Noncoding RNAs in Neural Stem Cell Development

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1. Introduction

Neural stem cells and neural progenitors/precursors (NSCs/NPs) are identified in both embryonic and adult central nervous system (CNS). NSCs can self-renew and give rise to neurons and glia. The development of NSCs is controlled by precisely orchestrated gene expression regulation. Recently, emerging evidence has shown the importance of noncoding RNA regulation in NSC self-renewal, proliferation, survival and differentiation. In this chapter, we will present new research of noncoding RNA functions in NSC development. We will highlight the future directions of applying noncoding RNAs in stem cell-based therapy for neurological diseases.

2. Noncoding RNAs

Noncoding RNAs (ncRNAs) are functional RNA molecules that do not show protein translation capability. ncRNAs consist of ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), piwi-interacting RNAs (piRNAs), microRNAs (miRNAs), and long noncoding RNAs (lncRNAs) and so on. ncRNAs have shown to play distinct but also conserved roles in normal development in invertebrates and vertebrates.

2.1 piRNAs

piRNAs are a group of small RNAs with size between 26-31 nucleotides (nt), which are only found in male and female germlines of invertebrates and within testes in mammalians (Houwing et al., 2007; 2009; Lander et al., 2001; Lau et al., 2006; Seto et al., 2007). piRNAs interact with piwi proteins to form RNA-protein complexes (Das et al., 2008; Houwing et al., 2007). The piRNA-protein complexes have been shown to silence transcription, specifically transposons (Brennecke et al., 2008; Das et al., 2008). Since piRNAs are mainly expressed during the germline stem cell development, they will not be discussed further in this chapter.

2.2 miRNAs

miRNAs are ~22 nt highly conserved small noncoding RNAs found in almost all eukaryotic cells (Khraiwesh et al., 2010) (Fig. 1). Like coding genes, miRNAs are mainly transcribed by the
RNA Polymerase II (Pol II) into long primary miRNA transcripts (pri-miRNAs). Pri-miRNAs are next cleaved by Drosha, a class 2 RNase III enzyme, to produce short hairpin stem-loop structures, known as precursor miRNAs (pre-miRNAs) (Ambros, 2008; Gregory et al., 2006). Pre-miRNAs are then processed into about 20-25 nt double-stranded mature miRNAs by RNase III enzyme Dicer (Lund and Dahlberg, 2006). The duplex undergoes unwinding and the strand with the weakest base pairing at its 5' terminus, together with Dicer and many associated proteins including Argonaute proteins, form an active RNA-induced silencing complex (RISC) (Neilson and Sharp, 2008; Rana, 2007). A miRNA recognizes and binds to the 3' untranslated region (3' UTR) of target messenger RNAs (mRNAs) by imperfect complementary sequence recognition (Neilson and Sharp, 2008; Wang et al., 2004). Once bound, the RISC can negatively regulate miRNA targets by degrading the mRNA or repressing its translation (Khraiwesh et al., 2010; Pratt and MacRae, 2009) (Fig. 1).

miRNAs are classified into intergenic and intragenic miRNAs, depending on their genomic location (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). Intergenic miRNAs are located at intergenic regions (between genes) in the genome. These miRNAs are usually transcribed using their own promoters. Intragenic miRNAs normally lie in intronic regions of coding genes with the same orientation. They are often transcribed together with the host genes.

Fig. 1. A scheme of microRNA biogenesis. microRNAs silence target coding genes by binding to the 3' untranslated region (3' UTR).

2.3 IncRNAs

The majority of the human genome has previously been considered as “junk” DNA, since only about 1.5% of the human genome, which occupies over 3 billion DNA base pairs,
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consists of protein-coding genes (Lander et al., 2001). A recent study of a large-scale complementary DNA (cDNA) sequencing project has shown that four fifths of transcripts of the human genome are RNA transcripts that don’t encode proteins (Kapranov et al., 2007). These RNA transcripts are normally longer than 200 nt, thus they are called long noncoding RNAs. Except no open reading frame (ORF) found within lncRNAs, they share many features with coding mRNAs such as 5’ capping. lncRNAs usually contain exons and introns (Carninci et al., 2005).

lncRNAs display different features of genomic location and orientation in the genome. Emerging evidence has demonstrated potential functions of lncRNAs in many biological events. lncRNAs have been shown to modulate gene transcription via different mechanisms. For example, some cis-antisense lncRNAs complementary to protein-coding transcripts regulate expression of the coding genes (Feng et al., 2006; Kotake et al., 2010; Pasmant et al., 2007; Tochitani and Hayashizaki, 2008; Yu et al., 2008); some lncRNAs modulate transcription factors by acting as co-regulators (Feng et al., 2006; Nguyen et al., 2001; Shamovskv et al., 2006; Wang et al., 2008; Willingham et al., 2005; Yang et al., 2001). Moreover, some studies have shown that lncRNAs are involved in epigenetic regulations such as chromatin and histone modification, and X-chromosome inactivation (Denisenko et al., 1998; Mancini-Dinardo et al., 2006; Mazo et al., 2007; Rinn et al., 2007; Sanchez-Elsner et al., 2006; Tsai et al., 2010; Wutz et al., 2002).

3. Noncoding RNAs and neural stem cell development

Noncoding RNAs such as miRNAs and lncRNAs participate gene expression regulation in many ways. The underlying mechanisms of noncoding RNA functions in normal development are beginning to be uncovered. In this book chapter, we will focus on reviewing functions of miRNAs and lncRNAs in neural stem cell development, such as NSC self-renewal, cell fate determination and survival.

3.1 miRNAs and self-renewal and proliferation of NSCs

The ability of self-renewal is essential for NSCs/NPs to perpetuate themselves to maintain an undifferentiated status during the embryonic stage and even in the adulthood (Gage, 2000; Shi et al., 2008; Temple, 2001). NSC proliferation and self-renewal are modulated by a complicated regulation network that consists of growth factors, epigenetic regulators, transcription factors and extrinsic signaling molecules from the NSC niche. Recent discovers have indicated that ncRNAs also play important roles in NSC self-renewal through a post-transcriptional regulation mechanism (Doe, 2008; Shi et al., 2008).

miRNAs have been shown to play essential roles in regulating NSC proliferation. Since Dicer is the key enzyme in miRNA processing, several studies have reported the global effects of miRNAs in NSC development by ablating Dicer and in turn blocking biogenesis of all miRNAs in the CNS using tissue specific Cre lines. Conditional deletion of Dicer from the mouse cerebral cortex using the Emx1-Cre line results in a significant reduction in cortical size and the cortical NP pool (De Pietri Tonelli et al., 2008; Kawase-Koga et al., 2010; Kawase-Koga et al., 2009). Dicer ablation from the mouse CNS using the Emx1-Cre and Nestin-Cre line causes a reduction of NSC numbers and abnormal differentiation (Andersson et al., 2010; Kawase-Koga et al., 2009) (Fig. 2A). Dicer-deficient NSCs display apoptosis when
mitogens are withdrawn from the culture medium (Fig. 2A). Because Dicer is also involved in maintaining the heterochromatin assembly, the defects of NSCs in Dicer knockout mice need to be carefully interpreted (Fukagawa et al., 2004; Kanellopoulou et al., 2005). Examining functions of individual miRNAs will help reveal precise roles of miRNAs in the NSC self-renewal and proliferation (Fig. 3).

Fig. 2. A. Dicer-deficient (Dicer-Ko) neural stem cells (NSCs) did not survive well in a differentiation culture medium without mitogens. Most Dicer-Ko neurospheres died after 48 hours in culture. Many differentiated cells (arrows) migrated away from the control neurosphere but not from the Dicer-Ko neurospheres. B. Under the differentiation condition without mitogens, passaged (p-1) Dicer-Ko NSCs gave rise to cells expressing neuronal (Tuj1+) and glial (GFAP+ and O4+) markers. However, their morphology was abnormal, as shown with shorter neurites and processes than controls (Kawase-Koga et al., 2010).

let-7, the first identified miRNA (Reinhart et al., 2000), has been shown to regulate NSC proliferation and differentiation by targeting the nuclear receptor TLX and the cell cycle regulator cyclin D1 (Zhao et al., 2010a) (Fig. 3). Overexpression of let-7b inhibits NSC proliferation and enhances differentiation, while knockdown of let-7b promotes NSC proliferation (Zhao et al., 2010a). It appears that the expression levels of let-7 in NSCs are controlled by a feedback regulation of Lin-28, a pluripotency factor that controls miRNA processing in NSCs (Rybak et al., 2008). Lin-28 binds to the let-7 precursor and inhibits its processing by Dicer. On the other hand, the expression of Lin-28 is repressed by let-7 and miR-125, allowing the maturation of let-7 (Fig. 4A). This feedback loop reveals an autoregulation between miRNA let-7 and miR-125, and transcription factor Lin-28 during NSC development (Rybak et al., 2008).

miR-124 is identified as a CNS-enriched miRNA and its expression is upregulated during neuronal differentiation (Lagos-Quintana et al., 2002) (Fig. 3). In the adult brain, NSCs are identified in the subventricular zone (SVZ). In cultured adult NSCs derived from the SVZ and in the SVZ in vivo, knocking down miR-124 results in an increase of NSC proliferation and a decrease of differentiation, while overexpressing miR-124 reduces the number of dividing precursors and enhances neuronal differentiation (Cheng et al., 2009). Moreover, miR-124 modulates NSC proliferation and differentiation by suppressing Sox9 expression in adult NSCs (Cheng et al., 2009). A recent study has shown that miR-124 regulates neuronal differentiation through a mutual inhibition mechanism of Ephrin-B1 (Arvanitis et al., 2010).
addition, miR-124 promotes differentiation of NPs by modulating a network of nervous system-specific alternative splicing through suppressing expression of PTBP1, which encodes a global repressor of alternative pre-mRNA splicing (Makeyev et al., 2007). Together, miR-124 plays a general role in promoting differentiation of embryonic and adult NSCs and NPs. It appears that miR-124 executes its function through repressing various targets.

Fig. 3. Many miRNAs are involved in neural stem cell (NSC) self-renewal and differentiation into neurons, astrocytes and oligodendrocyte precursor cells (OPCs) and oligodendrocytes.

Fig. 4. Feedback loop regulation of miRNAs and their target genes. A. Let-7 processing is inhibited by Lin-28, and the 3' untranslated region (3' UTR) of Lin-28 has binding sites for Let-7. B. TLX inhibits miR-9 expression, while miR-9 displays silencing effects on TLX.
miR-9 is another CNS-enriched miRNA. miR-9 is shown to inhibit NSC proliferation but promote differentiation through a feedback regulation of a nuclear receptor TLX (Zhao et al., 2009) (Fig. 4B). In human embryonic stem cell (ESC) derived NPs, miR-9 is shown to have a positive effect on proliferation but a negative effect on migration by directly targeting Stmn1, which increases microtubule instability (Delaloy et al., 2010). The opposite effect of miR-9 on proliferation is perhaps caused by differential physical contacts of miR-9 with target genes and the different culture systems.

In the CNS of Xenopus, miR-9 knockdown promotes the proliferation of NPs in the hindbrain, leads to an increased expression of cyclin D1 and a downregulation of p27Xic1 (Bonev et al., 2011). miR-9 targets Hairy1 and regulates proliferation of NPs (Bonev et al., 2011). In zebrafish, miR-9 promotes differentiation of NPs that give rise to neurons at the midbrain-hindbrain domain and controls the organization of the midbrain-hindbrain boundary by targeting several genes in the Fibroblast growth factor (Fgf) signaling, such as fgf8-1 and fgfr1 (Leucht et al., 2008). In the chick spinal cord, miR-9 specifies a subtype of motor neurons that project axons to the axial muscles from motor neuron progenitors by specifically targeting transcription factor FoxP1 (Otaegi et al., 2011).

In the mouse brain, miR-9 function is demonstrated by the generation of miR-9-2 and miR-9-3 double knockout mice. miR-9 double mutants show reduced cortical layers, disordered migration of interneurons, and misrouted thalamocortical axons and cortical axon projections, suggesting an important role of miR-9 in NP proliferation, differentiation and migration during brain development (Shibata et al., 2011). Moreover, it appears that miR-9 regulates multiple target genes, including Foxg1, Pax6 and Gsh2, which have shown to be essential in cortical development (Shibata et al., 2011). Therefore, miR-9 plays an important role in controlling differentiation of NSCs/NPs in different regions in the CNS (Fig. 3).

The major role of let-7, miR-124 and miR-9 is to inhibit NSC/NP proliferation and to induce their differentiation into specific cell types. miRNAs that promote proliferation of NSCs and NPs have also been identified (Fig. 3). miR-134 plays a role in enhancing proliferation of cortical NPs by targeting doublecortin (Dcx) and/or Chordin-like 1 (Chrdl-1) (Gaughwin et al., 2011). miR-25 is shown to be a major player in the miR-106-25 cluster in neural development. Overexpression of miR-25 but not miR-106b and miR-93 promotes adult NP proliferation (Brett et al., 2011). Interestingly, the expression of the miR-106-25 cluster is regulated by FoxO3, a transcription factor maintaining the NSC population (Renault et al., 2009).

During the retina development, otx2 and vsx1 genes are shown to control the division of retinal precursors and differentiation into bipolar retina neurons. In early retinal precursors, the expression of otx2 and vsx1 is inhibited, accompanied with a rapid precursor division. miR-129, miR-155, miR-214, and miR-222, which are highly expressed in the embryonic retina, have been identified to target and repress translation of otx2 and vsx1, by which they promote proliferation of retinal precursors (Decembrini et al., 2009).

miRNA expression is also controlled by epigenetic regulators in the NSC development. The expression of miR-137 is regulated by DNA methyl-CpG-binding protein (MeCP2) and transcription factor Sox2. miR-137 modulates adult NSC proliferation and cell fate determination by targeting Ezh2, a histone methyltransferase and polycomb group protein (Szulwach et al., 2010). Ectopic expression of miR-137 in adult NSCs enhances proliferation, while knockdown of miR-137 promotes differentiation of adult NSCs (Szulwach et al., 2010).
In addition, miR-184 expression is suppressed by methyl-CpG binding protein 1 (MBD1) and miR-184 promotes adult NSC proliferation by repressing the expression of Numb-like (Numbl) (Liu et al., 2010).

3.2 IncRNAs and proliferation of NSCs

The IncRNAs may also play a role in controlling NSC proliferation, even though studies of IncRNAs in NSC development are still sparse. Sox2 is a transcription factor and plays a key role in the maintenance of the undifferentiating state of embryonic and adult NSCs (Pevny and Placzek, 2005). Sox2 overlapping transcript (Sox2OT) is an IncRNA containing Sox2 gene and shares the same transcriptional orientation with Sox2 (Fig. 5A). Similar to Sox2, Sox2OT is stably expressed in mouse embryonic stem cells and down-regulated during differentiation. Sox2OT is expressed in the neurogenic regions of the adult mouse brain including olfactory bulb (OB), rostral migratory stream (RMS) and SVZ, and is dynamically regulated during vertebrate CNS development, implying its role in regulating NSC self-renewal and neurogenesis (Amaral et al., 2009; Mercer et al., 2008).

Fig. 5. Genomic location and potential functions of long noncoding RNAs (lncRNAs) (Bian and Sun, 2011). A. Sox2 overlapping transcript Sox2OT is an IncRNA containing Sox2 gene and shares the same transcriptional orientation with Sox2. B. Evf2 is transcribed from the intergenic region between the Dlx-5 and Dlx-6 loci, and is overlapped with Dlx-5/6 enhancer i (ei) and enhancer ii (eii) sequences. Evf2 acts as a transcriptional co-activator of Dlx-2 and activates the Dlx5/6 enhancer. C. Nkx2.2 antisense (Nkx2.2as) is an antisense IncRNA to Nkx2.2 gene and promotes Nkx2-2 expression.

3.3 Summary

Taken together, self-renewal and differentiation of NSCs and NPs are controlled by complex gene regulation networks that consist of both protein coding genes and noncoding miRNAs. During proliferation and differentiation of NSCs and NPs, one miRNA can have multiple target genes and features a feedback regulation with their targets (Figs. 3 and 4). The availability of physical contacts and the binding affinity of a miRNA and its targets perhaps determine interactions of the miRNA with the specific targets. The interactions of miRNAs
and their target genes eventually produce proper protein output of key factors that directly control self-renewal, proliferation and differentiation of NSCs/NPs.

3.4 NSC survival controlled by noncoding RNAs

Several reports have shown that miRNAs play a general role in controlling cell survival. Conditional deletion of Dicer from neural crest cells using Wnt1-Cre mouse line results in an increased apoptosis of neural crest-derived cells (Zehir et al., 2010). Ablation of Dicer from postmitotic neurons in the cortex and the hippocampus using calmodulin kinase II (CaMKII) promoter-driven Cre transgenic mice results in smaller cortex, enhanced cortical cell death (Davis et al., 2008). Emx1-Cre Dicer conditional knockout mice have shown an increased apoptosis, especially in the ventricular zone (VZ) and SVZ (De Pietri Tonelli et al., 2008). Our own work of cortical NSCs of Emx1-Cre Dicer conditional knockout mice using proteomic analysis by mass spectrometry and bioinformatic assays has indicated that Dicer deletion results in an increase of pro-cell-death and a decrease of pro-survival proteins in Dicer-deficient NSCs (Kawase-Koga et al., 2010) (Fig. 2A). Interestingly, an upregulation of fragile X mental retardation protein (FMRP), a proven target for miR-124, and Caspase3, a key cell apoptosis molecule, are observed in Dicer-deficient NSCs. On the other hand, proteins such as transforming growth factor-beta receptor type II (TGFβR2) and SOD1 are downregulated in Dicer-deficient NSCs (Kawase-Koga et al., 2010). These observations suggest that miRNAs perhaps control survival of NSCs by modulating the balance of protein output of genes regulating apoptosis and survival.

Neurotrophins and their receptors play important roles in the NSC proliferation, survival and differentiation. miR-128 is shown to target the truncated non-catalytic form of the human neurotrophin-3 receptor (NTRK3), which affects membrane remodeling and cytoskeletal reorganization. Overexpression of miR-128 in neuroblastoma cells leads to round cell body and shorter neurites, which is similar to knockdown of truncated NTRK3. miR-128 overexpression causes altered expression of genes involved in cell proliferation and apoptosis such as antiapoptotic factor Bcl-2, suggesting an important role of miR-128 on cell survival (Guidi et al., 2010). Moreover, miR-134 is shown to be required for inhibiting apoptosis initiated by Chrdl-1 in cortical progenitors (Gaughwin et al., 2011). Studies on an ethanol teratogenic culture model by exposing embryonic cortex-derived NPs in ethanol have revealed different roles of miRNAs during this pathological process (Sathyan et al., 2007). In NP cultures, miR-21 is suppressed by the ethanol exposure and the reduction of miR-21 causes cell apoptosis, suggesting an anti-apoptotic effect of miR-21 (Sathyan et al., 2007).

The BH3-only family is a group of pro-apoptotic regulators, including Bim, Hrk, Bmf, Puma and N-Bak, which induce cytochrome c release from mitochondria (Giam et al., 2008). Overexpression of miR-29b in neurons inhibits endogenous BH3-only proteins Bim, Puma and Bmf, and promotes neuronal survival (Giam et al., 2008; Kole et al., 2011). In the brain of the calorie-restricted mice, expression of three miRNAs, miR-181a-1*, miR-30e and miR-34a, is significantly downregulated with a corresponding upregulation of their target gene Bcl-2, a decrease of pro-apoptotic factor Bax and cleavage of Caspases (Khanna et al., 2011). Overexpressing these three genes results in an increased cell apoptosis, accompanied with a decrease in Bcl-2 expression (Khanna et al., 2011).
miRNAs also play an important role in neural tissue growth and organ development by regulating cell survival. In *Drosophila*, the Hippo pathway together with Yorkie transcriptional activator contribute to the regulation of tissue growth by stimulating cell proliferation and inhibiting apoptosis (Saucedo and Edgar, 2007). Recent studies have shown that Yorkie not only activates cyclin E and apoptosis inhibitor DIAP1, but also triggers the expression of *bantam* miRNA to promote proliferation and cell survival (Huang et al., 2005; Thompson and Cohen, 2006). A downregulation of *bantam* miRNA is found in dying Rim cells at the eye margin, and restoration of *bantam* miRNA to higher levels prevents apoptosis of these cells, suggesting a role of *bantam* miRNA in enhancing cell survival in eye development (Thompson and Cohen, 2006). In addition, as the largest miRNA family in *Drosophila*, miR-2/6/11/13/308 are required for inhibiting embryonic apoptosis by suppressing pro-apoptotic factors hid, grim, reaper and sickle (Leaman et al., 2005).

In the forebrain of *Xenopus*, miR-9 deletion results in apoptosis of NPs due to increased expression of p53 (Bonev et al., 2011). In addition, miR-24a is expressed in the retina of *Xenopus* (Walker and Harland, 2009). Overexpression of miR-24a in retinal cells prevents cells from death, while knockdown of miR-24a causes a reduction in eye size due to an increased apoptosis (Walker and Harland, 2009). miR-24a controls cell survival by a negative regulation of pro-apoptotic factors caspase9 and apa1.

In summary, miRNAs play critical roles in regulating survival of both NSCs/NPs and postmitotic neurons. miRNAs either promote cell survival or lead to apoptosis, depending on functions of their target genes.

### 3.5 NSC differentiation and cell fate determination mediated by noncoding RNAs

In the mammalian CNS, different neural cell types arise and migrate in a precise temporospatial manner. In the developing mouse brain, neurons arise first by embryonic day 12 (E12), neurogenesis peaks at E14 and ceases by E18. Astrocytes appear around E18, with their numbers peaking in the postnatal period. Oligodendrocytes are generated after birth when the neurogenesis is almost complete. Studies have shown that ncRNAs play an important role in regulating both neurogenesis and gliogenesis.

#### 3.5.1 Cell fate determination controlled by miRNAs

miRNAs play essential roles in NSC differentiation and the cell fate switch between neurons and glia (Cuellar et al., 2008; Hebert et al., 2010; Zheng et al., 2010). We have found that *Dicer*-deficient NSCs display abnormal differentiation, with shorter neurites in neurons and fewer processes in glial cells (Kawase-Koga et al., 2010) (Fig. 2B). Conditional deletion of *Dicer* from the mouse forebrain neurons using *CamKII-Cre* line results in neuronal degeneration and an increase in glial fibrillary acidic protein (GFAP)-positive astrocytes (Hebert et al., 2010). *Dicer* ablation in the dopaminceptive neurons in the basal ganglia using a dopamine receptor-1 (DR-1)-Cre line leads to astrogliogenesis, but not neurodegeneration (Cuellar et al., 2008). Interestingly, in the mouse spinal cord, conditional deletion of *Dicer* using *Olig1-Cre* line disrupts production of both oligodendrocytes and astrocytes (Zheng et al., 2010). These observations suggest that global loss of miRNAs in specific precursor cells affects production of distinct cell types.
miRNA expression profiling studies have shown that some miRNAs are preferentially expressed in neurons or glia. For example, miR-124 and miR-128 are highly expressed in neurons, while miR-23 is restrictively expressed in astrocytes. miR-26 and miR-29 display higher expression in astrocytes than in neurons; and miR-9 and miR-125 are evenly expressed in neurons and astrocytes (Smirnova et al., 2005). Overexpressing miR-124 in cultured NSCs and in embryonic cortical NPs using lenti-virus and in utero electroporation, respectively, promotes neurogenesis and stimulates cortical progenitor migration (Maiorano and Mallamaci, 2009). In cultured adult NSCs, overexpressing miR-124 enhances neuronal differentiation (Cheng et al., 2009). Ectopic expression of miR-124a and miR-9 in the embryonic stem cell-derived NPs results in a great reduction of GFAP-positive astrocytes compared to the control groups, while knockdown of miR-9, but not miR-124a, switches differentiation of NPs from neurogenesis to astrogliogenesis (Krichevsky et al., 2006). miR-124 and miR-9 promotes neurogenesis by targeting phosphololated signal transducer and activator of transcription 3 (STAT3), a transcription factor normally initiating astrogliogenesis (Bonni et al., 1997; Krichevsky et al., 2006).

miR-200 family members, including miR-200a, miR-200b, miR-200c, miR-141 and miR-429, are highly expressed in the developing olfactory bulb. Loss of function of the miR-200 family prevents normal differentiation of olfactory precursors into mature neurons (Choi et al., 2008). Foxg1, Zfhx1 and Lfng have been identified as the targets of the miR-200 family that affect neurogenesis of the olfactory bulb.

Specific miRNAs that promote gliogenesis have also been identified. Brain-enriched miR-125b is up-regulated in cultured interleulin-6 (IL-6)-induced human astrocytes. Loss of function of miR-125b causes an impaired proliferation of astrocytes, accompanied by an upregulation of a miR-125b target cyclin-dependent kinase inhibitor 2A (CDKN2A), which is a negative modulator for cell proliferation (Pogue et al., 2010). The miR-17-92 cluster displays enriched expression in cultured oligodendrocytes. Specific deletion of the miR-17-92 cluster from oligodendrocyte precursor cells (OPCs) results in a decreased number of Olig2-positive oligodendrocytes in the mouse brain (Budde et al., 2010). Overexpression of miR-17 and miR-19b in cultures increases the number of oligodendrocytes. The miR-17-92 cluster regulates oligodendrocyte development by targeting tumor suppressor Pten and activating its downstream Akt signaling pathway.

Moreover, miR-219 and miR-338 are identified in the oligodendrocyte lineage in the mouse spinal cord and brain. Overexpression of miR-219 and miR-338 in cultured OPCs and in the embryonic chick neural tube promotes differentiation of oligodendrocytes, while knockdown of these two miRNAs in OPC cultures and knockdown of miR-219 in zebrafish abolish oligodendrocyte maturation (Zhao et al., 2010b). Oligodendrocyte differentiation inhibitors Sox6 and Hes5 are identified as targets of miR-219 and miR-338 during oligodendrocyte development (Zhao et al., 2010b).

Lamin B1 (LMNB1) is reported to be associated with autosomal domination leukodystrophy disease (ADLD), a CNS demyelination disorder (Padia et al., 2006). Overexpression of Lamin B1 represses expression of oligodendrocyte-specific genes such as myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG), and leads to impaired oligodendrocyte maturation. A recent study has shown that Lamin B1 is post-transcriptionally regulated by miR-23, a glia-specific miRNA. Overexpression of miR-23
results in significantly increased number of oligodendrocytes and rescues the defects of oligodendrocyte differentiation caused by Lamin B1 (Lin and Fu, 2009).

### 3.5.2 Cell fate determination regulated by IncRNAs

Studies of IncRNA functions on NSC differentiation are emerging. Dlx-6 is a homeobox containing transcription factor and plays an important role in forebrain neurogenesis (Wang et al., 2010). *Embryonic ventral forebrain-1 (Evf1)* is a 2.7 kb IncRNA transcribed upstream of the mouse *Dlx-6* gene (Kohtz and Fishell, 2004). As an alternatively spliced form of *Evf1*, *Evf2* is transcribed from the intergenic region between the *Dlx-5* and *Dlx-6* loci, and is overlapped with the conserved *Dlx-5/6* intergenic enhancer (Feng et al., 2006; Zerucha et al., 2000) (Fig. 5B). Induced by the Sonic hedgehog (Shh) signaling pathway, *Evf2* has been proven to function as a transcriptional co-activator of *Dlx-2* and activates the *Dlx5/6* enhancer during forebrain development (Feng et al., 2006). Deletion of *Evf2* results in a reduction of GABAergic interneurons and impaired synaptic inhibition in the developing hippocampus (Bond et al., 2009).

*Nkx2.2 antisense (Nkx2.2as)* is an antisense IncRNA to *Nkx2.2* gene, which is expressed in the developing mammalian forebrain and is required for oligodendrocyte development (Price et al., 1992) (Fig. 5C). Ectopic expression of *Nkx2.2as* in cultured NSCs induces oligodendrocyte differentiation through an upregulation of the *Nkx2.2* mRNA level, suggesting that *Nkx2.2as* regulates NSC differentiation and promotes gliogenesis by modulating protein coding gene *Nkx2.2* expression (Tochitani and Hayashizaki, 2008).

*Retinal noncoding RNA 2 (RNCR2)*, an intergenic IncRNA also known as *Gomafu* and *Miat*, is an abundant polyadenylated RNA in the developing retina (Blackshaw et al., 2004). *RNCR2* is highly expressed in both mitotic and postmitotic retinal progenitors. Knockdown of *RNCR2* leads to an increase of amacrine cells and Müller glial cells in postnatal retina. Mislocalization of *RNCR2* from nuclear to cytoplasm photocopies the effects caused by *RNCR2* knockdown, suggesting that *RNCR2* is required for retinal precursor cell specification (Rapicavoli et al., 2010).

### 4. Noncoding RNAs as a tool for stem cell-based therapy

Because of the features of self-renewal and the ability to differentiate into many cell types in the CNS, applying NSCs for the treatment of neurological disorders, especially neurodegeneration diseases and injuries in the CNS, has become promising. Directing NSCs into specific cell types and transplanting these cells to replace damaged cells in the CNS have been proven to be successful in some mouse models (Kim and de Vellis, 2009).

Transplantation of NSCs into aged triple transgenic Alzheimer’s disease mouse model (3×Tg-AD) rescues the spatial learning and memory defects in these mice (Blurton-Jones et al., 2009). Parkinson’s disease (PD) results from a loss of dopaminergic neurons in the substantia nigra. It involves abnormalities in movement variably accompanied by sensory, mood and cognitive changes. Transplantation of undifferentiated human NSCs into PD primate models causes a significant behavioral improvement (Redmond et al., 2007). Directed differentiation of mouse ventral midbrain NSCs in the presence of Shh, FGF8 and Wnt5a produce 10-fold more dopaminergic neurons *in vitro* (Parish et al., 2008).
Transplantation of these pre-differentiated dopaminergic neurons into the brain of PD mouse models results in functional recovery (Parish et al., 2008). Implantation of human NSCs in the rat model of Huntington’s disease (HD) is shown improved motor function (McBride et al., 2004). Furthermore, delayed transplantation of adult mouse NSCs surrounding the lesion site of the spinal cord promotes remyelination and functional recovery after spinal cord injuries in rats (Karimi-Abdolrezaee et al., 2006).

Stem cell-based therapeutic applications for neurological disorders also face problems. First, the molecular mechanisms that control NSC proliferation and differentiation into distinct cell types are still unclear. Second, to succeed in clinical applications, transplanting sufficient numbers of NSCs and specific neuronal cell types is critical. Third, to achieve functional recovery from neurological disorders, transplanted cells need to acquire connections with neighbor neurons and restore neural circuitry. Although little studies of using ncRNAs for therapeutic treatment have been done, the emerging reports of ncRNA functions in NSC proliferation and cell fate determination have shown promising future directions. Moreover, due to the technical advances in ncRNA in vitro synthesis and delivery, particularly miRNAs, manipulating ncRNA expressions in NSCs will provide a new means for stem cell based therapies for neurological diseases.

5. Acknowledgment

We thank members of the Sun lab for providing thoughtful comments. Owing to space limitations, we apologize for being unable to cite many excellent papers in this field. This work was supported by the Ellison Medical Foundation (T. S.), an award from the Hirschl/Weill-Caulier Trust (T. S.) and an R01-MH083680 grant from the NIH/NIMH (T. S.).

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This book is a collective work of international experts in the neural stem cell field. The book incorporates the characterization of embryonic and adult neural stem cells in both invertebrates and vertebrates. It highlights the history and the most advanced discoveries in neural stem cells, and summarizes the mechanisms of neural stem cell development. In particular, this book provides strategies and discusses the challenges of utilizing neural stem cells for therapy of neurological disorders and brain and spinal cord injuries. It is suitable for general readers, students, doctors and researchers who are interested in understanding the principles of and new discoveries in neural stem cells and therapy.

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