets are repressed more than twofold in OLs relative to OPCs. Interestingly, ZFP238 and FoxJ3, without reported functions in OPCs before, are new transcription factors that have roles in OPC–OL transition. Both miR-219 and miR-338 specifically target the 3′-UTR of the transcriptional repressor ZFP238/RP58, which is crucial for cell division patterning and neuronal survival in the developing cortex [40]. ZFP238 appear to be an inhibitor of OL differentiation because its overexpression in neural progenitor cells represses Olig2 expression and inhibits the formation of RIP^+ mature OLs [17]. The overexpression of either of these OPC-expressed transcription factors significantly inhibits normal OL differentiation and increases the expression of the OPC marker NG2. When cotransfected with luciferase into HEK293 cells, a miR-219 mimic represses ~50% of the translation of luciferase linked to the 3′-UTRs of FoxJ3, PDGFRα, and ZFP238. The 3′-UTRs of OL differentiation–inhibiting genes have binding sites where miRNAs bind to take effect. Site-directed mutagenesis of both miRNAs and target genes and miRNA antisense inhibitors confirmed the results. Apart from Sox6 and Hes5, miR-138 can also target SOX4 (an OL differentiation inhibitor similar to SOX6) and UHRF1 [14] (Fig. 2).

Interestingly, miR-219 was found to be involved in regulating fast neurotransmission and synaptic plasticity in the brain by targeting a component of the *N*-methyl-D-aspartate (NMDA) receptor signaling cascade subunit, calcium/calmodulin-dependent protein kinase II [41]. Precursor, immature, and mature OLs in the white matter, especially in the compact myelin sheath of the cerebellum and corpus callosum, have also been shown to exhibit NMDA-evoked currents [42–44]. Therefore, the NMDA receptor, through activation during ischemia and mediation of calcium accumulation, damages the OLs, ultimately resulting in the eventual loss of the myelin sheath [43]; miR-219 may play a role in modulating NMDA receptor-mediated OL differentiation and myelination dysfunction, even though NMDA receptor signaling in OPCs is not required for oligodendrogenesis and myelination [45].

Cumulatively, by inhibiting the genes that maintain OPCs in their undifferentiated state using miRNAs, a positive feedback loop is set up, accelerating the transposition of OPCs from the proliferation to the differentiation state. That is, OL-induced miRNAs link the cessation of OPC proliferation to the initiation of OL differentiation, which provides a new pathway for promoting OL remyelination after injury [14].

Regulating Other Inappropriate but OL-Related Genes Expression

Apart from the specific miRNAs required for OL differentiation, the regulation of other inappropriate but OL-related genes are also necessary for OL differentiation, myelin formation, and maintenance, especially miR-9, miR-145, miR-184, and miR-23 (details are in Figs. 1a, 2, and 3, and in Section 3.3).

MiR-9 was detected in neural progenitor cells and OL lineage cells. Gene Ontology revealed that the demyelination of sciatic nerve is associated with miR-9. The best candidate target of miR-9 is peripheral myelin protein 22 (PMP22), which is primarily expressed in myelinating Schwann cells. The existence of PMP22 mRNA, but not protein, was confirmed in OLs by Western blot and qRT-PCR, and it is increasing approximately twofold during OL differentiation [31].

In the in vitro binding assays, miR-9 represses luciferase activity by ~50%, whereas miR-9 and the luciferase reporter with the 3′-UTR of PMP22 were cotransfected into HeLa cells. The 3′-UTR of PMP22 has two repeat 'AACCAAA' sequences that correspond to the nucleotide pairing with the seed sequence of miR-9. If these two repeat sequences were deleted, miR-9 would lose the ability to downregulate PMP22 protein expression. Similarly, miR-29a also binds and inhibits PMP22 reporter expression, and regulates myelin gene expression in Schwann cells [8]. MiR-9 also suppresses nuclear receptor TLX (a tailless gene involved in the division of neural stem cells) expression, negatively regulates neural stem cell proliferation, and accelerates neural differentiation [46]. The present study provides a model for controlling the balance between neural stem cell proliferation and differentiation by forming a negative regulatory loop with TLX. MiR-9 is downregulated during OL differentiation. The loss of miR-9 suppresses proliferation and promotes migration of human neural progenitor cells [47], which will increase the number of OPCs (Fig. 1a).

In addition, two of the top ten differentially expressed miRNAs from the early to middle stage transition of OPCs, miR-199a-5p and miR-145 show strong target bias towards C11Orf9 (chromosome 11 open reading frame 9) [15]. This gene is speculated to be the human analog of mouse MRF (myelin gene regulatory factor), a protein that has been shown to be critical for OL maturation and myelin production [48].

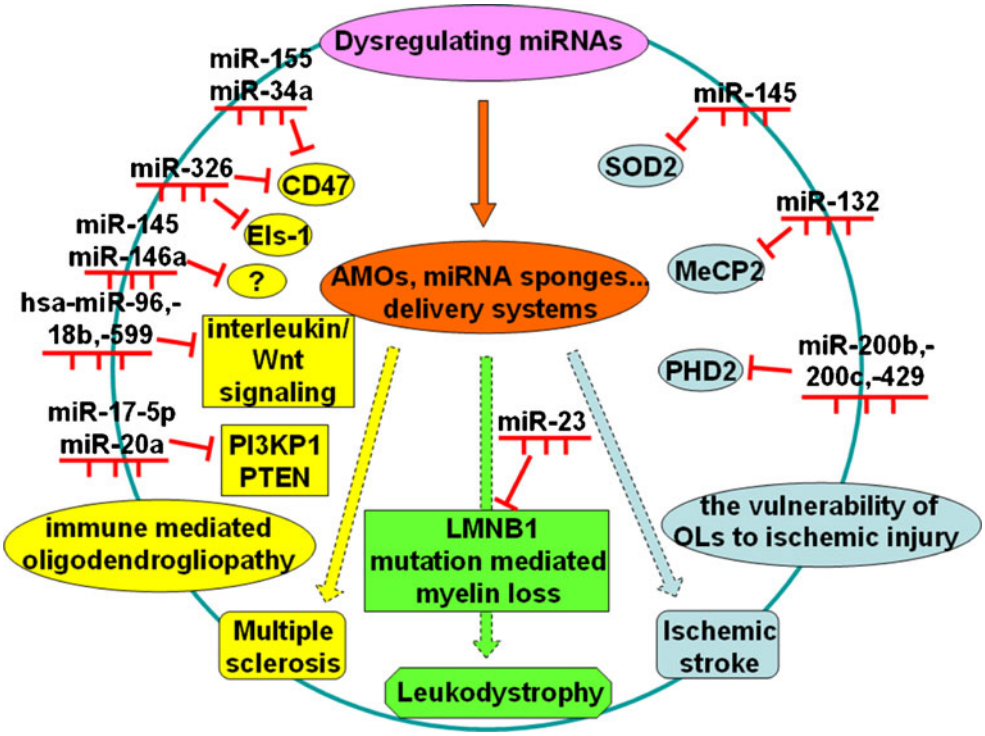

Fig. 3 Dysregulating miRNA (s) implicated in demyelination-related diseases and their potential therapeutic perspectives via the targeted delivery systems

Suppressing the expression of non-OL lineage (such as neurons and astrocytes) genes is an important way to increase the number of OPCs and promote OL differentiation. At the upstream of OPCs, neural progenitor cells could be prevented from differentiating into neurons and astrocytes by finely changing the related genes without altering normal physiologic function. For example, miR-184 enhances oligodendrogenesis by downregulating the expression of the genes that facilitated astrocyte differentiation, such as Bcl2-like 1 (Bcl2l1), a gene highly expressed in astrocytes and co-expressed with glial fibrillary acidic protein (GFAP) [15] (Fig. 1a). Furthermore, miR-219 and miR-338 also target and inhibit a number of genes known to regulate neuronal differentiation, such as proneuronal differentiation factors NeuroD1, Isl1, and Otx2 [17] (Fig. 1a). Therefore, transfection of miR-184 and/or miR-219 analogs into neural progenitor cells may help reduce astrocyte and neuronal differentiation and promote the production of OL cultures with increased purity [15].

Together, these studies prove that miRNAs are important regulators of oligodendrogenesis and are a resource for future studies on miRNA functions in OLs. For example, the transfection of related miRNA mimics or analogs to pathological conditions may promote the production of myelinating OLs and partly or fully rescue demyelination defects in related diseases. Clearly, further exploration of the interaction networks that link miRNAs and their predicted targets with OL differentiation, maturation, and myelin maintenance might provide broad insights into new therapeutic agents for adult-onset autosomal dominant leukodystrophy (ADLD) and other demyelinating diseases such as MS.

MiRNAs Serve as Diagnostic and Therapeutic Molecules in Demyelination-Related Diseases

MiRNAs are highly expressed in cells of the immune and nervous system, and they play an important role in neuroimmunology. The dysregulation of miRNA expression and function has been suggested to be associated with a number of diseases because of the important functions of miRNAs in OL differentiation and myelination. Recently, several studies have investigated the roles of miRNAs in demyelination-related diseases, such as MS, ischemic stroke, and leukodystrophy. These diseases are potential diagnostic and therapeutic applications of miRNAs, despite the complexity of changed miRNA profiles and the interaction of miRNA–mRNA network [49]. Furthermore, tissue type-specific miRNAs spectrums of various pathologic conditions were detected in body fluids, including serum, plasma, and cerebrospinal fluid, which would increase the clinical detection rate of diseases, and provide an effective means of diagnosing diseases at the early stages [50]. In the current review, several demyelination-related diseases are outlined, wherein miRNAs play a possibly irreplaceable role (Table 1 and Fig. 3). Although the focuses of the present studies are not OLs, this information can provide new insights into the miRNAs in OL-related diseases for further investigation.

Table 1 A list of dysregulating miRNA(s) implicated in demyelination-related diseases

Demyelination-related diseases	Pathogenesis	Implicated miRNA(s)	Likely target/pathway(s)	Potential mechanisms	References
Multiple sclerosis	Immune-mediated oligodendrogliopathy	miR-326↑	Els-1	Promote TH-17 differentiation	[58]
		miR-155, -34a, -326↑	CD47	Release macrophages/microglia from inhibitory control, and then promote myelin phagocytosis	[59]
		miR-145, -155, -146a	/	Discriminate MS from controls, has a high accuracy, specificity, and sensitivity	[57, 63]
		miR-18b, -599	interleukin/Wnt signaling	Mark the status of relapse	[64]
		miR-96	interleukin/Wnt signaling	Mark the status of remission	
		miR-17-5p, -20a↑	PI3KP1/PI3K/Akt pathway PTEN	Inhibited MS-associated T cell activation genes	[67]
Ischemic stroke	The vulnerability of OLs to ischemic injury	miR-145↑	SOD2	Mediate inflammation, transcription, neuroprotection, receptors function, and ionic homeostasis	[70]
		miR-132↓	MeCP2	Increase susceptibility to preconditioning ischemia	[76]
		miR-200b,-200c,-429↑	PHD2	Improve neural cell survival	[78]
Leukodystrophy	LMNB1 Mutation mediated myelin loss	miR-23↑	LMNB1	Regulate OL maturation and myelin maintenance	[80]

Multiple Sclerosis

MS is the most frequent chronic inflammatory demyelinating disease of the CNS. Oligodendrogliopathy is the primary event in MS pathogenesis and immune reactions are merely secondary phenomena [51]. Both OL recruitment and maturation defects are major causes of poor remyelination and axonal injury in MS. A differentiation block in oligodendroglial progenitors is a major determinant of remyelination failure in chronic MS lesions [52]. In MS, myelin-specific T cells are normally associated with the destruction of myelin and axonal damage, and the infiltrating myelin-specific T cells stimulate oligodendrogenesis in the adult CNS [53]. Enhancing endogenous tissue repair mechanisms, such as myelin repair, gliogenesis, and neurogenesis, is an important and increasingly realistic, therapeutic goal in MS. Aside from the above fundamental knowledge about miRNAs in OL differentiation and myelination, little is known about the direct relationship of miRNAs linking OLs to MS pathogenesis. However, recent studies have indirectly suggested that miRNA dysregulation (the expression and genetic analysis of miRNAs involved in CD4+ cell activation) may contribute to inflammatory demyelinating pathogenesis of MS and highlights the possibility to define different disease entities with specific miRNA profiles [54]. For example, 1145 miRNA expression was measured in peripheral blood mononuclear cells (PBMCs) from 19 MS patients and 14 controls, and the whole genome mRNA profiling was also performed in the same subjects wherein 104 miRNAs were identified as deregulated in MS patients, including novel putative deregulated miRNA and mRNA transcripts [55]. The results revealed novel pathogenic steps and identified potential biomarkers for MS. Several miRNAs identified as important participants in inflammatory demyelinating pathogenesis of MS are introduced (Table 1 and Fig. 3).

MiR-326

Interleukin 17 (IL-17)-producing T helper cells (T_H-17 cells) are increasingly recognized as key participants in various autoimmune diseases including MS. Mice with fewer T_H-17 cells are less susceptible to experimental autoimmune encephalomyelitis (EAE, an animal model of MS). T_H-17 cells have been found in lesions of brain tissues of patients with MS [56]. Large amounts of miR-326 has been found preferentially expressed in T_H-17 cells than in naive CD4+ T cells; its function is to positively regulate T_H-17 cell differentiation and affect the T_H-17 cell population through the translational inhibition of its functional target endogenous Ets-1 protein, a negative regulator of T_H-17 differentiation [details in 13 and 72]. MiR-326 knockdown alleviates EAE, whereas overexpression aggravates it. In the

brain white matter lesions of active MS patients, miR-326 expression was remarkably upregulated compared with those of the age-matched controls [57]. This result correlated with disease severity and IL-17 production [56]. The expression of miR-326 was also increased in the acute phase of EAE mice, which produced more IL-17-expressing $CD4^+$ T cells, but decreased to normal levels when the disease remitted. These results imply that miR-326 is a T_H-17 cell-associated miRNA that functions in MS pathogenesis, thereby providing a new target for manipulating the generation of T_H-17 cells in related diseases. Although the expression of miR-326 is upregulated during the transition from $A2B5^+$ OPCs into premyelinating $GalC^+$ OLs [31], further exploration of the function of miR-326 in OPCs or myelinating OLs is important for understanding the cell differentiation network in demyelinating diseases. MiR-326 may serve as a new and valuable target for clinical applications in patients with MS [58].

Moreover, by analyzing the expression level of 365 different mature miRNAs in active and inactive white matter lesions of the MS brains, miRNA profiles have been established, new insights into pathogenesis have been obtained, and potential targets for therapy of MS have been unveiled [59]. Among the significantly altered miRNAs, some show differential regulation in active versus inactive lesions, such as miR-326, -34a, and -155, are prominently upregulated in active MS lesions. The transcripts of the regulatory protein CD47 (a "do not eat me" signal involved in the inhibition of macrophage activity via interaction with signal regulatory protein α (SIRP)-α [59]) have been revealed to be highly downregulated by ~50% in active lesions compared with control white matter through immunohistochemistry and qRT-PCR techniques. This interaction between special miRNAs and CD47 releases macrophages/microglia from inhibitory control and then promote myelin phagocytosis, the pathologic hallmark of active MS lesions. Activated microglia and the release of molecules, which are detrimental to OL, have been suggested as mechanisms by which innate immunity causes demyelination in MS [60]. CD47 colocalized with both GFAP and proteolipoprotein, indicating that this broadly expressed protein is displayed by both astrocytes and OLs. However, further analysis of the local regulation of miRNAs in OLs in active and newly formed MS lesions is needed. MiR-155 targeted both its highly conserved and less conserved binding sites in the 3′-UTR of CD47 by screening the database Targetscan 5.0 [61, 62; www.targetscan.org/]. Furthermore, miR-326 and miR-34a also targeted the 3′-UTR of CD47. Therefore, the act of targeting these miRNAs might represent a potential therapeutic strategy to calm lesion activity.

MiR-145, miR-18b, miR-599, and miR-96

Using human miRNA microarray and the Geniom Real Time Analyzer (GRTA) platform, 866 human miRNA expression patterns in blood cells of relapsing-remitting MS (RRMS) patients were investigated. About 165 miRNAs were identified as significantly deregulated, demonstrating that unique miRNA signatures in RRMS patients allow accurate differentiation from healthy controls [63]. The most significant deregulated miRNA in human serum albumin (hsa), miR-145, that is upregulated during the transition from OPC to OL [15, 31], discriminates MS from the controls with an accuracy of 89.7%, a specificity of 89.5%, and a sensitivity of 90.0%. In addition, the combination of miR-155, miR-146a, and miR-142-3p yielded the best results, with a sensitivity of 77.8% and a specificity of 88.0% in predicting disease [57]. These data suggest that miRNA expression signatures may serve as potential biomarkers for the diagnosis and prognosis of MS and that the dysregulation of individual miRNA expression, and even more miRNA expression profiles, may play a critical role in the complex pathogenesis of MS, especially immune-mediated oligodendrogliopathy. Future studies should clarify whether these small molecules are also suited for the differentiation of specific courses or different pathogenetic subtypes of MS [63]. Another study revealed that miR-18b and miR-599 may be relevant to relapse time, and that miR-96 may be involved in remission [64]. The genes targeted by miR-96 are involved in immunological pathways, such as interleukin signaling, and in other pathways, such as Wnt signaling. The research elucidated the molecular mechanisms of MS and opened up a new therapeutic approach to explore and highlight some candidate biomarker targets in MS. Interestingly, miRNAs can also impair neurosteroidogenesis in MS and EAE. Some neurosteroid synthetic enzyme–specific miRNAs (miR-338, miR-155, and miR-491) have been identified, which suppress neurosteroidogenic enzymes and allopregnanolone in the white matter of MS patients. Allopregnanolone treatment of the EAE mouse model limited the associated neuropathology, including neuroinflammation, myelin, and axonal injury, and reduced neurobehavioral deficits [65]. Excitingly, by comparing the miRNA expression levels in MS subjects and normal samples, Otaegui et al. revealed new methods for diagnosing subjects afflicted with MS in a new patent [66], including tendencies of miRNA deregulation in MS. The new methods can be used to assess the subject in terms of experiences in remission or relapse status, as well as for designing gene therapy for MS patients.

MiR-17–92 Cluster

The miR-17–92 cluster, especially miR-19b, has a critical role in controlling the number of oligodendroglial cells by targeting the negative regulator of cell proliferation PTEN, as described above (Fig. 1). Furthermore, the available evidence indicates that miR-17 and miR-20a, two members

of the miR-17–92 cluster, are significantly under-expressed in the whole blood of MS patients, which not only facilitated astrocyte differentiation, but also promoted MS-associated T cell activation genes via the PI3K/Akt/mTOR pathway, and then induced the immune-mediated oligodendrogliopathy of the disease [67]. MiR-17-5p involved in auto-immunity was upregulated in the $CD4^+$ cells from MS patients. The functional experiments revealed that miR-17-5p potential target genes, phosphatidyl-inositol-3-kinase regulatory subunit 1 (PI3KR1) of the PI3K/Akt pathway and PTEN (an inhibitor of PI3K), were downregulated upon stimulation of $CD4^+$ cells with anti-CD3/CD28 in vitro (Fig. 1b). The responses to stimulation of the deregulated miRNAs were distinct, providing further insights into the importance of miRNA-dependent regulatory mechanisms in the immunopathogenesis of MS. However, the potential coordinated expression of miRNAs in MS needs further investigation [68].

The latest study reported the aberrant expression of four miRNAs, which had been previously reported to be critically involved in T_H-17 differentiation (e.g., miR-326, miR-155), the regulation of immune tolerance (miR-142-3p, miR-146a), or innate immunity (miR-146a). Excitingly, glatiramer acetate treatment seemed to normalize the levels of miR-146a and miR-142-3p but not miR-155 and miR-326 expression in RRMS patients [57], which accelerated the speed of restoring the expression of deregulated miRNAs in MS.

Collectively, the foregoing works have demonstrated that miRNAs play important roles in the inflammatory pathogenesis of MS, and that the myelin maintaining mature OLs are a presumed target of the T cell immune-mediated demyelinating process in this disease, providing a new way to promote myelin repair and serve as diagnostic and therapeutic molecules for MS. Further investigation of the role of OLs in the pathogenesis of MS along with miRNAs and the above fundamental knowledge is needed.

Ischemic Stroke

The vulnerability of OLs to ischemic injury may contribute to functional myelin loss in diseases of the central white matter [69]. Oligogenesis and the maturation of OPCs play an important role in functional recovery after ischemic stroke. Several studies have confirmed that miRNA profiles could be induced and developed as biomarkers for diagnosis and prognosis of cerebral ischemic stroke using brain (hippocampus) and peripheral blood cells [7, 70–74], providing new insights into the recovery approach of ischemic stroke.

Through transient focal ischemia in a middle cerebral artery occlusion and reperfused rat model, some temporal-expressed miRNAs highly expressed in the ischemic brain could simultaneously be detected in the blood samples, and their target genes were found to be regulated in parallel [71]. Different patterns of miRNA expression occurred in the brain and the blood 24 h after brain ischemia, compared with the untouched control animals [72]. The top five upregulated miRNAs (miR-298, -200b, -205, -107, and -423-5p) regulate multiple pathologic conditions in both brain and blood, such as ischemic stroke, intracerebral hemorrhage, and kainate seizures [72]. In the hippocampus of rats, the changes in miRNA expression profile were described after 20 min of global ischemia, followed by either 30 min or 24 h of reperfusion [73]. In the normal hippocampus, 286 miRNAs were detected, of which the let-7 family accounted for 32%. The number of related miRNAs dynamically changed according to the time of ischemia and reperfusion [73]. The results suggest that several miRNAs may be involved in regulating the normal physiological activity of hippocampus and in its response to brain ischemia and reperfusion injury. Moreover, these results contribute in identifying the targets and biological roles of these miRNAs and in developing new strategies to treat brain ischemia. Several studies have indicated that some specific miRNAs are critical in the pathogenesis of ischemic stroke, such as miR-145,-132,-200, and -182.

MiR-145

As described in MS, the upregulated miR-145 during the transition from OPC to OL also participated in ischemic stroke etiology and pathology. The antagomir-mediated prevention of significantly upregulated miR-145 expression has been investigated to lead to an increased protein expression of its downstream target, superoxide dismutase-2 (SOD2), in the post-ischemic brain [70]. In contrast, the mRNA expression of Drosha, Pasha, Exportin-5, and Dicer, which modulate miRNA biogenesis, were not altered, indicating that miRNAs play essential roles in regulating mRNA transcription and translation in the post-ischemic brain, but focal ischemia has little effect on the miRNA synthetic machinery. Their potential targets are mRNAs that are known to mediate inflammation, transcription, neuroprotection, receptors function, and ionic homeostasis. Interestingly, a recent study found that human SOD1 overexpression increases the proliferation of OPCs and accelerates their differentiation to mature OL in vitro. Furthermore, SOD1 overexpression reduces oxidative stress-mediated death in OPCs. These results suggest that miR-145 regulates SOD as a therapeutic target for increasing OPC response for OL replacement and remyelination after injury [75].

MiR-132

In the ischemic mouse model, distinct changes in the miRNA expression were observed in the preconditioned, ischemic, and tolerant brain cortices [76]. MiR-132, which

regulates MeCP2 (methyl-CpG binding protein 2, also expressed in glial cells) expression, decreased in the preconditioned cortex. The downregulation of miR-132 is consistent with the preconditioning ischemia and it induces a rapid increase in the MeCP2 protein, but not the mRNA levels, in the mouse cortex. An increased susceptibility to preconditioning ischemia in was shown in MeCP2 knockout mice, which suggests that miRNAs and MeCP2 could serve as effectors of ischemic preconditioned (IPC)-induced tolerance. In addition, recent studies have shown that MeCP2 is not restricted to neuronal cells, but also expressed in embryonic and adult stages in all glial cell types, including astrocytes, OPCs, OLs, and microglia (see details in ref. [76, 77]). Further studies are required to understand how MeCP2 contributes to ischemic tolerance and its role in the vulnerability of OLs to ischemic injury.

MiR-200 and miR-182 Clusters

Two miRNA clusters, miR-200 (miR-200a, -200b, -200c, -141, and −429) and miR-182 (miR-182, -183, and -96), were upregulated early after ischemic preconditioning [78]. Among them, miR-200c showed target bias in $A2B5^{+}$ $GalC^{-}$ cells [31]. Furthermore, miR-200b, -200c, and -429 targeted the oxygen-dependent enzyme prolyl hydroxylase 2 (PHD2, downregulated) and had the best neuroprotective effect. Following focal cerebral ischemia, significant changes were found in the miRNA transcriptome, independent of an effect on the expression of miRNA machinery. The changes implicated miRNAs in the pathologic cascade of events that included blood–brain barrier disruption (miR-15a) and caspase-mediated cell death signaling (miR-497). The early activation of miR-200 family members improved neural cell survival via PHD2 mRNA silencing and subsequent HIF-1α (hypoxia-inducible factors-1α, a well-established transcription factor rapidly induced by hypoxia) stabilization. The pro- (miR-125b) and anti-inflammatory (miR-26a, -34a, -145, and let-7b) miRNA may also be manipulated to influence stroke outcomes positively.

Furthermore, pathway analysis shows that mitogen-activated protein kinase and mammalian target of rapamycin (mTOR) signaling were the top two Kyoto Encyclopedia of Genes and Genomes pathways targeted by the upregulated miRNAs. Wnt and GnRH (gonadotropin-releasing hormone) signaling were the top two pathways targeted by the downregulated miRNAs after ischemic preconditioning. Interestingly, the altered miRNAs had common targets, such as 8 upregulated miRNAs that target FXR1 (Fragile X mental retardation 1) and ten downregulated miRNAs that target MeCP2 [70].

The alterations in the miRNAs and their downstream mRNAs might play a role in the induction of ischemic tolerance. These miRNAs may be useful in future research and therapeutic applications. Successfully implemented miRNA therapeutics will direct the future of gene therapy and offer new therapeutic strategies by regulating large sets of genes in related pathways of the ischemic stroke cascade. However, the specific role of each miRNA in the different intracellular processes in the ischemic brain and the relevance of their temporal and spatial expression patterns need further investigation, which may lead to novel strategies for therapeutic interventions. Future studies will show whether the modulation of specific miRNAs in OL lineage can be used as a therapeutic option to prevent post-ischemic pathophysiologic events of OLs and to promote plasticity and regeneration. Future studies are also needed to evaluate the possible use of miRNAs as biomarkers in ischemic stroke and related demyelinating pathologies of the disease.

Leukodystrophy

To date, ADLD is the only human disease linked to lamin B1 (LMNB1) mutation. LMNB1 is one of the major components of the nuclear envelope and is essential for maintaining nuclear integrity, gene expression, and many other functions. The duplication of the gene LMNB1 causes severe demyelinating phenotype in the brains of adult-onset ADLD patients [79].

What is the mechanism by which elevated LMNB1 gene dosage leads to myelin loss in ADLD? Recent works have shown that LMNB1 overexpression suppresses OL-specific genes, such as MBP, proteolipid protein (PLP), and MOG. The LMNB1 expression level is crucial in regulating the progress of OL maturation and myelin formation, uncovering a novel function for nuclear structural proteins [80]. Western blot and qRT-PCR confirmed that LMNB1 is developmentally regulated during OL maturation. The LMNB1 expression pattern shows an inverse correlation with other myelin-specific proteins, such as CNP, suggesting a potential role for LMNB1 in the regulation of OL development.

To test whether miRNAs play a role in the developmental regulation of LMNB1, Lin and Fu [80] sought to identify miRNAs that targeted mouse LMNB1 mRNA. Using a luciferase reporter and Western blot analysis, miR-23 was identified as a negative regulator of LMNB1 that could counteract the defects caused by increased LMNB1 dosage. The knockdown of the endogenous miR-23 with synthetic antisense probe results in increased LMNB1 levels in OLs. LMNB1 overexpression leads to altered MBP and PLP subcellular localization and reduced OL-(CNP) and myelin-(MBP, PLP, MAG) specific proteins in vivo. MiR-23 could also restore OL maturation defects caused by LMNB1 overexpression via a dosage-dependent biological effect, which indicates that LMNB1 has a threshold effect on OL development and myelin production. When constructs carrying

LMNB1 with mutated miR-23 binding sites were used, no restoration was detected. The LMNB1 and miR-23 overexpression experiments revealed that OL development, but not astrocyte, was specifically suppressed by LMNB1 and enhanced by miR-23. That is, miR-23 acts as a positive regulator of OL development, whereas LMNB1 is a suppressor of OL maturation, which raises the possibility that a finely regulated LMNB1 expression might be important in the control of OL development and myelin formation in vivo. These results indicate a role for miR-23 in downregulating LMNB1 expression, especially in adults. MiR-23 is important for the transitioning of OPCs into mature OLs. Further exploration of the mechanisms that link miR-23 and LMNB1 to OL maturation and myelin maintenance might provide insights into new therapeutic agents for ADLD and other demyelination-related diseases [5, 80].

Conclusion and Prospects

MiRNAs play essential roles in regulating OL differentiation and myelination by inhibiting negative genes that maintain the undifferentiated state of OPCs, increasing the number of OPCs and OLs, and suppressing the inappropriate or transiently required high-level expression of genes in OPCs and OLs. The potential clinical implications of miRNAs as diagnostic and prognostic indicators of disease type and status and as a novel therapeutic target in demyelination-related diseases, such as MS, ischemic stroke, and leukodystrophy, are quite valuable. Thus, better understanding of miRNA mechanisms might shed light not only on the pathogenesis of OL-related diseases, but also on potential approaches for managing or suppressing the pathogenesis of these diseases. The global patterns of miRNA expression might be more revealing than the analysis of single miRNAs. However, the mere knowledge of the physical interaction networks between specific mRNAs and miRNAs that can be used to predict putative causal regulatory interactions in myelinating diseases is insufficient [81]. Other factors, such as Egr1, Sox11, and ErbB4 [3, 82], that inhibit OL differentiation may also interact with unknown but related miRNAs; thus, further studies are needed.

MiRNA-based therapies could involve the administration of a specific miRNA mimic to downregulate target genes or antisense probes for the blocking of certain miRNAs to increase the expression of target genes in OLs. However, because the manipulation of one miRNA may affect multiple mRNAs and one mRNA may be regulated by several miRNAs, guarding against off-target effects is important. Furthermore, significant challenges to successful therapy include the development of a targeted delivery system, prolongation of the presence of miRNAs, and reducing the risk of triggering a cellular immune response with RNA therapy. Different approaches have been developed to inhibit endogenous miRNA activity, including the modification and mimic of miRNAs, such as anti-miRNA oligonucleotides (AMOs) [83] and artificial miRNA sponges [84]. A very promising approach of AMOs may be the use of locked nucleic acids (LNAs). These molecules comprise a class of bicyclic conformational analogs of RNA that exhibit high binding affinity with complementary RNA molecules and high stability in blood and tissues in vivo [85]. Recent reports on LNA-mediated miRNA silencing in primates supported the potential of LNA-modified OLs in studying miRNA functions in vivo and in the future development of miRNA-based therapeutics [86]. Present clinical studies about miRNAs in demyelination-related diseases are limited to non-OL lineage cells; therefore, the miRNAs involved in myelinating diseases that target OLs need further investigation.

We concluded that miRNAs might act as promising diagnostic and prognostic biomarkers for disease type and status, and as novel therapeutic targets in OL differentiation and demyelination-related diseases. Further understanding of the functions of miRNAs in specific disease types, including miRNA expression profiling, predicated targets, and the interaction of miRNA–mRNA networks, is urgently needed. Consequently, the novel era of OL differentiation and demyelination-related diseases is coming.

Acknowledgments This work was supported by grants from the National Natural Science Foundation of China (grant no. 31071056) and the Natural Science Foundation Project of CQ CSTC (2011jjA10026).

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