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REVIEW

The Role of MicroRNAs in Neural Stem Cells and Neurogenesis

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ABSTRACT

Neural stem cells give rise to neurons through the process of neurogenesis, which includes neural stem cell proliferation, fate determination of new neurons, as well as the new neuron's migration, maturation and integration. Currently, neurogenesis is divided into two phases: embryonic and adult phases. Embryonic neurogenesis occurs at high levels to form the central nervous system. Adult neurogenesis has been consistently identified only in restricted regions and occurs at low levels. As the basic process for embryonic neurodevelopment and adult brain maintenance, neurogenesis is tightly regulated by many factors and pathways. MicroRNA, short non-coding RNA that regulates gene expression at the post-transcriptional level, appears to be involved in multiple steps of neurogenesis. This review summarizes the emerging role of microRNAs in regulating embryonic and adult neurogenesis, with a particular emphasis on the proliferation and differentiation of neural stem cells.

KEYWORDS: MicroRNA; Neural stem cell; Embryonic neurogenesis; Adult neurogenesis

INTRODUCTION

Neural stem cells (NSCs) are a class cells capable of self-renewing and giving rise to different neural lineages, such as neurons, astrocytes, and oligodendrocytes (Gage, 2000). To progress from NSC to functional neurons requires the process known as neurogenesis, which includes the proliferation of the neural stem/progenitor cells (NSPCs), the differentiation of neurons, and the integration of new neurons into the existing neural circuitry. As the basic process of neuron generation, neurogenesis plays important roles during the embryonic development and adult nervous system maintenance. In the embryo, the neuroepithelial cells within the neural tube are the common source of new neurons (Kintner, 2002). During the development of the brain, neurogenesis in the embryo occurs at a high level, forming the central nervous system (CNS). In the adult brain, the newborn neuronal cells are derived from adult NSCs. To date, adult NSCs have been persistently found in the subventricular zone (SVZ) and the hippocampal subgranular zone (SGZ). Two types of NSCs have been identified based on their morphology, cellular markers, and proliferative capacity (Zhao et al., 2008). In the SVZ, a population of $GFAP^+$ and $CD133^+$ radial glia-like progenitors, type-B cells, has been hypothesized to be the primary source of NSCs, which subsequently generate DCX^+ PSA^-NCAM^+ neuroblasts through intermediate progenitors. The neuroblasts then migrate to the olfactory bulb (OB) and differentiate into $GABA^+$ $dopamine^+$ neurons. In the SGZ, a population of $GFAP^+$ $Sox2^+$ and $Nestin^+$ radial cells was found to be the reservoir of NSCs and may generate the non-radial $Sox2^+$ $GFAP^-$ cells, which actively self-renew and subsequently differentiate into neurons. Compared with embryonic neurogenesis, adult neurogenesis is largely restricted to niches in which both proliferation and neuronal differentiation are under tight regulation. Moreover, the frequency of neurogenesis in the adult brain occurs at much lower levels. Altogether, NSCs are the cellular basis of neurogenesis, and neurogenesis is a fundamental process for both embryonic neurodevelopment and adult brain maintenance.

The progression from an NSC to a mature neuron is tightly regulated by various signaling pathways and factors. For

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example, the Wnt signaling pathway regulate NSC proliferation and differentiation both in embryonic development and adult neurogenesis (Lie et al., 2005; Wexler et al., 2009). In addition, the disruption of the Sonic Hedgehog (Shh) signaling pathway decreases the level of NSC proliferation (Lai et al., 2003). TLX (also known as NR2E1, nuclear receptor subfamily 2 group E member 1), an orphan nuclear receptor, plays an essential role in regulating the activity of NSCs (Shi et al., 2004; Liu et al., 2008; Zhang et al., 2008). In addition, some transcription factors, such as AscH, Neurog2, and Tbr2, influence the differentiation of NSCs (Ozen et al., 2007; Jessberger et al., 2008; Brill et al., 2009). Some epigenetic factors, such as MBD1, MeCP2, and Gadd45b, are also closely associated with adult neurogenesis (Zhao et al., 2003; Smrt et al., 2007; Ma et al., 2009).

In addition to the above-mentioned signaling pathways and factors, neurogenesis is regulated by a novel class of modulators named microRNAs (miRNAs). MiRNAs are endogenously encoded single-stranded RNAs that can post-transcriptionally regulate gene expression by targeting mRNAs for cleavage or translational repression (Bartel, 2004). Thus far, hundreds of miRNAs have been identified in mammals (miRBase, http://www.mirbase.org/). These molecules are derived from independent transcription units or the introns of protein-coding genes with single or clustered distributions in the genome. A miRNA gene is transcribed into a primary transcript called the pri-miRNA. The pri-miRNA is then cleaved in the nucleus by the RNAase III endonuclease Drosha to produce a 60-70 nt stem-loop intermediate known as the pre-miRNA. The pre-miRNA is subsequently exported to the cytoplasm, where it is processed by Dicer to produce a shorter, double-stranded RNA duplex. The Dicer cleavage process is coupled with the binding of the mature miRNA to the RNA-induced silencing complex (RISC) (Rische and Klug, 2012). The miRNA then directs the RISC to its target mRNA by a perfect match in the so-called seed region of 6-8 nt at the 5′ end (Bartel, 2009).

The interaction between the miRNA and mRNA leads to the downregulation of protein expression by translational repression, mRNA degradation, or the promotion of mRNA decay. More than 60% of all mammalian mRNAs are under the control of miRNAs. Because the size and binding specificity of miRNAs are limited, a single miRNA always targets hundreds of mRNAs, and one mRNA can be targeted by multiple miRNAs. Therefore, miRNAs often act as fine-tuning devices rather than as primary gene regulators. Nevertheless, miRNAs may impact physiological processes by regulating the key cellular proteins in a single or related pathway, because many signaling pathways have been identified in the proliferation and differentiation of NSCs.

In the mouse brain, approximately 70% of the miRNAs are detectable. In mice, Dicer deficiency results in the abnormal development of the CNS and the failure to form appropriate cellular and tissue morphogenesis in the cortex and hippocampus; it also affects neurogenesis and gliogenesis (Davis et al., 2008; Kawase-Koga et al., 2009; Huang et al., 2010). The deficiency of Ago2, a component of the RISC, results in defects in neural tube closure and mis-patterning of the forebrain (Liu et al., 2004). Together, these observations indicate that miRNAs biogenesis pathway plays important roles in the development of the central nervous system.

To date, many miRNAs that are specifically or richly expressed in mammalian brain have been identified. For example, miR-124, which accounts for 25%−48% of all brain miRNAs, is the best studied (Lagos-Quintana et al., 2002). miR-9 is a brain-specific miRNA, abundantly expressed in neurogenic regions in embryos and adults (Deo et al., 2006). Let-7 and miR-137 are richly expressed in both embryonic and adult brains (Miska et al., 2004). miR-124a, miR-125b, miR-128, miR-132, and miR-219 are abundantly detected in the fetal hippocampus (Lukiw, 2007). During the differentiation of embryonic stem cells, the expression dynamics of a set of highly expressed neural miRNAs were also evaluated (Smirnova et al., 2005). For instance, miR-124 and miR-128 tend to be expressed in neurons, whereas miR-23 expression is mostly restricted to astrocytes. The expression of miR-26 and miR-29 is higher in astrocytes than in neurons. Indeed, the spatial and temporal expression characteristics of miRNAs indicate their important roles in NSC proliferation and differentiation.

Many miRNAs are associated with the pathogenesis of neurological diseases. For example, miR-19b, miR-302* and miR-323-3p contribute to the pathology of Fragile X syndrome by repressing the expression of fragile X mental retardation protein (FMRP) (Yi et al., 2010). miR-181b plays a role in schizophrenia by targeting visinin-like 1 (VSNL1) and glutamate receptor subunit (GRIA2) (Beveridge et al., 2008). In patients with Alzheimer's disease, the expression of miR-9 and miR-128 is upregulated, while miR-15a and miR-107 are downregulated (Lukiw, 2007; Wang et al., 2008). These studies strongly support the important roles of miRNAs in neurogenesis. In the present review, we summarize the effects of miRNAs on NSC proliferation and differentiation at both the embryonic and adult levels (Fig. 1).

miRNAs INVOLVED IN EMBRYONIC NEUROGENESIS

Many miRNAs are dynamically regulated during neural development or are partially expressed in the brain. miRNAs are speculated to act as developmental switches by timely regulating key related genes. The essential roles of miRNAs in cortical neurogenesis in the embryonic brain have been identified by an increasing number of studies.

One well-characterized brain specific miRNA, miR-124, which is expressed at increased levels during brain development, has been proposed to promote neuronal differentiation in several ways. For example, miR-124 induces neurogenesis by suppressing small C-terminal domain phosphatase 1 (SCP1) expression during CNS development (Visvanathan et al., 2007). SCP1 is restricted to non-neuronal tissues and recruited to repressor element 1 (RE1)-containing neural genes by repressor element-1 silencing transcription factor (REST). The balance between the anti-neural activity of SCP1 and the pro-neural activity of miR-124 is important for inducing neurogenesis

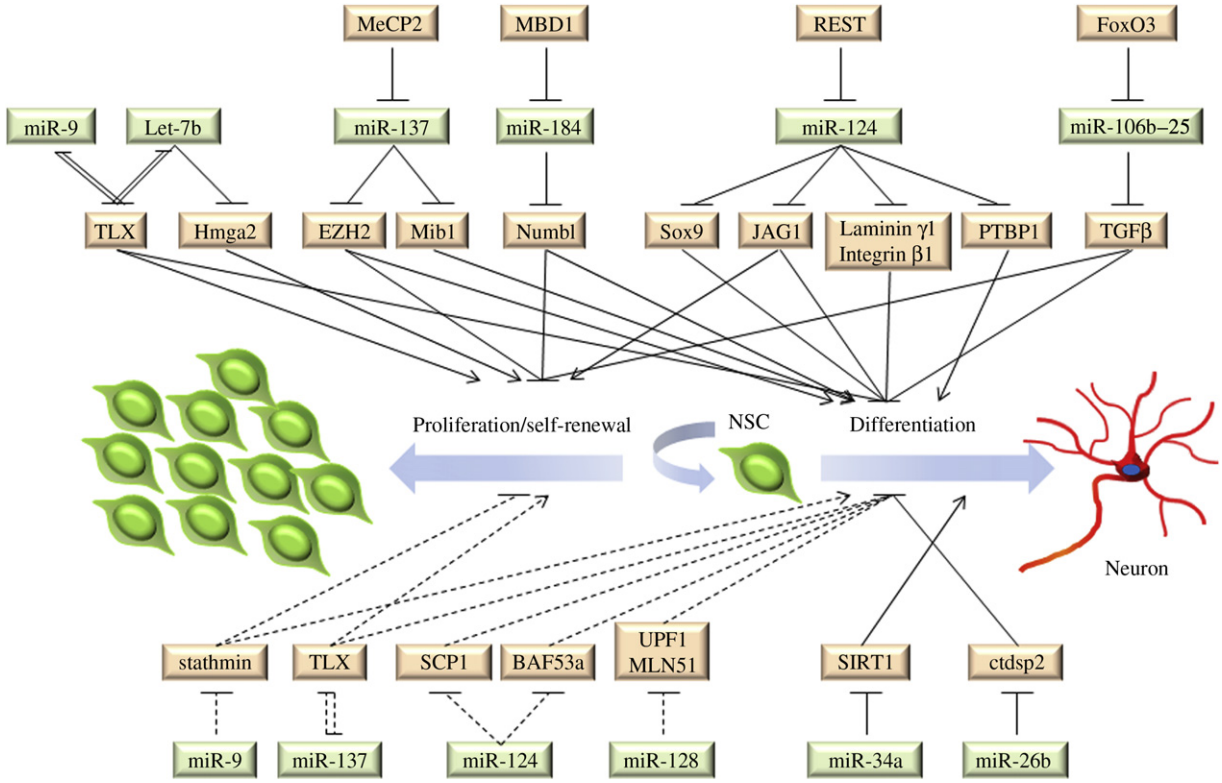

Fig. 1. miRNAs are key regulators in NSC proliferation and differentiation in the embryo and adult brain.
Inhibitory: ⟊; stimulatory: ⟶; found in embryo: - - - -; found in adult: ——.

during CNS development. In addition to SCP1, the other two components of the REST complex, MeCP2 and CoREST, have miR-124-binding sites in their 3′ UTR (Wu and Xie, 2006). Simultaneously, REST inhibits miR-124 expression by binding to the miR-124 genomic loci in non-neuronal cells and neural progenitors (Conaco et al., 2006). Indeed, SCP1 expression does not completely abolish the neurogenic potential of miR-124, indicating there are other targets of miR-124.

In 2009, Yoo et al. found that miR-124 mediates neuronal differentiation by repressing the neural progenitor-specific BAF complex 53 kDa subunit (BAF53a), which is replaced by the neuron-specific BAF (BAF53b) during neuronal differentiation in the mouse embryo (Yoo et al., 2009). Moreover, the overexpression of REST reactivates BAF53a expression, while the co-transfection of miR-124 and REST-expression plasmids inhibits BAF53a reactivation in neurons. These results indicate that REST-mediated repression of miR-124 plays an essential role in the exchange of the BAF53a subunit with BAF53b during neuronal differentiation. Additionally, the persistent expression of BAF53a inhibits dendritic outgrowth in neurons upon KCl treatment. Similarly, Yu et al. (2008) found that the overexpression of miR-124 in differentiating mouse P19 cells promotes neurite extension by altering the level or localization of Cdc42 and Rac1, the activation of which attenuates neurite outgrowth.

In addition to miR-124, miR-9 is one of the most fascinating miRNAs in the brain. In human neural progenitor cells, miR-9 expression is activated during neurosphere formation and is upregulated before terminal differentiation. The loss of miR-9 function suppresses the proliferation and promotes the migration of human embryonic neural progenitors by targeting stathmin, which increases microtubule instability in migrating neuroblasts (Delaloy et al., 2010). In the mouse embryonic brain, the overexpression of miR-9 stimulates neural differentiation and migration by targeting TLX (Zhao et al., 2009).

Another miRNA that plays an essential role in controlling embryonic NSC fate determination is miR-137 (Sun et al., 2011). The overexpression of miR-137 inhibits cell proliferation and promotes the neural differentiation of embryonic NSCs. The influence of miR-137 on neuronal differentiation is mediated by repressing the histone lysine-specific demethylase 1 (LSD1), which is a transcriptional co-repressor of TLX (Sun et al., 2010). Notably, TLX negatively regulates miR-137 expression by recruiting LSD1 to the genomic regions of miR-137. Therefore, TLX is an upstream regulator and LSD1 is a downstream effector of miR-137. TLX, miR-137 and LSD1 form a regulatory loop to keep the balance between NSC proliferation and differentiation during neural development.

miR-128 is a brain-enriched and neuron-specific miRNA. The expression of miR-128 is upregulated during brain development. The overexpression of miR-128 promotes neural differentiation and increases the average dendritic spine length of NSCs. The functional targets of miR-128 are involved in the nonsense-mediated decay (NMD) pathway, including up-frameshift1 (UPF1), a RNA helicase and metastatic lymph node 51 (MLN51), the exon-junction complex core component (Bruno et al., 2011). NMD degrades abnormal mRNAs harboring premature termination codons and many normal

transcripts with a stop codon in a context that may be recognized as premature (Chan et al., 2007; Bhuvanagiri et al., 2010). In summary, miR-128 is upregulated in differentiating neuronal cells and elicits the upregulation of neural-related transcripts that have NMD-inducing features.

Tripartite motif-containing 32 (TRIM32) has been shown to inhibit NSC proliferation and to promote neuronal differentiation. Let-7a, a member of the let-7 family, can be activated by TRIM32. Therefore, let-7a also plays a role in the neuronal differentiation of embryonic neural progenitors (Schwamborn et al., 2009).

Although some functions of miRNAs have been revealed, the precise roles of many miRNAs in the proliferation, differentiation and migration of embryonic NSCs remain largely unknown, because most miRNAs are short lived or expressed at low levels. With the development of new technologies, such as deep sequencing (Ling et al., 2011), more miRNAs and new functions will be identified in the future.

miRNAs INVOLVED IN ADULT NEUROGENESIS

The roles of miRNAs in the proliferation and differentiation of adult NSCs have been extensively studied. Mounting evidence indicates that miRNAs fine-tune the progression of adult neurogenesis.

The let-7 family of miRNAs is enriched in both embryonic and adult brains (Sempere et al., 2004; Wulczyn et al., 2007), and the expression of let-7 is upregulated during NSC specification. Zhao et al. (2010) found that let-7b targets the stem cell regulator TLX and the cell cycle regulator cyclin D1, thereby inhibiting the proliferation and promoting the differentiation of NSCs. In addition, let-7b regulates the proliferation and self-renewal of NSCs by targeting high mobility group-AT-hook 2 (Hmga2), which promotes the NSC self-renewal in the central and peripheral nervous systems of fetal and young mice (Nishino et al., 2008).

In the adult brain, miR-9 regulates NSC proliferation and differentiation by binding to the 3′ UTR of *TLX* mRNA (Zhao et al., 2009). During neural differentiation, miR-9 is upregulated while TLX is downregulated. The overexpression of miR-9 reduces NSC proliferation and promotes neural differentiation. Interestingly, TLX also inhibits the expression of miR-9 pri-miRNA by acting as a transcriptional repressor. This regulatory loop between miR-9 and TLX may play a critical role in the rapid transition from NSC to differentiated cells.

The neural-specific miRNA miR-124 is upregulated during the transition from the transit-amplifying cell stage to the neuroblast stage in the SVZ. The knockdown of miR-124 maintains neural progenitors as dividing precursors, whereas the overexpression of miR-124 promotes precocious neural maturation *in vivo* (Cheng et al., 2009). These results suggest that miR-124 acts as a primary determinant of neuronal differentiation. Currently, several downstream targets have been identified. Laminin $\gamma 1$ and integrin $\beta 1$, which are highly expressed in neural progenitors but repressed upon neuronal differentiation, are downregulated by miR-124 (Cao et al., 2007). PTBP1 (PTB/hnRNP I), which is a global repressor of mRNA alternative splicing in non-neuronal cells, is directly targeted by miR-124. The downregulation of PTBP1 leads to the accumulation of correctly spliced PTBP2, which in turn favors neuronal differentiation (Makeyev et al., 2007). Another target inhibited by miR-124 is the SRY-box transcription factor Sox9, which controls adult neurogenesis (Cheng et al., 2009). In the rat stroke model, miR-124 inhibits the proliferation of neural progenitor cells and promotes their differentiation into neurons by targeting the JAG1-notch signaling pathway (Liu et al., 2011). Recently, the generation of functional neurons by introducing miR-124 and related transcription factors into human fibroblasts has further highlighted the importance of miR-124 in neuronal differentiation (Ambasudhan et al., 2011; Yoo et al., 2011).

The actions of miR-137 on adult NSC proliferation and neuronal differentiation have been studied. It has been reported that miR-137 modulates the proliferation and differentiation of adult NSCs by targeting EZH2, a histone H3 lysine 27 methyltransferase (Szulwach et al., 2010). The overexpression of miR-137 promotes the proliferation of adult NSCs, whereas the repression of miR-137 enhances the differentiation of adult NSCs. Additionally, miR-137 also regulates neuronal maturation by inhibiting dendrite formation through binding Mind bomb 1 (Mibl) (Smrt et al., 2010). Mibl is an ubiquitin ligase that is known to be important for neurodevelopment. The overexpression of Mibl rescues the neuronal maturation deficits associated with miR-137 overexpression.

Another miRNA that plays a significant role in NSC proliferation and differentiation is miR-184. Methyl-CpG binding protein 1 (MBD1) directly represses the expression of miR-184 in adult NSCs. Furthermore, miR-184 suppresses the expression of Numb1 by targeting its 3′ UTR (untranslated region). Increased levels of miR-184 promote the proliferation but inhibit the differentiation of adult NSCs. Thus, MBD1, miR-184, and Numb1 form a regulatory pathway that maintains the balance between the proliferation and differentiation of NSCs (Liu et al., 2010).

In NSPCs isolated from adult mice, the expression of miR-106b−25 clusters has been identified. The overexpression of miR-25 or the entire miR-106b−25 cluster promotes adult NSPC proliferation and neuronal differentiation (Brett et al., 2011). The potential targets of miR-25 are involved in the TGFβ and insulin/IGF signaling pathways. The effects of miR-25 on NSPCs may be mediated by these two pathways. Moreover, the expression of miR-106b−25 is regulated by FoxO3, which is important for self-renewal, proliferation, and differentiation of NSCs (Paik et al., 2009; Renault et al., 2009). Therefore, miR-106b−25 may have crucial roles in the maintenance of adult neurogenesis.

Another recently reported miRNA involved in NSC differentiation is miR-34a. The miR-34 family members have been identified as direct p53 targets. Aranha et al. (2011) reported that miR-34a regulates NSC differentiation in the following ways: miR-34a increases the length of neurites by targeting sirtuin 1 (SIRT1), and miR-34a promotes astrocytic differentiation in a SIRT-independent manner.

Also, one miRNA that has been reported in human neurogenesis is miR-125b, which is abundantly expressed in animal

brains and is upregulated during neuronal differentiation. The overexpression of miR-125b significantly promotes the neurite outgrowth of two human cell lines, SH-SH5Y cells and ReNcell VM cells. More than 160 genes were predicted to be the targets of miR-125, and several targets were found to repress neuronal gene expression (Le et al., 2009).

Recently, miR-26b was reported to promote the differentiation of NSCs into neurons by targeting *ctdsp2* (RNA polymerase II C-terminal domain small phosphatase 2), which is an important component of REST/NRSF (neuron-restrictive silencer factor) suppressing the expression of neurogenesis related genes. It is noteworthy that miR-26b is located in an intron region of the *ctdsp2* gene. Therefore, the co-expression of miR-26b and *ctdsp2* constitutes an intrinsic negative feedback loop to govern the differentiation of NSCs to neurons (Dill et al., 2012; Han et al., 2012).

The above studies have highlighted the significant roles of miRNAs in the regulation of adult neurogenesis. As a class of autonomous cellular molecules, miRNAs may play even more crucial roles in adult neurogenesis than we expected. A full understanding of the miRNA regulatory network will ultimately provide more precise targets for therapeutic applications.

CONCLUDING REMARKS

Neurogenesis is a complex process that is tightly regulated at multiple levels in a time- and stage-dependent manner. miRNAs, as post-transcriptional regulators, play important roles in NSC proliferation and differentiation. Although a significant amount of literature has presented data on the regulation of NSCs and neurogenesis by miRNAs, two aspects have not received adequate attention. First, while the downstream targets of miRNAs have been heavily focused upon, the upstream regulators of miRNAs have been studied less. Second, the crosstalk between miRNAs has not been well studied. The emerging studies indicate that the regulation of neurogenesis requires the interplay between miRNAs and transcriptional regulators. In the future, insights into the global network of regulators of NSCs, including miRNAs, will help to elucidate the mechanism of neurogenesis. Furthermore, unraveling the full roles of miRNAs will help us to identify new molecular markers and offer novel therapeutic targets for neurological disorders.

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