Published in final edited form as:

Curr Opin Cell Biol. 2013 April ; 25(2): . doi:10.1016/j.ceb.2012.12.007.

MicroRNAs: regulators of neuronal fate

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Abstract

Mammalian neural development has been traditionally studied in the context of evolutionarily conserved signaling pathways and neurogenic transcription factors. Recent studies suggest that microRNAs, a group of highly conserved non-coding regulatory small RNAs also play essential roles in neural development and neuronal function. A part of their action in the developing nervous system is to regulate subunit compositions of BAF complexes (ATP-dependent chromatin remodeling complexes), which appear to have dedicated functions during neural development. Intriguingly, ectopic expression of a set of brain-enriched microRNAs, miR-9/9* and miR-124 that promote the assembly of neuron-specific BAF complexes, convert the nonneuronal fate of human dermal fibroblasts towards post-mitotic neurons, thereby revealing a previously unappreciated instructive role of these microRNAs. In addition to these global effects, accumulating evidence indicate that many microRNAs could also function locally, such as at the growth cone or at synapses modulating synaptic activity and neuronal connectivity. Here we discuss some of the recent findings about microRNAs' activity in regulating various developmental stages of neurons.

Introduction

MicroRNAs (miRNAs) are endogenous 20~24 nucleotide RNAs that bind to target motifs in mRNAs of protein-coding genes to direct posttranscriptional silencing either through transcript degradation or translational repression [1,2]. Predominantly these binding motifs are found in the 3' untranslated regions (UTRs) of target mRNAs, although examples of their presence in the coding regions of genes have been documented [3]. A single microRNA can have up to several hundreds of targets; and conversely, a gene can have target sites for different miRNAs synergizing the downregulation by multiple miRNAs [4-6]. So far more than one thousand microRNAs have been denoted in the human genome and the number is ever increasing, illustrating the potential of microRNAs as important players in gene regulation. In addition, aberrant expressions of miRNAs have been linked to various pathologies including tumors, highlighting their function in safeguarding normal cell growth and development [7]. This review mainly focuses on recent advances in our understanding of the role of miRNAs in neural development and miRNA-mediated neuronal conversion of mammalian cells.

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Essential functions of microRNAs in neural development

MicroRNAs are abundantly expressed in the nervous system, with about half of known species detected in the human brain [8], implicating their significant contribution in neural development and function. Earlier studies utilizing genetic deletions of Dicer, a core component of the biogenesis of mature microRNAs demonstrated essential roles of miRNAs in diverse neural cell types at different stages of development. Conditional deletion of Dicer in neural progenitors in Emx1-Cre or Nestin-Cre mice resulted in markedly thinner cortices, increased apoptosis and disorganized cortical layering, indicating their essential function in neural progenitor expansion as well as neuronal differentiation [9-11]. Selective deletion of Dicer in specific neuronal types (including Purkinje cells (Pcp2-Cre), spinal cord neurons (VACHT-Cre), dopaminergic neurons (DAT-Cre) have consistently resulted in apoptosis of neurons [12-14]. Furthermore, in many cases, such as shown in spinal cord neurons, mutant mice exhibited marked decrease in activity and movement with an array of hallmarks reminiscent of spinal muscular atrophy (SMA), implicating miRNA deregulation in neurodegenerative disorders [14]. In addition, studies from deleting other components of microRNA biogenesis including Dgcr8 have largely yielded similar phenotypes including increased apoptosis and microcephaly, further illustrating the importance of miRNAs during neural development [15]. It should be noted that since the generation of several non-canonical miRNAs may bypass the requirement of Dgcr8 but not Dicer, comparison of the phenotypes of the two knockouts should reveal the contribution of noncanonical miRNAs, as recently shown in rodent neurons [15].

Specific MicroRNAs and their roles in neuronal differentiation, maturity and function

While interfering with miRNA machinery has demonstrated essential roles of miRNAs in brain development, recent development of various experimental techniques that allow selective overexpression or inhibition of individual miRNAs has been instrumental in delineating the role of specific miRNAs. We here review recently published literature of miRNAs with specific functions in different stages of neuronal development (Table 1).

A number of miRNAs have been demonstrated to regulate proliferation and differentiation of neural stem cells (NSCs). MiR-184 was found to be expressed in the adult neural stem cells in the subventricular zone and dentate gyrus, and promote their expansion. Its expression was negatively regulated by methyl-CpG binding protein 1 (MBD1), an epigenetic transcriptional repressor whose deficiency results in impaired adult neurogenesis [16]. In this study Numbl (Numbl), a molecule previously known to regulate stem cell asymmetric division, was identified as a target of miR-184, as its expression restored the imbalance between NSC proliferation and neuronal differentiation caused by either overexpressing miR-184 or abrogating MBD1 [16]. However, it is not clear exactly how Numbl functions in this context. Nevertheless, this study clearly demonstrated a cross-talk between epigenetic mechanisms and miRNAs in adult neurogenesis, and it would be interesting to examine whether similar genetic networks operate in embryonic brain development.

The evolutionarily conserved let-7 family miRNAs also regulate NSC self-renewal and differentiation. Overexpression of let-7a in neural stem cells induced neuron formation while blocking its activity retained them as proliferative Nestin-positive progenitors [17]. The activity of let-7a is enhanced along neuronal differentiation, as a result of binding of TRIM32, a mammalian homologue of *Drosophila* Brat and Argonaute 1 (Ago1) [17]. Another family member, let-7b was found to promote cell cycle exit and neuronal differentiation [18,19]. Several functional targets of let-7b have been identified, including

high mobility group AT-hook 2 (Hmga2) [18], a known regulator of neural stem cell self-renewal, cyclin D1 [18], and an orphan nuclear receptor TLX [19], an neural stem cell fate determinant [20].

In proliferating neural stem cells, TLX forms a repressor complex with lysine specific demethylase 1 (LSD1) and transcriptionally repress miR-137 that is enriched in neurons [21]. Intriguingly, miR-137 targets LSD1, creating a double negative regulation involving TLX-miR-137-LSD1 as NSCs differentiate into post-mitotic neurons. miR-137 has also been shown to be regulated by SRY-box 2 (Sox2), a core transcription factor sustaining NSC self-renewal, and methyl CpG binding protein 2 (MeCP2) in adult mice [22]. The same group also reported that Ezh2 to be a target of miR-137 in adult NSCs, and suggested that reduced Ezh2 expression (resulting in the decrease in H3K27 level) may contribute to the Rett syndrome-like phenotype in MECP2 mutant mice. However, the *in vivo* relevance of this finding remains unclear. Additionally, miR-137 functions to inhibit dendritic morphogenesis and functional maturation of neurons through silencing of an ubiquitin ligase mindbomb homolog 1 (Mib1) [23].

miR-9, a highly expressed miRNA in the brain, promotes neuronal fate determination in the developing CNS as well as influencing neuronal subtype specification and regulating axonal growth, branching and targeting [24-33]. Reported targets of miR-9 that are important for neuronal differentiation include NEFH, TLX, Foxg1, Gsh2, SIRT1, and REST/NRSF [24-29]. miR-9 was recently demonstrated to promote neural stem cell differentiation by targeting TLX whereas in neural stem cells, TLX represses miR-9 expression [33]. In addition, miR-9 was also found to form a double negative feedback loop with Hes1, an essential molecule for NSC homeostasis [31]. Moreover, Otaegi *et al.* recently reported a role of the interplay between miR-9 and its target Foxp1 in establishing motor neuron identity and columnar architecture of developing chick spinal cord [30]. Furthermore, a role of miR-9 in regulating axon length and branching through Map1b, an important protein for microtubule stability, in mouse cortical neurons has been described [32]. Taken together, mounting evidence supports a multifaceted role of miR-9 during neuronal differentiation.

miR-124 is another highly abundant brain-enriched miRNA that has been studied extensively. While most loss-of-function and overexpression studies in vertebrates supports miR-124 as a promoter of neuronal differentiation and an inhibitor of progenitor self-renewal (discussed further below), independent reports that studied miR-124 knockout in *Drosophila* showed that it is grossly dispensable for neuronal differentiation, but rather required for proliferation of neural stem cells [34-36]. Several possibilities could underlie the apparent divergence in the function of miR-124. Notably in flies miR-124 is expressed in proliferating neuroblasts, whereas in vertebrates, its expression starts when double-cortin positive neurons appear [34,35,37,38]. Similarly, in human embryonic stem cell to neuron differentiation system, miR-124 expression was largely restricted to differentiated neurons [39]. Thus the different expression pattern could underlie the distinct activities of miR-124 in different species. Second, the targets of miR-124 could have evolved differentially in different organisms. Consistent with this, Anachronism, a target of miR-124 in neural stem cells in flies has no mammalian homologue [35]. In this review, we focus on studies using vertebrate systems where miR-124 has repeatedly been shown to promote cell cycle exit and neuronal differentiation.

Earlier studies showed that overexpression of miR-124 in HeLa cells shifted their transcriptome towards that of neurons and suggested the pro-neuronal activity of miR-124 [40]. To gain insight of miR-124's function *in vivo*, two recent studies examined the role of miR-124 in the subventricular zone in mice [37,38]. In both studies ectopic expression of miR-124 in SVZ cells resulted in early exhaustion of neural progenitors and premature

neuronal differentiation while knockdown of miR-124 rendered the opposite effects, thus establishing miR-124 as a neuronal fate determinant in the adult SVZ. Whether miR-124 has a role in neuron/glia lineage specification remains unclear, as the two reports arrived at different conclusions. This discrepancy may be due to the different knockdown efficacy and duration achieved owing to different techniques employed; a genetic loss-of-functional approach thus would be much needed to clarify this issue (which has been hindered so far due to the presence of 3 loci encoding miR-124). In one study Sox9, an important regulator of NSCs [41], and Jag1, a Notch ligand, were identified as targets of miR-124 [37]. In addition to Sox9 and Jag1, many other targets of miR-124 have been documented, whose repression are essential for establishment of neuronal programs, including PTBP1, SCP1, Ephrin-B1, and BAF53a (in conjunction with miR-9*) [4,37,42-44]. In particular, BAF53a, a subunit of a neural progenitor-specific BAF (npBAF) complexes [45]), is repressed and replaced by a homologous BAF53b as a part of neuron-specific BAF (nBAF) complexes. This subunit switching is important for dendritic arborization [46]. Yoo *et al.* demonstrated that miR-9* and miR-124 synergistically target BAF53a in neural progenitors during neuronal differentiation allowing BAF53b to be expressed in post-mitotic neurons [4]. The expression of miR-124 and miR-9* has been shown to be regulated by REST [24], thus miR-124 and miR-9/9* appear to lie in the center of a triple negative genetic circuit regulating mitotic exit of neural progenitors and the onset of neuronal differentiation [38].

Besides its role in promoting neuronal fate acquisition, miR-124a is also required for hippocampal axonogenesis and retinal cone survival through Lhx2 suppression in mice [47] and constraining synaptic plasticity through CREB in *Aplysia* [48]. A similar role of miR-124 for regulating synaptic function in mammals will need to be confirmed.

In addition to neuronal fate acquisition, an increasing number of miRNAs, some of which have been described above, are implicated in dendritic morphogenesis and synaptic development in neurons. Interestingly, many of them including miR-134 displayed localization in dendrites or synapses, consistent with their purported role in generating rapid and local responses in an activity dependent manner [49]. Physiological functions, *in vivo* targets and regulators of such miRNAs are extremely fascinating topics, and have recently been reviewed elsewhere [50].

MicroRNAs and neuronal reprogramming

Because miRNAs typically mediate gene silencing, they are traditionally thought as fine-tuners of gene expression. Consistent with this view, most published papers on cell fate switches used transcription factor based cocktails, rather than miRNAs [51]. Recently, however, several studies reported the potency of microRNAs in influencing neuronal cell fates [52,53]. In one study, miR-9/9* and miR-124 alone were shown to be able to convert human fibroblasts towards neurons and more so with as few as one transcription factor [53]. Synergism between mi-9/9* and miR-124 is crucial for this conversion, since expression of individual miRNAs was insufficient to induce neurons. Further addition of neurogenic factors enhanced both the efficiency and the maturity of the reprogrammed neurons, while expression of these factors alone was far less ineffective at generating neurons. These results clearly demonstrated an instructive role of these miRNAs in induction of neuronal fates, and highlighted the cooperation between miRNAs and transcription factors in neuronal reprogramming. What are the crucial targets of the miRNAs that mediate this transdifferentiation? Yoo *et al.* attempted to address this question by persistently expressing some of the known targets of miR-9/9* and miR-124, including SCP1, PTBP1 and BAF53a during reprogramming. However, none of these targets could completely block neuronal conversion when overexpressed singly. These data suggested that miR-9/9* and miR-124 could act synergistically and programmatically on multiple targets to exert their neurogenic

activities. Fig. 1 depicts a model of the neurogenic activity of these miRNAs, illustrating multiple negative pathways that eventually lead to repression of factors that oppose neuronal fate acquisition.

MicroRNAs in specific neuron subtype identity

A hallmark of mammalian nervous system is its extraordinary diversity of neuronal subtypes. Do miRNAs play any important roles in specification of neuronal subtypes? In a study using *in vitro* differentiation of mouse ES cells to dopaminergic neurons (DNs), miR-133b has been suggested to play important role in DN formation and function by targeting Pitx3 [13]. However, genetic deletion of miR-133b in mouse does not affect DN formation nor function [54], a discrepancy that may be possibly explained by compensatory actions by other miR-133 family miRNAs. In addition, miR-7a has recently been shown to regulate Pax6 in adult neurogenesis and affect dopaminergic fate in the olfactory bulb [55].

A significant advance in our understanding of neuron cell type specific miRNA expression came from a recent study using an ingenious miRNA tagging and affinity-purification (miRAP) method that is targeted to specific neuronal subtypes through the Cre-loxP technique in mice [56]. Using miRAP, the authors were able to identify a large number of miRNAs with distinct profiles in glutamatergic, gabaergic neurons and subtypes of gabaergic neurons, providing experimental support that the identity of neuronal subtypes could be related to differentially expressed microRNAs. Indeed, even between closely related interneurons types such as parvalbumin (PV) neurons versus somatostatin expressing (SST) neurons, more than half of the miRNAs profiled are differentially expressed. Interestingly, miR-124 and miR-9/9* were found to be highly expressed at relatively constant levels among all subtypes, consistent with their pan-neuronal neurogenic activities. Based on the potency of microRNAs in influencing cell fates, combining the neurogenic activity of miR-9/9* and miR-124 with subtype-specific microRNAs to reprogram into specific neuronal types remains an intriguing possibility to be tested (Fig. 2).

Conclusions and Future Perspectives

MicroRNAs are emerging pivotal regulators of neurogenesis. In addition to being involved in neuronal differentiation, as reviewed here, miRNAs have also been shown to play significant roles in other neural lineages such as oligodendrocytes [57,58]. Even within the neurons, increasing numbers of miRNAs are being identified to regulate various stages of neuronal development and function. Expression of miRNAs as well as the targets of miRNAs are dynamically regulated, both spatially and temporally, contributing to the diversity and plasticity of our brain. MiRNA profiling in distinctive neuronal populations have just started, and it would be exciting to see even more refined classification of miRNAs expression pattern with better cell isolation techniques. In addition, the link between miRNAs deregulation and neurological diseases are just beginning to be revealed and certainly merit further studies.

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Figure 1.

A model of neuronal reprogramming mediated by miR-9/9*-124 and neurogenic factors. MiR-9/9* and miR-124 in non-neuronal cells are repressed synergistically by neurogenic repressors such as REST complex, non-neuronal BAF complexes and others. The top panel is an example of REST repressing neuronal genes such as miR-9/9* and miR-124. Forced expression of miR-9/9* and miR-124 results in the break of genetic network involving repression of multiple factors including REST complexes, BAF53a, and PTBP1, which normally suppress neurogenesis. The synergism of miRNAs suggests that these miRNAs work programmatically on multiple targets during the process of neuronal conversion. In addition, it is possible that miRNAs may target factors whose silencing potentiates the activity of neurogenic transcription factors (NFs) (bottom panel).

Figure 2.

A hypothetical scheme of production of subtype-specific neurons by reprogramming. Leveraging on the known pan-neuronal activity of miR-9/9* and miR-124, additional miRNAs specific to neuronal subtypes may lead to neuronal reprogramming into their respective neuronal subclasses.

Summary of specific miRNAs discussed in the text, with their known functions and targets in neural development.

Table 1

miRNA	species	Function	Targets	Reference
miR-184	mouse	Promotion of adult neural stem cell proliferation and inhibition of neuronal differentiation	Numb1	16
let-7b	mouse	Impairment of neural progenitor expansion and enhancement of cell cycle exit and neuronal	Hmga2, TLX, and Cyclin D1	18,19
miR-137	mouse	Inhibition of neural stem cell proliferation and promotion of neuronal differentiation	LSD1, Ezh2	21,22
	mouse	Inhibition of dendritic development	Mib1	23
miR-9	mouse	Promotion of neuronal fate and inhibition of neural progenitor division	TLX, Foxg1, Gsh2, SIRT1, REST/NRSF, Hes1	24-29, 31,33
	chick	Regulation of motor subtype determination	FoxP1	30
	mouse	Regulation of axon development	Map1b	32
miR-124	mouse	Promotion of neuronal fate and inhibition of neural progenitor division	PTBP1, Sox9, SCP1, Ephrin-B1, JAG1, BAF53a	4, 37, 42-44
	mouse	hippocampal axonogenesis and retinal cone survival	Lhx2	47
	Aplysia	Regulation of synaptic activity	CREB	48
miR-134	rat	Regulation of dendritic spine morphogenesis	Limk1	49
miR-133	mouse	regulation of Dopaminergic neurons formation	Pitx3	13
miR-7a	mouse	regulation of Dopaminergic neurons formation in the olfactory bulb	Pax6	55