MicroRNA Regulation of CNS Myelination

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Introduction

MicroRNAs (miRNAs) are a class of small (~22 nt) noncoding RNAs that are capable of posttranscriptionally silencing mRNAs that contain sequences complementary to the miRNAs' 7–8 bp "seed" sequence (Bartel, 2004; Wu and Belasco, 2008). Because single miRNAs are predicted to often target up to hundreds of individual transcripts, miRNAs are able to broadly affect the overall protein expression state of the cell. This capability can translate into global effects on cellular health and differentiation state. Recently, several reports have identified crucial roles for miRNAs in controlling the production, differentiation, and health of myelinating cells of the mammalian nervous system. In this chapter, we will discuss how individual miRNAs regulate these various processes and how miRNA production in general is required for several stages of myelin generation and maintenance.

Oligodendrocytes Require miRNAs at Various Stages of Development

To study the overall role of miRNAs in biological processes, researchers have knocked out enzymes required for normal miRNA processing, such as Dicer1 (Bartel, 2004). However, *Dicer1*$^{-/-}$ mice die embryonically (Bernstein et al., 2003). Therefore, to study the requirement of mature miRNAs in postnatal processes, Cre-mediated recombination has been used to disrupt Dicer1 function in specific cells of interest. In this way, functional miRNAs have been shown to be required at all stages of oligodendrocyte (OL) generation and myelination in the mammalian CNS.

The knockout of Dicer1 function in uncommitted neural precursors, by driving Cre expression from the *Nestin* promoter, leads to a reduction in overall OL lineage cell number: both mature OLs and immature OL precursor cells (OPCs) (Kawase-Koga et al., 2009). This effect may represent a reduction in OPC generation from neural precursors, as opposed to OPC proliferation, because disruption of Dicer1 function in specified OPCs and OLs, by expressing Cre from either the *Olig1* or *Olig2* promoter, does not reduce OPC number *in vivo* (Dugas et al., 2010; Zhao et al., 2010). However, OPCs that lack Dicer1 do fail to differentiate normally because OL differentiation and myelin formation are significantly disrupted in *Olig1-Cre*, *Olig2-Cre*, and *CNP1-Cre Dicer*$^{Flox/Flox}$ mice, and OPCs purified from these animals fail to differentiate normally *in vitro*. Finally, mature miRNA production is not only necessary during development but is also required to maintain healthy myelin: Disrupting Dicer1 function specifically in fully mature OLs, by driving tamoxifen-inducible Cre expression from a *PLP* promoter, leads to the eventual degradation of fully formed CNS myelin (Shin et al., 2009).

In summary, these results indicate that mature miRNA activity is required at various stages of OL development:

- In the initial production of fate-specified OPCs;
- In the differentiation of mature OLs and generation of compact CNS myelin during development; and
- In the maintenance of functional myelin sheaths in older animals (Fig. 1).

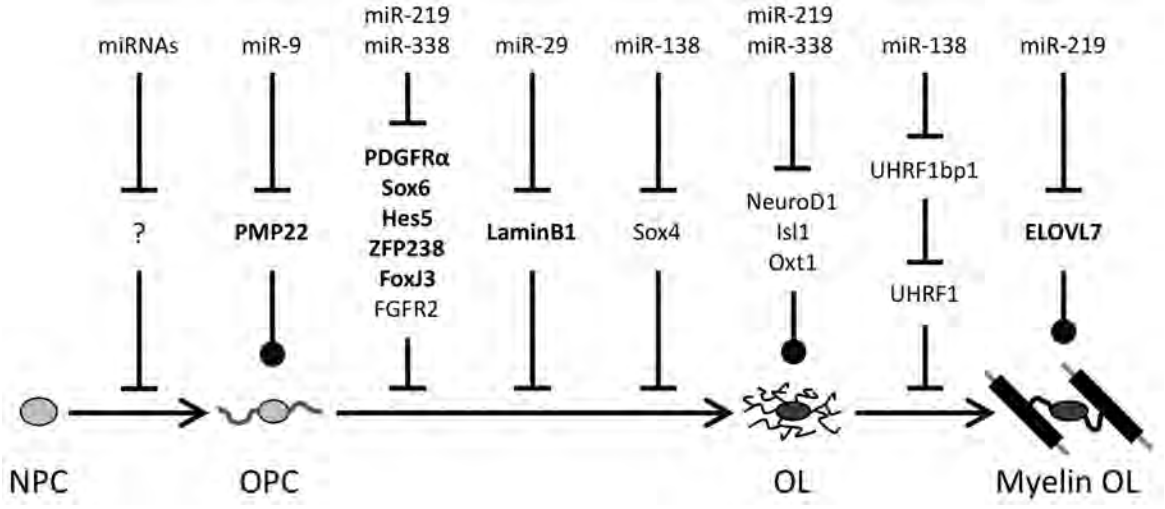


Figure 1. miRNA regulation of CNS myelination. Specific miRNAs involved in regulating various stages of OL differentiation and myelination are shown (or miRNAs in general in the neural precursor to OPC transition). Targets of miRNAs are shown; confirmed targets are in bold and predicted targets in plain text. miR-9 is more highly expressed in OPCs, whereas all other miRNAs shown are more highly expressed in OLs. Inhibition of expression or stage transition is shown by lines with bars; expression that may be detrimental to cell health or function is shown by lines with circles.

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Influences of Individual miRNAs on Oligodendrocyte Biology

Having determined that mature miRNAs are required for normal OL generation and myelin formation, several labs have subsequently investigated the roles of individual miRNAs in promoting functional CNS myelination. These experiments have identified specific miRNAs that promote the formation and maintenance of healthy CNS myelin by three distinct mechanisms:

- The suppression of OPC-expressed genes to promote differentiation;
- The overall suppression of inappropriate non-OL lineage gene expression in OPCs and OLs; and
- The suppression of genes transiently required at high levels during myelin sheath formation.

miRNA promotion of OL differentiation

miR-219

Several labs have noted that miR-219 is the most highly expressed/strongly induced miRNA in differentiating OLs (Lau et al., 2008; Dugas et al., 2010; Zhao et al., 2010) In addition, miR-219 expression appears to be restricted to the vertebrate CNS and to be restricted to mature OLs within the CNS (Wienholds et al., 2005; Dugas et al., 2010), indicating that miR-219 is highly enriched within OLs relative to all other vertebrate tissues. Functionally, miR-219 alone is both necessary and sufficient to promote normal OPC differentiation into OLs *in vitro* and *in vivo*.

miR-219 appears to exert its effects, at least in part, by suppressing the production of several OPC-expressed proteins that normally hinder OL differentiation: PDGFRα, the receptor for the OPC mitogen PDGF, is directly suppressed by miR-219, as are the differentiation-inhibiting transcription factors Sox6, Hes5, ZFP238, and FoxJ3 (Dugas et al., 2010; Zhao et al., 2010). These data indicate a model whereby miR-219 links the initiation of OL differentiation to the suppression of OPC proliferation. By strongly inducing miR-219 at the outset of OL differentiation, the OPC is able to simultaneously suppress the production of several genes that normally maintain the OPC in a proliferative, undifferentiated state, thereby facilitating the rapid state change from proliferating precursor to postmitotic, differentiated OL. Indeed, this model of miRNA function as increasing the gain of a developmental state change has been postulated previously (Reinhart et al., 2000; Bartel, 2009) and, therefore, the results observed for miR-219 may represent one general mode of action of miRNAs in development.

miR-338

miR-338 has also been detected as an miRNA that is strongly induced during OL differentiation and been shown to target the proliferation-promoting genes *Sox6*, *Hes5*, and *ZFP238* (Lau et al., 2008; Dugas et al., 2010; Zhao et al., 2010). However, miR-338 expression appears to be less widespread *in vivo*, as strong expression of miR-338 is detected only in the spinal cord, and significantly weaker expression has been detected in the brain (Dugas et al., 2010; Zhao et al., 2010). In addition, only Zhao et al. (2010) were able to detect a functional role for miR-338 in promoting OL differentiation, whereas Dugas et al. (2010) did not. These differing results may potentially be explained by the fact that miR-338 is also predicted to target FGFR2. Altering miR-338 activity may produce significant effects only on OPCs cultured in the presence of FGF for this reason: FGF is a mitogen for OPCs, but FGF was present only in experiments performed by Zhao et al. but not Dugas et al. In addition, Dugas et al. used only miR-338-5p in experiments, whereas Zhao et al. used both miR-338-5p and miR-338-3p. It is the 3p strand that targets FGFR2 and ZFP238. Therefore, the 3p strand of miR-338 may significantly contribute to the promotion of OL differentiation observed by Zhao et al. Despite these caveats, miR-338 appears to function similarly to miR-219: to increase the rate of OL differentiation by inhibiting the production of OPC-expressed proliferation-promoting genes.

miR-23

miR-23a and miR-23b are both induced ~5× during OL maturation, and overexpression of either can enhance OL differentiation (Lau et al., 2008; Lin and Fu, 2009). miR-23 represses Lamin B1 expression, which is normally downregulated during OL differentiation; overexpression of Lamin B1 inhibits the normal morphological differentiation of OLs, and *Lmnb1* duplication in humans leads to late loss of healthy myelin in autosomal dominant leukodystrophy (Padiath et al., 2006; Lin and Fu, 2009). Therefore, miR-23 appears to also influence OL differentiation by reducing the expression of a gene that inhibits normal OL maturation.

miR-138

miR-138, which is also induced in differentiating OLs, appears to play an interesting role in regulating OL differentiation, which proceeds in a series of distinct temporal stages (Baumann and Pham-Dinh, 2001; Dugas et al., 2006). Whereas miR-219 promotes all stages of OL differentiation, miR-138 specifically promotes the early stages (CNP^+ and MBP^+) of OL differentiation while suppressing the later (MOG^+) stage (Dugas et al., 2010). This

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intermediate MBP+/MOG– stage corresponds to the point at which early differentiating OLs extend processes to contact axons and initiate myelin sheath formation; by the time OLs are MOG+, they have lost the ability to form new myelin sheaths (Watkins et al., 2008). Potentially, miR-138 could play a central role in prolonging this intermediate stage of OL differentiation, which would extend the time frame in which a newly differentiating OL could form the contacts that will produce mature myelin sheaths. How miR-138 accomplishes this remains unclear, but potentially, miR-138 could simultaneously target one set of genes that represses the initiation of OL differentiation and another set of genes that promotes the late stage of differentiation. Candidates include *Sox4*, which pairs with *Sox6* to inhibit early OL differentiation (Stolt et al., 2006), and *UHRF1bp1*, a putative binding partner of *UHRF1*, which itself has been shown to specifically inhibit the late phase of OL differentiation (Dugas et al., 2006).

miRNA suppression of inappropriate gene expression

Often, the expression patterns of genes targeted by an miRNA are inversely correlated with the expression of the targeting miRNA. For example, as miR-219 expression levels rise in differentiating OLs, the expression of several genes targeted by miR-219 falls (Dugas et al., 2010). However, this is not always the case.

miR-9

In one of the earliest studies of miRNA expression in OL-lineage cells, miR-9 was one of two OPC-enriched miRNAs identified whose expression positively correlated with its predicted targets (Lau et al., 2008). This correlated expression pattern may indicate miR-9's role in repressing inappropriate gene expression: miR-9 may be expressed to silence the "leaky" expression of genes that should not be produced in OL-lineage cells and would therefore be required at highest levels when its targeted genes are also being most highly expressed. In fact, this appears to be the case for at least one gene miR-9 directly targets: *PMP-22*. PMP-22 is normally produced only in PNS-myelinating Schwann cells, yet *PMP-22* mRNA expression is observed in OPCs (Baumann and Pham-Dinh, 2001; Dugas et al., 2006; Cahoy et al., 2008). Lau and colleagues (2008) demonstrated that miR-9 directly prevents the inappropriate production of PMP-22 protein in OL-lineage cells. These data illustrate that miRNAs play an additional role as "guardians of the transcriptome" by preventing inappropriately expressed mRNAs from being translated into functional proteins that could detrimentally affect the health of the cell.

miR-219 and miR-338

In addition to the role miR-219 and miR-338 play in promoting OL differentiation, Zhao et al. (2010) point out that these miRNAs may also play a role in inhibiting the production of neurogenic factors in OLs, such as NeuroD1, Isl1, and Otx1—all targets of one or both of these miRNAs. The fact that miR-219 and miR-338 could be involved both in extinguishing OPC gene expression and blocking inappropriate neurogenic gene expression illustrates the fact that miRNAs, by virtue of the wide variety of genes they are capable of targeting, can simultaneously influence distinct aspects of a cell's gene expression program.

miRNA suppression of genes transiently required during differentiation

miR-219

miR-219, in addition to its role in promoting OL differentiation and putative role in preventing inappropriate neurogenic gene expression, contributes to the regulation of gene expression in fully mature OLs. Robust OL expression of miR-219 has been consistently detected in adult (P50-60) mice, and the expression of miR-219 is lost when Dicer1 function is specifically ablated in mature OLs in mice containing a tamoxifen-inducible *PLP-CreER*^{T} gene (Shin et al., 2009; Dugas et al., 2010). Interestingly, disruption of OL-expressed Dicer1 at P14-18 leads to a strong reduction in miR-219 levels in mature OLs by P30, but at this age, mutant mice look normal and begin to show functional deficits only by P60-90, with reduced CNS myelin observed at P180. So why the delay, and what is the reason for the eventual loss of healthy CNS myelin? In these mice, Dicer1 function is disrupted only in mature OLs, so OL specification and differentiation should not be adversely affected. Instead, these results indicate a role for mature miRNAs in maintaining healthy myelin, with the caveat that miRNAs in mature OLs are more acutely required after myelin has been fully formed by P45-60 (Baumann and Pham-Dinh, 2001) as opposed to during the initial generation of myelin sheaths.

This dichotomy may be explained by the fact that a prominent target of miR-219 is *ELOVL7* (Shin et al., 2009). ELOVL7 is an enzyme expressed at high levels in OLs that is involved in the production of very-long-chain fatty acids (VLCFAs) (Cahoy et al., 2008; Tamura et al., 2009). VLCFAs are incorporated into proteolipid protein (PLP) as an integral component of the fatty myelin sheath, but overproduction of VLCFAs can lead to demyelinating diseases such as X-linked adrenoleukodystrophy (Dubois-Dalcq et al., 1999). Potentially, high levels

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of ELOVL7 activity could be required during active myelin sheath formation, when large amounts of lipid-rich membranes are being produced, but this production would need to be tempered once full axonal myelination is completed. miR-219 may serve this role in mature OLs by reducing the amount of functional ELOVL7 produced from the highly transcribed *ELOVL7* locus.

In general, these data indicate that another role for miRNAs in OLs (and likely in other cell types) may be to moderate the expression of proteins that are required at high levels for the transformation from immature to fully differentiated phenotype but whose continued high-level expression in stable, mature cells may be detrimental. As a consequence of the continued expression of transcription factors that specify the mature state of the cell, expression of these genes would persist but could be regulated by miRNAs' suppression of targeted genes.

miRNA in glioma

The role of miRNAs in promoting OL differentiation by inhibiting OPC-expressed proliferation-promoting genes may also indicate that miRNA misregulation could contribute to glioma proliferation; if OL-expressed miRNAs inhibit proliferation, then loss of these miRNAs would create a permissive environment for tumorigenesis. In fact, in analyzed medulloblastoma samples, OL-expressed miR-219, miR-138, and miR-192 are all downregulated relative to normal control tissue expression (Ferretti et al., 2009). Cumulatively, these data indicate that reintroducing OL-enriched miRNAs into active CNS tumors, especially those expressing characteristics of the OPC-OL lineage, could prove efficacious for blocking tumor progression and/or driving tumor regression.

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