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MicroRNA and Transcriptional Crosstalk in Myelinating Glia

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Abstract

Several recent studies have addressed the important role of microRNA in regulation of differentiation of myelinating glia. While Schwann cells and oligodendrocytes in the peripheral and central nervous systems, respectively, exhibit significant morphological and regulatory differences, some aspects of transcriptional and microRNA regulation are shared between these two cell types. This review focuses on the intersection of microRNAs with transcriptional regulation in Schwann cell and oligodendrocyte differentiation. In particular, several microRNAs have been shown to modulate expression of critical transcription factors, and in turn, the regulation of microRNA expression is enmeshed within transcriptional networks that coordinate both coding gene and noncoding RNA profiles of myelinating cells. These hubs of regulation control both myelin gene expression as well as the cell cycle transitions of Schwann cells and oligodendrocytes as they terminally differentiate. In addition, some studies have begun to highlight the combinatorial effects of different microRNAs that establish the narrow range of gene regulation required for efficient and stable myelin formation. Overall, the integration of microRNA and transcriptional aspects will help elucidate mechanistic control of the myelination process.

Myelin is required to enable saltatory conduction of action potentials, and also to provide long term trophic support that maintains axonal integrity (reviewed in Nave, 2010; Nave and Trapp, 2008). Myelin is synthesized by two different cell types—Schwann cells and oligodendrocytes—in the peripheral and central nervous systems, respectively. These systems are developmentally and functionally independent, and specific molecular pathways exhibit significant differences. Disruptions in nerve myelination can occur as a result of a broad spectrum of genetic, inflammatory, metabolic, and infectious disorders. For example, hereditary peripheral neuropathies are among the most common genetic diseases affecting the nervous system (Saporta et al., 2011). Myelin stability is notably sensitive to subtle changes in gene expression, as gene dosage effects are a major cause of myelin disorders, such as Charcot-Marie-Tooth (CMT) disease and Pelizaeus-Merzbacher disease.

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Since the role of microRNAs in myelinating glia has been a very active area of investigation, several excellent reviews have recently covered various aspects of microRNA regulation (Barca-Mayo and Lu, 2012; Dugas and Notterpek, 2011; He et al., 2011; Zheng et al., 2012). Initial studies of microRNA function in myelinating glia have focused on microarray profiling and using knockouts of Dicer, which is required for formation of mature microRNAs. In these studies, microRNAs have emerged as important modulators of gene expression patterns in oligodendrocytes and Schwann cells. Moreover, the roles of microRNAs are not limited to development of myelin but also extend to myelin maintenance and response to injury. The purpose of this review is to highlight connections between microRNAs and transcriptional regulators of Schwann cell and oligodendrocyte development. This review will take advantage of newly emerging genomic patterns of transcription factor binding established by ChIP-Seq analysis (Bujalka et al., 2013; Gokey et al., 2012; Srinivasan et al., 2012; Yu et al., 2013), and which highlight transcriptional control of microRNAs themselves.

Despite the significant differences between PNS and CNS myelination, transcriptional regulation of these two cell types affect some common target genes (e.g. Proteolipid protein and myelin basic protein), and accordingly there are shared transcriptional pathways. For example, Sox10 is required throughout Schwann cell and oligodendrocyte development, and its expression is also maintained in mature myelinating cells, (Bremer et al., 2011; Stolt et al., 2002; Stolt and Wegner, 2010). The importance of Sox10 regulation in myelinating glia is also shown by the identification of dominant negative Sox10 mutations that cause a complex syndrome affecting both central and peripheral myelin called PCWH (peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease) (Inoue et al., 2004; Inoue et al., 2002). However, there are very significant differences in the transcriptional regulation in the two cell types (Emery, 2010; Meijer and Svaren, 2013; Mitew et al., 2013; Svaren and Meijer, 2008). For example, the Egr2/Krox20 transcription factor is induced by axonal signals in myelinating Schwann cells and is required for initiation and maintenance of myelination in the peripheral nervous system (4,5). Both SOX10 and EGR2 continue to be expressed in myelinating Schwann cells through adulthood, and inducible deletion of either factor in mature Schwann cells leads to demyelination (6,7). However, Egr2 loss does not affect CNS myelination, but instead a series of other transcription factors are uniquely required for oligodendrocyte myelination, including Olig2 and the recently discovered MyRF transcription factor (Bujalka et al., 2013; Emery et al., 2009; Mitew et al., 2013). MyRF is comparable in some respects to Egr2/Krox20 since it is uniquely induced in myelinating oligodendrocytes.

Analysis of MicroRNA function through Dicer deletion in myelinating glia

Knockouts of Dicer have been performed in oligodendrocytes using cre driver genes driven by Plp (Shin et al., 2009), Olig1 (Zhao et al., 2010), Olig2 and Cnp (Dugas et al., 2010). These studies have shown that Dicer is required for differentiation of both cell types as well as cell cycle exit (Dugas et al., 2010; Zhao et al., 2010). The failure to differentiate is reflected by a loss of mature myelin markers such as Myelin Basic Protein (MBP). Two highly expressed microRNAs in oligodendrocytes were investigated further, and miR219 was able to induce formation of PGDFR α positive OPCs that also express Olig2 in chick

embryo transfections, but miR338 expression also promoted expression of MBP (Zhao et al., 2010). However, ectopic introduction of miR338 and miR219 in cultured oligodendrocytes was able to partially rescue the phenotype and promote myelin gene expression (Dugas et al., 2010; Zhao et al., 2010). Moreover, morpholino analysis of miR219 (but not miR338) caused a reduced number of mature oligodendrocytes in zebrafish studies (Zhao et al., 2010).

Similar studies have implicated microRNAs in the regulation of peripheral nerve myelination. In mouse models where Dicer was specifically ablated in Schwann cells (using Desert Hedgehog-cre or Myelin Protein Zero-cre), the Schwann cells remained viable and underwent the normal radial sorting process, albeit somewhat delayed (Bremer et al., 2010; Pereira et al., 2010; Yun et al., 2010). However, Schwann cells lacking Dicer fail to produce myelin and instead continue to proliferate. The knockouts display decreased myelin gene expression and lower levels of the Egr2/Krox20 transcription factor. The studies variably show some induction of immature (or nonmyelinating) Schwann cell markers in the absence of Dicer, such as p75/Ngfr, Sox2, Notch1, and c-Jun. Interestingly, the use of Dhh-cre failed to show any proliferation changes at P4 (Pereira et al., 2010), but in a separate study with the same cre driver, increased proliferation was observed at P22 when there are very few dividing cells in control nerve (Bremer et al., 2010). The use of Mpz-cre revealed increased proliferation as early as P7, with a more pronounced increase at P14 (Yun et al., 2010). The promyelinating Schwann cell markers Oct6/Pou3f1 and Brn2/Pou3f2 mRNAs were reduced (P4) in one study (Bremer et al., 2010), consistent with slightly lower Oct6/Pou3f1 protein at P5 (Pereira et al., 2010). Many of the Schwann cells eventually arrest at a promyelinating stage after P20, and accordingly immunohistochemistry revealed an increased number of Oct6/Pou3f1 positive Schwann cells at P22 in the Mpz-cre study (Yun et al., 2010). Another important observation is that the major neuregulin/Akt-regulated pathway that regulates myelination (Nave and Salzer, 2006) is notably reduced in the absence of Dicer in early postnatal timepoints (Bremer et al., 2010; Pereira et al., 2010). Finally, there is some persistent development of myelin profiles despite the lack of Dicer, which is enhanced in dorsal roots compared to ventral roots and sciatic nerve (Pereira et al., 2010), suggesting that Dicer may not be absolutely required for myelin initiation. However, the residual profiles are still much thinner than in control nerve. Studies using Dicer shRNA in cultured Schwann cells revealed increased proliferation as well as reduced expression of Egr2 and major myelin genes (Verrier et al., 2010).

Studies have not only probed the involvement of Dicer in developmental control, but have also addressed the role of Dicer in myelin maintenance. Using a tamoxifen-inducible cre driven by the Plp promoter, induced deletion of Dicer beginning at P14 was shown to cause hindlimb ataxia at ~2 months, and demyelination in the CNS apparent at 6 months, along with astrogliosis, microgliosis, and increased APP accumulation consistent with axon degeneration (Shin et al., 2009). A similar study was performed using the same cre driver to analyze the role of Dicer in peripheral myelin maintenance. Somewhat surprisingly, there was no apparent changes in Schwann cell morphology reported, up to 14 weeks later after cre activation (Viader et al., 2011). This may reflect a more stable mechanism of myelin maintenance which is less activity dependent in Schwann cells.

Microarray Profiling and Target Identification

Microarray analyses have identified a series of microRNAs that are developmentally regulated during maturation of oligodendrocytes (Dugas et al., 2010; Lau et al., 2008; Zhao et al., 2010) or Schwann cells (Bremer et al., 2010; Gokey et al., 2012; Pereira et al., 2010; Yun et al., 2010). Some microRNAs have been found to be specific to oligodendrocytes (e.g. miR219, as compared to other cell types) or oligodendrocytes and Schwann cells (miR138 and miR338). Target identification of individual microRNAs has so far relied on target prediction programs which indicate putative targets, which can be further refined by integrating with previous microarray analyses of developmentally regulated gene expression in oligodendrocytes and Schwann cells (Lau et al., 2008; Viader et al., 2011), since expression levels of target mRNAs are often inversely correlated with microRNA levels (Guo et al., 2010). Validation of microRNA targets in myelinating glia has so far not utilized specific knockout mice for individual microRNAs, but instead has relied upon use of microRNA blockers or mimics in cultured cells as well as 3' UTR luciferase assays. One recent bioinformatics analyses has been performed using Schwann cell microRNA and mRNA expression data (Chang et al., 2013). While such approaches are valuable, there are some cases where microRNAs can change protein levels without affecting steady state levels of the target mRNA (Hoffmann et al., 2014).

As a result of these efforts, some targets of specific microRNAs have begun to be identified (Bremer et al., 2010; Pereira et al., 2010; Verrier et al., 2010; Yun et al., 2010). Target analysis for miR338 identified Hes5 and Sox6, which are antagonists of oligodendrocyte maturation (Zhao et al., 2010). Other predicted targets include proneural transcription factors, suggesting that microRNAs may maintain lineage specification by inhibiting inappropriate transcription factor expression. Targets of miR219 in oligodendrocytes appear to be Sox6 and the PDGFR α gene (Dugas et al., 2010), which encodes a receptor for the major mitogen (PDGF) for OPCs. Since Sox6 is targeted by both miR219 and miR338, this is consistent with its previously described role in inhibiting oligodendrocyte differentiation (Stolt et al., 2006). Accordingly, Sox6 is expressed in OPCs but not in mature oligodendrocytes (Zhao et al., 2010). Similarly, analysis of microRNAs in Schwann cells indicated that miR138 is induced during myelination, and 3' UTR assays showed that it could reduce expression of Sox2, c-jun, and cyclinD1 (Yun et al., 2010), which are downregulated during peripheral nerve myelination. Since the demonstrated effect of a single microRNA can be modest (20-50%), it seems likely that more profound expression level changes may be mediated by combinatorial microRNA targeting, such as miR338/219 targeting Sox6 (described above). Another example of this is cyclin D1, which has been shown to be targeted by miR138 and -34a (see below).

Recent studies have extended microarray profiling to analysis of peripheral nerve after nerve injury (Adilakshmi et al., 2012; Viader et al., 2011; Yu et al., 2012), where Schwann cells demyelinate, resume proliferation, and also begin to secrete factors that stimulate nerve regeneration leading to eventual remyelination (Arthur-Farraj et al., 2012; Jessen and Mirsky, 2008; Stassart et al., 2013). These data show an injury-induced decline in miR338 and miR138, and in addition show that approximately half of microRNAs expressed in Schwann cells are dynamically regulated. Interestingly, the same study used an inducible

Plp-cre to ablate Dicer in mature mice. As noted above, loss of Dicer had little effect on myelin maintenance, and also the early stages of nerve injury. However, recovery of myelination after nerve crush injury was defective (Viader et al., 2011). These data indicate that Dicer is not required for the demyelination accompanying nerve injury, but rather for remyelination.

Transcriptional regulation of microRNAs

While the targets of microRNA action are being elucidated, it is also important to identify how such microRNAs are regulated, and how this regulation relates to the established transcriptional regulators of oligodendrocyte and Schwann cell development. Many microRNAs reside within introns of genes with which they are co-transcribed, or are processed from longer transcripts that may encompass one or more closely linked microRNAs (Finnegan and Pasquinelli, 2013; Graves and Zeng, 2012). Therefore, transcriptional regulation of the host gene (or a longer noncoding transcript) can be a determinant of microRNA levels, although post-transcriptional processing also plays a major role. Therefore, it is likely that most microRNAs are integrated within the same transcriptional networks that regulate expression of coding genes. One important finding in this regard was that the miR219 and miR338 levels in oligodendrocytes are dependent upon *Olig1* for their expression (Zhao et al., 2010), and there is also *Myrf* binding in the miR219-2 locus (Bujalka et al., 2013) that would account for its induction during CNS myelination. To address transcriptional regulation of microRNA expression in Schwann cells, we recently used siRNA analysis followed by microarray analysis in the S16 Schwann cell line to identify a set of microRNAs whose expression is dependent upon *Sox10* (Gokey et al., 2012). Interestingly, one of the most *Sox10*-dependent microRNAs is miR338. In addition to its putative role in oligodendrocyte development, miR338 is highly expressed in Schwann cells (Bremer et al., 2010; Yun et al., 2010). In addition, miR338 was recently identified as a biomarker of Schwann cell origin in a transmissible cancer model in Tasmanian devils (Murchison et al., 2010). A similar *Sox10* dependence of miR338 expression was observed by siRNA analysis in the CG4 oligodendrocyte cell line (Gokey et al., 2012).

Most of the *Sox10*-dependent microRNAs identified in our study are constituents of microRNA clusters known as oncomirs (Gokey et al., 2012), and the best studied locus (containing miR17/92) has been termed “oncomir-1” for its association with many different types of cancer (Figure 1). This oncomir has been duplicated in the mammalian genome to form two paralogs; miR106a/363 and miR106b/25 (Mogilyansky and Rigoutsos, 2013). These microRNAs have been independently linked to tumorigenesis in different tissues, and common targets include *Pten* and the p21 Cdk inhibitor (Ivanovska et al., 2008; Plasterk, 2006; Uren et al., 2008). *Sox10* siRNA treatment of the S16 Schwann cell line resulted in downregulation of the three pri-miRNA transcripts and several of the individual microRNAs in the clusters were also found to be *Sox10*-dependent using microRNA-specific detection assays. Consistent with findings in other systems, we found that downregulation of *Sox10* resulted in increased levels of p21 expression (but not *Pten*) as well as decreased proliferation in primary rat Schwann cells and the S16 Schwann cell line (Gokey et al., 2012).

The oncomir-associated microRNAs are similarly highly enriched in rodent oligodendrocytes compared to astrocytes and neurons, although they are also expressed in microglia (Budde et al., 2010; Jovine et al., 2013). The miR17/92 oncomir cluster is highly expressed in human oligodendrocytes (de Faria et al., 2012) and also in oligodendrocytes derived from human stem cells (Letzen et al., 2010). Interestingly, a recent paper identified that the Plp 3' UTR is targeted by the miR20a component of the oncomir cluster (Wang and Cambi, 2012). Since oncomirs decline during development (see below), this may contribute to induction of Plp during differentiation.

Repression of oncomir microRNAs during myelination

While some microRNAs are induced during differentiation (e.g. miR219, -338, -138), several microRNAs are high in premyelinating Schwann cells and oligodendrocytes but then decrease during myelination. In Schwann cells, at least some of these microRNAs rebound after peripheral nerve injury (Adilakshmi et al., 2012; Viader et al., 2011; Yu et al., 2012). For example, oncomir expression is very high at early myelination timepoints (e.g. P0), but declines as myelination proceeds, consistent with the general decrease in Schwann cell proliferation (Gokey et al., 2012). Similarly, other studies have demonstrated reduction of oncomir expression in mature oligodendrocytes (de Faria et al., 2012; Lau et al., 2008). While Sox10 regulation provides an explanation for high oncomir expression in embryonic and perinatal Schwann cells, the mechanism for the decline in postnatal development has not been elucidated. In general, downregulation of microRNAs could be due to active repression, or simply because the relevant transcriptional activators may decline during terminal differentiation.

Intriguingly, a study of macrophage differentiation revealed that Egr2 actively represses miR17/92 oncomir expression to promote cell cycle arrest and differentiation (Pospisil et al., 2011). This mechanism involved direct binding to a GC-rich sequence upstream of the 17/92 cluster, and furthermore was shown to involve the Jarid1b histone demethylase, which directly interacts with Egr2 and catalyzes demethylation of histone H3K4me3 which is normally associated with transcriptional initiation sites. Consistent with this idea, the observed developmental downregulation of oncomirs in postnatal peripheral nerve parallels the developmental induction of Egr2 (Zorick et al., 1996). Finally, we find that the 17/92 and 106a/363 oncomirs are induced in peripheral nerve of Egr2-deficient mice (unpublished data), consistent with the increased Schwann cell proliferation in these mice (Le et al., 2005; Zorick et al., 1999).

Moreover, the same study found that oncomir-associated microRNAs inhibit Egr2 expression in differentiated macrophages (Pospisil et al., 2011), suggesting a mutual antagonism that could constitute a bistable switch regulating proliferation and Egr2 induction. Experiments in this system showed that mutation of the two target sites in the Egr2 3' UTR resulted in elevated expression levels of a reporter construct. Moreover, inhibitors of miR17-5p and -20a resulted in elevated levels of the reporter. Conversely, expression of the 17/92 oncomir downregulated the Egr2 3'UTR reporter and Egr2 protein levels (Pospisil et al., 2011).

MicroRNAs target transcription factors in myelinating glia

The targeting of *Egr2* illustrates the potential complexity of microRNA regulation (Figure 2). In addition to the inverse correlation of oncomir expression with *Egr2*, it has been suggested that *Egr2* is also targeted by two microRNAs that increase during the myelination period: miR138 (Adilakshmi et al., 2012) and miR140 (Viader et al., 2011). Analysis of miR140 suggested that it limits induction of *Egr2* so that it does not become overexpressed. Both of these microRNAs decline after nerve injury, which may facilitate re-expression of *Egr2* as the injured nerve remyelinates. Interestingly, another microRNA (miR709) is induced after nerve injury, and similarly targets *Egr2* (Adilakshmi et al., 2012). This microRNA may therefore be involved in the post-injury reduction in *Egr2* expression.

Recently, an analysis of Sox2 function in oligodendrocytes revealed another example of repression. Sox2 is widely known as a pluripotency factor in stem cell biology, but it continues to be expressed (along with SoxB family member Sox3) in oligodendrocyte progenitors until the initiation of myelination (Hoffmann et al., 2014). In a Sox2 (and/or Sox3) knockout there is an induction of miR145, which is more highly expressed in OPCs compared to mature oligodendrocytes. One target of miR145 is Sox9 which is coexpressed with Sox10, and plays a partially redundant role in oligodendrocyte development (Finzsch et al., 2008). While this does not explain the defective oligodendrocyte development in Sox2 and Sox3 (and double knockouts), other targets of miR145 are Myrf and Med12, suggesting that miR145 in OPCs prevent premature expression of Myrf. Med12, a mediator subunit, was recently shown to interact with Sox10 and is required for development of Schwann cell and oligodendrocytes (Vogl et al., 2013). Profiling of human stem cell differentiation to oligodendrocytes has also identified another microRNA—miR199—that could target the 3' UTR of the Myrf gene (Letzen et al., 2010). Accordingly, the induction of Myrf correlates with decline of miR145 and miR199 during oligodendrocyte differentiation (Figure 2).

In addition to repression of transcription factor mRNAs, other nuclear processes are microRNA targets. Nuclear lamins are important constituents of the nuclear envelope, which control nuclear localization and export. Duplication of the Lamin b1 gene was shown to cause autosomal dominant leukodystrophy, associated with severe myelin loss. Interestingly, lamin B1 is regulated by mir23, and overexpression of miR23 stimulates oligodendrogenesis in vitro (Lin and Fu, 2009; Lin et al., 2013). Moreover, miR23a-overexpressing mice have increased myelination, which is also associated with reduced Pten expression. MicroRNAs have also been shown to trigger epigenomic repression of target loci, and induction of miR709 after nerve injury is associated with an increased repressive H3K27 trimethylation after nerve injury (Adilakshmi et al., 2012).

Integration of ChIP data with microRNA expression profiles

Studies of microRNA expression profiles have also been complemented by analysis of binding of transcription factors using chromatin immunoprecipitation (ChIP). The application of massive parallel sequencing to ChIP samples (i.e. ChIP-Seq) has revealed binding patterns of Sox10 in peripheral nerve (Srinivasan et al., 2012) as well as Olig2 (Yu et al., 2013) and Myrf (Bujalka et al., 2013) in cultured rat oligodendrocytes. While in vitro

differentiation protocols are widely used in analysis of oligodendrocyte differentiation, a challenging aspect of performing ChIP analysis in Schwann cells is that most transcriptional regulation is driven by axonal signaling during myelination (Taveggia et al., 2005; Voyvodic, 1989). While the S16 Schwann cell line has been used for ChIP analysis (Jang et al., 2006; Jang and Svaren, 2009; Jones et al., 2012; Jones et al., 2007; Jones et al., 2011; LeBlanc et al., 2006), it would be ideal to perform ChIP analysis in peripheral nerve tissue where axonal signaling is intact. Therefore, ChIP analysis has been performed in rodent sciatic nerve, in which approximately 80-90% of the nuclei are Schwann cells (Jang et al., 2006; Jones et al., 2007; LeBlanc et al., 2006; LeBlanc et al., 2007; Mager et al., 2008). Of these, >50% are myelinating Schwann cells, whereas the remaining are nonmyelinating (Remak) Schwann cells (Topilko et al., 1997; Zorick et al., 1996). Importantly, the presence of neuronal nuclei in the spinal cord allows physical separation from Schwann cell nuclei in sciatic nerve. Finally, analysis of some transcription factors can also be performed in spinal cord, since Sox10 expression is confined to Schwann cells and oligodendrocytes (and progenitors) in peripheral nerve and spinal cord, respectively.

One interesting case where ChIP-Seq data have been helpful is miR338, which is Sox10 dependent. This microRNA is located within an intron of the *Aatk* gene (apoptosis-associated tyrosine kinase). Interestingly, in vivo ChIP-Seq in P15 rat sciatic nerve revealed a strong Sox10 peak within another intron of the *Aatk* gene, approximately 2.5 kb upstream of miR338 (Gokey et al., 2012). The binding site is in a highly conserved region of intron 3 (Figure 3), and contains a consensus dimeric Sox10 binding sequence. Similar analysis of P15 rat spinal cord identified the same Sox10 binding site suggesting an analogous regulatory mechanism in oligodendrocytes. Analysis of rat expressed sequence tags (ESTs) for this locus indicated two transcription start sites for the *Aatk* gene. The annotated promoter lies >10 kb upstream of the Sox10 binding site, but several ESTs from brain initiate within intron 3 immediately downstream of the major Sox10 binding peak in this locus. Expression of the alternative transcript was sharply reduced in S16 cells lacking Sox10, whereas the transcript from the original annotated promoter was unchanged (Gokey et al., 2012). While regulation by Sox10 may explain expression of miR338 in myelinating glia, what causes the induction during myelination? The same ChIP-Seq studies identified high levels of Egr2 binding proximal to the Sox10 binding site, and analysis of MyRF binding reveal binding of this factor to the region in differentiated oligodendrocytes (Bujalka et al., 2013).

Consistent with the Sox10 regulation of oncomir expression, ChIP analysis in spinal cord identified strong Sox10 binding to a well conserved site ~10 kb upstream of the miR17/92 cluster (Figure 4), consistent with the high level of expression of this oncomir in oligodendrocytes (Budde et al., 2010). The same peak was identified in Sox10 binding analysis of the S16 Schwann cell line (Gokey et al., 2012), indicating that Sox10 may similarly activate expression of the oncomir in Schwann cells. However, our ChIP-Seq analysis of miR17/92 has identified a novel Sox10 binding site just upstream of 17/92 in sciatic nerve (Figure 4). Consistent with the repression of miR17/92 expression by Egr2 in macrophage development, we see significant binding of Egr2 in the CpG island just upstream of 17/92 in sciatic nerve during active myelination (P15), corresponding to the

binding sites of repressive Egr2 binding defined in macrophage differentiation (Pospisil et al., 2011).

Regulation of Proliferation by microRNAs

Overall, the Dicer knockouts indicate that microRNA expression is required for terminal differentiation and cell cycle exit (Dugas and Notterpek, 2011; He et al., 2011). Since proliferation appears to be a pathway regulated by microRNAs, are there specific microRNA pathways that may mediate cell cycle exit? One proposed negative regulator of proliferation is miR34a, based on its targeting of cyclin D1 and Notch1 (Bremer et al., 2010; Viader et al., 2011). Notch1 has previously been shown to antagonize the myelination process and promote proliferation, and its expression is diminished as Schwann cells terminally differentiate (Woodhoo et al., 2009). Similarly, Notch1 antagonizes differentiation and promotes proliferation in oligodendrocytes (Zhang et al., 2009), and is accordingly reduced in myelinating oligodendrocytes (Cahoy et al., 2008). While miR34a expression has not been reported in oligodendrocytes, Notch1 can also be targeted by miR138 and miR146 (Mei et al., 2011), which are induced during oligodendrocyte differentiation (Dugas et al., 2010; Lau et al., 2008).

In mouse models with a peripheral nerve-specific deletion of Dicer, Schwann cells fail to produce myelin and instead continue to proliferate and express markers of immature Schwann cells such as Sox2 and c-Jun (Bremer et al., 2010; Pereira et al., 2010; Yun et al., 2010). As noted above, miR138 is induced during myelination, and 3' UTR assays showed that it could reduce expression of Sox2 and cyclinD1 (Adilakshmi et al., 2012; Yun et al., 2010). Oligodendrocyte cell cycle regulation may involve similar targets of miR138 and miR338, but lack of cell cycle exit in the Dicer knockouts could reflect continued expression of factors that normally prevent premature differentiation. This includes repression of Sox6 and Hes5 as noted above for microRNAs 219/338, as well as inhibition of MYRF by miR145.

The continued proliferation in Dicer knockouts suggested that aggregate functions of microRNAs are primarily anti-proliferative in myelinating glia. However, some studies of oncomir-associated microRNAs reveal a pro-proliferative role. Mice deficient in miR17/92 die shortly after birth due to incorrect lung and heart development (Ventura et al., 2008), but tissue-specific deletion of the miR17/92 oncomir cluster in oligodendrocyte development (using Cnp-cre) revealed 25% fewer Olig2 positive oligodendrocytes in P0 brain after Cnp-cre mediated deletion of the 17/92 cluster. This lower number of oligodendrocytes was also found in a similar Cnp-cre mediated deletion of Dicer in perinatal brain. Using cultured oligodendrocytes, loss of miR17/92 reduced proliferation of oligodendrocyte precursor cells, which was attributed to effects on Pten and Akt signaling (Budde et al., 2010). Overall, these studies suggest that Dicer ablation causes a modest proliferative defect in embryonic development, followed by a failure to exit the cell cycle at later postnatal timepoints.

These data are consistent with studies of microRNA function in many systems in which microRNAs exert both pro- and anti-proliferative effects. The later proliferative phenotype in the Dicer knockouts may relate to the generally long half-life of microRNAs. Most of the

in vivo studies of Dicer function have utilized a cre recombinase that is expressed in either the oligodendrocyte or Schwann cell lineage. If one assumes that cell cycle exit depends on reduction of pro-proliferative microRNAs and also increases in anti-proliferative microRNAs, the cre-induced loss of Dicer would preferentially prevent induction of anti-proliferative microRNAs whereas pro-proliferative microRNAs may persist for a longer time. Therefore, the use of an embryonically induced Dicer knockout would preferentially expose the roles of anti-proliferative microRNAs. Consistent with this argument, profiling studies of oncomir-associated microRNAs after Dhh-cre-mediated deletion of Dicer show that they are not decreased at E17, and only show a slight decrease at P4 (Bremer et al., 2010).

If microRNAs also play pro-proliferative roles, another microRNA-mediated mechanism for cell cycle exit may be loss of pro-proliferative microRNAs during myelination, as noted above for the oncomir clusters. One of the most common targets of oncomir action is the p21 (Cdkn1a) CDK inhibitor (Ivanovska et al., 2008; Plasterk, 2006; Uren et al., 2008). Interestingly, while the p21 transcript is highly expressed at birth (top 15% of all genes (Buchstaller et al., 2004)) and is induced throughout myelination (Verheijen et al., 2003), p21 protein cannot be detected in peripheral nerve until after postnatal day 7 when most proliferation has ceased (Atanasoski et al., 2006). In addition, the developmental decline in oncomir expression would allow Egr2 induction, which itself is involved in cell cycle arrest (Decker et al., 2006; Le et al., 2005; Parkinson et al., 2004; Topilko et al., 1994; Zorick et al., 1999).

Summary

A number of studies have probed the role of microRNAs in myelinating glia. Most have focused on profiling and target identification and analysis of microRNA function using Dicer knockouts in oligodendrocytes and Schwann cells. It is anticipated that future studies will allow analysis of single microRNAs using traditional mouse knockouts, and the growing applicability of genome editing will allow targeted ablation of individual microRNAs and/or their target sites in 3' UTRs. In addition to their effects on transcript stability and translation, which has been the focus of this review, there is evidence for other types of effects including modulating chromatin structure of target loci (Adilakshmi et al., 2012). Target identification remains a challenge for microRNA studies, and perhaps the use of proteomics will facilitate such approaches. Finally, it seems likely that more profound microRNA-induced expression level changes may be due to coordinated action of several microRNAs on a target mRNA. Therefore, it is anticipated that this redundancy may complicate studies that traditionally use a reductionist approach to analyze functions of single microRNAs. Nevertheless, the important aspects of microRNA function are beginning to emerge which are a major contributor to development of myelinating glia.

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Potential oncomir targets in myelinating glia: Pten, Cdkn1a, Egr2, Plp

Figure 1. Structure of Oncomir Clusters

The figure indicates the structure of the 3 oncomir loci, and human chromosome numbers are indicated. The microRNAs are color coded to mark related family members with similar/identical seed sequences. Putative targets listed are discussed in the text.

Figure 2. Combinatorial Targeting of pro-myelinating transcription factors by microRNAs
 The diagram indicates proposed roles of various microRNAs (boxed) in either immature/premyelinating Schwann cells or oligodendrocytes, with a shift in microRNA expression in myelinating cells as shown in the right side of the diagram. Arrows indicate direct activation of microRNA expression based on ChIP-Seq data, and targets of microRNA repression are indicated in each state. Depicted targets focus on transcription factors that are differentially expressed in either stage, and the p21 CDK inhibitor and myelin gene, Plp, are also shown.

Figure 3. Sox10 and Egr2 binding within the host gene for miR338

The UCSC genome browser view shown in the figure displays peaks of Sox10 and Egr2 binding based on Homer (Valouev et al., 2008) analysis of the ChIP-Seq data from sciatic nerve and spinal cord (Srinivasan et al., 2012) (unpublished data). The annotated transcript initiates at the right end of the locus, but an alternate transcript initiates within intron 3 just downstream of the Sox10 binding site. The position of miR338 within an Aatk intron is indicated.

Figure 4. Sox10 and Egr2 binding upstream of the miR17/92 oncomir cluster

The UCSC genome browser view shows Egr2 and Sox10 binding data around the oncomir miR17/92 cluster as described for Figure 2.