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Epigenetic regulation of neural stem cell property from embryo to adult

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ABSTRACT

Neural stem cells (NSCs) have the ability to self-renew and give rise to neurons and glial cells (astrocytes and oligodendrocytes) in the mammalian central nervous system. This multipotency is acquired by NSCs during development and is maintained throughout life. Proliferation, fate specification, and maturation of NSCs are regulated by both cell intrinsic and extrinsic factors. Epigenetic modification is a representative intrinsic factor, being involved in many biological aspects of central nervous system development and adult neurogenesis through the regulation of NSC dynamics. In this review, we summarize recent progress in the epigenetic regulation of NSC behavior in the embryonic and adult brain, with particular reference to DNA methylation, histone modification, and noncoding RNAs.

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1. Introduction

Neural stem cells (NSCs) are defined as cells that have the capacity for self-renewal and differentiation into 3 cell types: neurons and 2 types of glial cells, astrocytes, and oligodendrocytes. Neurogenesis, the process by which neurons are generated from NSCs, occurs not only in the embryonic but also in the postnatal/adult brain. Neurogenesis takes place in particular zones throughout organism's lifespan and has been implicated in various brain functions, such as learning and memory (Bond et al., 2015; Christian et al., 2014; Gotz and Huttner, 2005; Greig et al., 2013; Hsieh and Eisch, 2010; Kriegstein and Alvarez-Buylla, 2009; Lledo et al., 2006; Miller and Gauthier, 2007; Ming and Song, 2011). Therefore, neurogenesis is an important process for both brain development and brain function.

During embryonic brain development, NSCs show unique features as they produce each type of cell in the brain. The onset of neurogenesis and gliogenesis is spatiotemporally restricted in the developing brain, in which NSCs generate each type of cell in a developmental stage-dependent manner: neurons are produced first, followed by glial cells. This change in the differentiation capacity of NSCs as development proceeds is tightly regulated through cooperation between extracellular factors and epigenetic mechanisms. In addition, NSCs that have been maintained until adulthood mainly produce neurons, and this adult neurogenesis consists of multiple steps. NSCs generate neurons via the production of transiently amplifying neuronal precursor cells (NPCs) through asymmetrical division. Thus, adult neurogenesis starts from the activation of NSC proliferation, which is followed by production of NPCs and their differentiation into neurons. Moreover, newly generated neurons are integrated into preexisting neuronal circuitry, thereby contributing to brain functions. Survival and maturation of newborn neurons are thus also key steps for adult neurogenesis to occur properly. In addition to embryonic neurogenesis, accumulating research in adult neurogenesis has been highlighting the importance of epigenetic regulation to control these processes.

Epigenetics can be defined as heritable influences on chromatin and gene function that are not accompanied by a change in DNA sequence in the progeny of cells or individuals (Allis et al., 2015). DNA methylation, histone modification, and noncoding RNAs are predominant epigenetic factors and have been studied for the last 2 decades. These studies have demonstrated that distinct epigenetic factors communicate with each other and play an essential role for the regulation of NSCs in collaboration with extracellular cues. In this review, we introduce representative examples of epigenetic mechanisms involved in the dynamics of embryonic and adult NSCs.

1.1. Epigenetic mechanisms regulating neurogenic and gliogenic competence of NSCs during development

Although the 3 types of cells in the brain are generated from NSCs during central nervous system (CNS) development, NSCs do not initially have the potential to generate these cell types. NSCs acquire this competence sequentially as development proceeds. After NSCs expand their pool by self-renewing in early brain development, they first produce neurons, followed by astrocytes and oligodendrocytes (Fig. 1). Many factors that induce NSC differentiation into each lineage have been identified, including Wingless/int (Wnt, neurons) (Hirabayashi et al., 2004; Muroyama et al., 2004; Zhou et al.,

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2006a), leukemia inhibitory factor (astrocytes) (Bugga et al., 1998; Nakashima et al., 1999a,b; Namihira and Nakashima, 2013), and Sonic hedgehog (Shh, oligodendrocytes) (Lu et al., 2000; Tekki-Kessaris et al., 2001). Recent studies have indicated that the way in which NSCs respond to these factors alters during development and that epigenetic mechanisms restrict the responsiveness of NSCs to them at each stage of development. This contributes to the sequential production of neurons and glial cells during development. In this section, we describe how NSCs sequentially acquire the competence to generate cells in each lineage during development, focusing on the epigenetic mechanisms in the order of histone modification, DNA methylation, and noncoding RNAs.

1.2. Histone modification regulating differentiation of NSCs

The remodeling of chromatin structure via histone modifications is an important means of modulating gene expression. Chromatin is composed of multiple nucleosomes, each consisting of 147 base pairs of DNA wrapped around histone proteins. Single nucleosomes contain 2 copies of each histone variant: histone 2A, histone 2B, histone 3 (H3), and histone 4. The amino acid residues in the N-terminal tails of histone proteins are subject to multiple posttranscriptional modifications, including acetylation, methylation, phosphorylation, and ubiquitination (Bernstein et al., 2007; Kouzarides, 2007; Margueron and Reinberg, 2010; Ruthenburg et al., 2007; Tessarz and Kouzarides, 2014). Histone modifications affect the access of transcription factors to their binding sites, thereby influencing gene activation and repression. For instance, lysine methylation of histone tails is associated with both activation and repression of gene expression; the effect of histone methylation on gene expression differs according to the position in the histone tail and the number of methylation in the lysine residues. H3 methylation at lysine 4 (K4), K36, and K79 leads to transcriptional activation, whereas H3 methylation at K9 and K27 is associated with transcriptional silencing. Both neurogenic and gliogenic gene promoters undergo various histone modifications, which ensure the sequential production of each cell type at appropriate stages of development.

Neuronal differentiation precedes glial differentiation of NSCs during development, and NSCs terminate neuronal production gradually as glial differentiation commences. The reduction of neuronal differentiation results from a change in the responsiveness of NSCs to Wnt/β-catenin signaling. Activation of Wnt/β-catenin signaling induces neuronal differentiation during midgestation (neurogenic phase) through up-regulation of the proneural basic helix-loop-helix (bHLH) transcription factor neurogenin1 (Neurog1) (Hirabayashi et al., 2004; Sun et al., 2001). However, *Neurog1* expression is no longer induced in NSCs at late gestation, when astrogliogenesis from NSCs begins, even when Wnt/β-catenin signaling is activated. This different responsiveness of NSCs to Wnt/β-catenin signaling at each developmental stage is attributed to a change in histone modification status on the *Neurog1* promoter.

The polycomb group (PcG) complex catalyzes H3 K27 trimethylation (H3K27me3), leading to transient transcriptional repression through alteration of local heterochromatin configuration (Ng and Gurdon, 2008; Ringrose and Paro, 2007). The PcG consists of 2 complexes, polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 (PRC2). PRC2 is responsible for the initiation of gene silencing by catalyzing H3K27me3, which provides a mark for PRC1 to be recruited for gene silencing. PRC1 maintains H3K27me3 and takes part in compacting chromatin states (Cao and Zhang, 2004; Shen et al., 2008).

One of the PRC2 components, enhancer of zeste 2 (Ezh2), an enzyme responsible for H3K27me3, is highly expressed in NSCs in the gliogenic phase and prevents Wnt signaling–mediated expression of

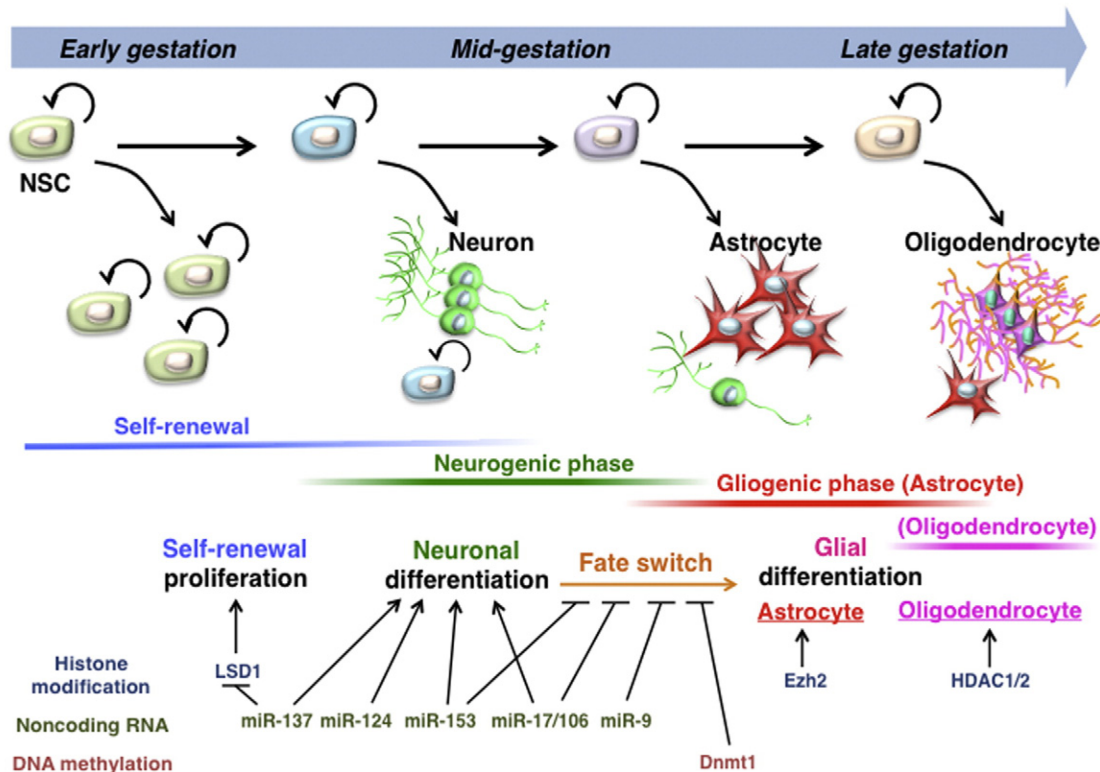

Fig. 1. Schematic diagram of developmental stage-dependent NSC differentiation in the embryonic brain. During early embryonic stages, NSCs undergo self-renewing symmetric divisions, leading to the expansion of their pool. NSCs first acquire the potential to differentiate into neurons at midgestation. They then acquire the ability to differentiate into glial cells from late to perinatal stages. Representative epigenetic regulators of NSCs during embryonic brain development are depicted. Semicircular arrows indicate the self-renewal and proliferation of NSCs. →, promotion; ⊣, inhibition.

neuronal genes at the onset of astrogliogenesis (Hirabayashi et al., 2009; Pereira et al., 2010) (Fig. 1). Consistent with the loss of neurogenic competence of NSCs during development, *Neurog1* gains H3K27me3 in the promoter, and its expression becomes repressed as NSC differentiation leans toward astrocyte production. Ezh2 directly targets *Neurog1* and suppresses its expression by catalyzing H3K27me3 in the promoter region (Hirabayashi et al., 2009). This eventually limits the responsiveness of NSCs to Wnt signaling and contributes to switching the competence of NSCs from neurogenic to gliogenic. In fact, NSC-specific conditional deletion of *Ezh2* before the onset of astrocyte differentiation prolongs neuronal production and delays the production of astrocytes (Pereira et al., 2010).

Polycomb repressive complexes also affect layer formation of the cortex. Newly generated neurons migrate to the outside of the cortical plate along with radial processes of NSCs, which extend to the pial surface, and establish the cortical layer in an inside-out order: deep layer (DL) neurons are generated first, followed by upper layer (UL) neurons (Greig et al., 2013; Molyneaux et al., 2007). The Fez transcription factor family member zinc-finger 2 (Fezf2) plays a key role in the differentiation of NSCs into DL neurons, and its expression governs the period for the production of DL neurons (Chen et al., 2005a, 2005b; Molyneaux et al., 2005). Ring1B, one of the components of PRC1, represses the expression of Fezf2 and contributes to the termination of DL neuron production. Deletion of *Ring1B* in NSCs prolongs the expression of Fezf2, resulting in increased production of DL neurons (Morimoto-Suzki et al., 2014).

Histone acetylation also affects appropriate layer formation by regulating the fate propensity of NSCs from DL neurons to UL neuron production. Acetylation of histone N-terminal tails induces relaxation of the nucleosomes by decreasing the interaction of the positively charged histone tails with the negatively charged DNA. Histone deacetylases (HDACs) condense chromatin by removing acetyl groups from histone tails, which prevents the access of transcription factors to their cognate sites and leads to transcriptional repression. Blocking HDAC activity in the neurogenic phase, using the HDAC inhibitor suberoylanilide hydroxamic acid, decreases the production of DL neurons and increased UL neurons from NSCs (Yuniarti et al., 2013). This finding suggests that histone acetylation participates in the transition from DL neuron to UL neuron production during cortical development.

As in neuronal differentiation, bHLH transcriptional factors play a critical role in oligodendrocyte differentiation. Shh signaling is highly activated in the ganglionic eminence of the ventral telencephalon, where oligodendrocytes are induced by the Shh-induced bHLH transcriptional factors Olig1 and Olig2 (Lu et al., 2000; Tekki-Kessaris et al., 2001). These 2 factors play essential roles in oligodendrocyte differentiation and maturation. Compound *Olig1* and *Olig2* knock-out (KO) mice displayed complete loss of oligodendrocytes (Zhou and Anderson, 2002). Olig2 is essential for the initiation of oligodendrocyte differentiation, whereas Olig1 is required for the maturation of oligodendrocytes and the remyelination of axons after oligodendrocyte ablation (Arnett et al., 2004; Dai et al., 2015; Liu et al., 2007; Xin et al., 2005). bHLH transcription factors activate expression of downstream targets by forming dimers with ubiquitously expressed bHLH E-proteins such as E12 and E47. However, the negative HLH factors hairy and enhancer of split (Hes) and inhibitor of differentiation (Id) inhibit bHLH transcription factors by competing with them for dimerization with E-proteins. Wnt signal activation induces expression of the negative HLH factors Id2 and 4, limiting the generation of oligodendrocytes (Ye et al., 2009). The activation of Wnt signaling declines at the onset of oligodendrocyte differentiation, implying that Wnt signaling influences the timing of oligodendrocyte differentiation during brain development (Langseth et al., 2010).

HDACs modulate the inhibition of oligodendrocyte production by Wnt signaling and ensure oligodendrocyte development. HDAC activity is essential for the development and maturation of oligodendrocytes: treatment with HDAC inhibitor attenuates oligodendrocyte differentiation (Marin-Husstege et al., 2002), and HDAC1 and HDAC2 double KO mice show severe defects in oligodendrocyte production and maturation (Ye et al., 2009) (Fig. 1). Wnt signaling stabilizes β-catenin, which then induces downstream targets of Wnt signaling, such as Id2 and 4, by forming a complex with transcription factor 7–like 2 (TCF7L2), a downstream effector. HDAC1 and 2 bind competitively to TCF7L2 with β-catenin, thereby inhibiting expression of the *Id2* and *4* genes (Ye et al., 2009). Another HDAC partner that promotes differentiation of oligodendrocytes, Yin Yang 1 (YY1), has been identified through a binding motif analysis in the promoters of up-regulated genes involving treatment with HDAC inhibitor. The *Id4* and *TCF7L2* promoters contain a YY1 binding site, and YY1 inhibits expression of these genes by recruiting HDAC1 and 2 to their promoters (He et al., 2007) (Fig. 1).

1.3. Role of DNA methylation in regulating gliogenic competence of NSCs

After the production of neurons, NSCs start to give rise to astrocytes. The later onset of astrogliogenesis results from a dramatic change of DNA methylation in astrocyte-specific promoters. DNA methylation predominantly occurs at the cytosine residue of CpG dinucleotides in the mammalian genome. DNA methylation in gene promoters has been associated with gene repression and stem cell differentiation in various tissues, including the CNS. *Glial fibrillary acidic protein* (*Gfap*) and *S100β* are representative genes that are expressed specifically in astrocytes. Their promoters are highly methylated in NSCs from early to midgestational stages, limiting the responsiveness of NSCs to astrocyte differentiation–inducing factors. Members of the interleukin 6 family of cytokines, such as leukemia inhibitory factor and ciliary neurotrophic factor, have been identified as astrocyte-inducing factors. These factors induce astrocyte differentiation of NSCs by activating the Janus kinase (JAK) signal transducer and activator of transcription 3 (STAT3) pathway. Astrocyte-specific genes, including *Gfap* and *S100β*, have STAT-binding sites in their promoters, and their expression is induced by activation of the JAK-STAT pathway during the gliogenic phase. However, NSCs in the neurogenic phase fail to express these genes even if the JAK-STAT pathway is activated. This is because STAT3-binding sites are methylated in NSCs, which interferes with the binding of STAT3 to the promoter region, resulting in the inhibition of astrocyte-specific gene expression during the neurogenic phase (Bonni et al., 1997; He et al., 2005; Nakashima et al., 1999a,b; Namihira and Nakashima, 2013; Rajan and McKay, 1998). Methylated DNA in astrocyte-specific genes undergoes demethylation during development, enabling NSCs to respond to astrocyte-inducing factors. Thus, demethylation of astrocyte-specific gene promoters explains, at least partially, how NSCs switch their competence from neurogenic to gliogenic. In support of this model, deletion of the gene encoding DNA methyltransferase 1 (Dnmt1), an enzyme responsible for the maintenance of DNA methylation after DNA replication, causes precocious astrocyte differentiation of NSCs in the neurogenic phase (Fan et al., 2005) (Fig. 1).

These findings raise further question of how DNA demethylation of astrocyte-specific genes occurs. Demethylation of DNA occurs via 2 distinct processes. One of these is passive demethylation, which progresses in a DNA replication–dependent manner as Dnmt1 is excluded from the methylation target site. Because neurons are the first cell type produced by NSCs during development—meaning that neurons are the first cell type with which NSCs make contact, other than NSCs themselves—we hypothesized that some signal from neurons triggers DNA demethylation and confers the competence to produce astrocytes on remaining NSCs. Newly generated immature neurons express the ligands of Notch, Delta-like 1 and JAGGED1, and they activate Notch signaling, which induces expression of nuclear factor IA (NFIA) in remaining NSCs (Namihira et al., 2009; Namihira and Nakashima, 2013). NFIA then binds to the astrocyte-specific gene promoters, which leads to the dissociation of Dnmt1 from the

promoters, thus preventing Dnmt1 from maintaining DNA methylation. This partially accounts for how DNA demethylation of astrocyte-specific gene promoters is induced during development and also why astrocyte production begins following the neurogenic phase. However, the precise molecular mechanism of how binding of NFIA leads to the dissociation of Dnmt1 from astrocyte-specific gene promoters remains elusive. It has been shown that, during DNA replication, Dnmt1 is recruited to methylation target sites by Uhrf1 (ubiquitin-like containing PHD and RING finger domains 1; also known as Np95), which is highly expressed by NSCs in the developing brain (Bostick et al., 2007; Murao et al., 2015; Sharif et al., 2007). Therefore, Uhrf1 may be involved in the mechanism by which NFIA induces exclusion of Dnmt1 from the target site. In any case, a more detailed mechanism to explain DNA demethylation in astrocyte-specific genes must await further investigation.

Another mechanism for DNA demethylation is active demethylation, which occurs in a replication-independent manner, and this is mediated by Ten-eleven translocation (TET) family proteins. TETs convert 5′-methylcytosine (5mC) to 5′-hydroxymethylcytosine (5hmC) in a Fe (II)– and α-ketoglutarate–dependent fashion (Pastor et al., 2013; Wu and Zhang, 2010). 5hmC is thought to serve as an intermediate in the active DNA demethylation process (Kohli and Zhang, 2013). TET proteins further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). These oxidized cytosines can then be successively excised by thymine-DNA glycosylase (TDG) followed by replacement with unmodified 5mC via the base excision repair pathway (He et al., 2011; Ito et al., 2011; Kohli and Zhang, 2013; Zhang et al., 2012).

During embryonic brain development, high levels of 5hmC are detected in the cortical plate, and the levels increase with neuronal differentiation (Hahn et al., 2013). This enrichment of 5hmC inversely correlates with H3K27me3. 5hmC is observed in the gene body of neuronal function–related genes, such as *sex-determining region Y* (*SRY*)*-related HMG box 5* (*Sox5*), *B-cell chronic lymphocytic leukemia/lymphoma 11B* (*Bcl11b*), and *myelin transcription factor 1-like* (*Myt1l*). Concomitant with their expression, these genes lose H3K27me3 from their promoter and gain 5hmC marks in the gene body during the progression of neuronal differentiation. This unique mode of occupancy of 5hmC and H3K27me3 is indicative of its mechanistic role in neuronal differentiation of NSCs. In fact, knockdown (KD) of Ezh2 promotes the neuronal differentiation of NSCs, and overexpression of Tet1 and Tet2 further enhances the Ezh2 KD-promoted neuronal differentiation. This suggests that formation of 5hmC and loss of H3K27me3 cooperate to promote neuronal differentiation during the neurogenic phase (Hahn et al., 2013). Interestingly, enrichment of 5hmC during this time does not lead to substantial DNA demethylation, suggesting that 5hmC functions as a stable epigenetic mark and takes part in regulating gene expression.

Similar to the enrichment of 5hmC, 5caC also displays transient accumulation in the genome specifically during differentiation of NSCs (Wheldon et al., 2014), and the accumulation of 5caC becomes more obvious as NSCs are stimulated to differentiate into glial cells. Indeed, TDG KD increased 5caC and 5fC levels during glial differentiation. In particular, glial gene promoters such as *Gfap*, *Olig1*, and *Olig2* acquire 5caC and 5fC during glial differentiation. Given that TDG excises both 5caC and 5fC and leads to DNA demethylation, this enrichment of 5caC in the promoters of astrocyte-specific genes such as *Gfap* suggests the possibility that DNA demethylation of astrocyte-specific genes also occurs through active DNA demethylation. Whatever the mechanism, these findings support the idea that DNA demethylation warrants the timing of gliogenesis in the developing brain.

1.4. Role of noncoding RNAs in embryonic NSC differentiation

Noncoding RNAs are transcribed from DNA but not translated into proteins. Among these, microRNAs (miRNAs) and long noncoding RNA (lncRNA) play a variety of roles in fine-tuning gene expression by transcriptional and posttranscriptional regulation. miRNAs are known to contribute to posttranscriptional repression of gene expression and have been explored extensively in recent years. These studies have shown that, in addition to histone modification and DNA methylation, noncoding RNAs also participate in the mechanisms that ensure the sequential production of distinct neural cell types from NSCs during development.

Chicken ovalbumin upstream promoter-transcription factor I and II (COUP-TFI/II) induces competence transition of NSCs from neurogenic to gliogenic during development. The expression of COUP-TFI/II is high in the gliogenic phase, and this facilitates the DNA demethylation of the *Gfap* promoter (Naka et al., 2008). A recent study has identified miR-17/106, whose expression in NSCs increases in the neurogenic phase and gradually decreases along with the transition to the gliogenic phase, as a downstream effector of COUP-TFI/II (Naka-Kaneda et al., 2014). miR-17/106 targets p38 (also known as MAPK14) to regulate the transition of NSC competence and plays an essential role in determining the responsiveness of NSCs to gliogenic signals (Fig. 1). Overexpression of p38 or suppression of miR-17/106 in embryonic stem cell–derived neurogenic NSCs induces precocious astrocytic differentiation in the presence of astrocyte differentiation-inducing factors. Therefore, the COUP-TFI/II–miR-17/106–p38 axis is a critical regulator for the neurogenic-to-gliogenic NSC competence transition.

Moreover, miR-153 also regulates the acquisition of gliogenic potential of NSCs (Tsuyama et al., 2015) (Fig. 1). miR-153 targets messenger RNAs (mRNAs) for NFIA and B, 2 essential regulators for the initiation of gliogenesis that act by inducing demethylation of astrocytic gene promoters. Gain- and loss-of-function experiments using miR-153 in vitro and in vivo suggest that miR-153–mediated fine tuning of NFIA and B expression is essential for the acquisition of gliogenic potential by NSCs during CNS development.

In addition to these miRNAs, a very recent study has revealed that miR-9, which shows specific enrichment in the brain, is also involved in fate specification of NSCs (Zhao et al., 2015) (Fig. 1). miR-9 is induced by Neurog1, and it directly targets mRNA of *Lif receptor beta* (*Lifr-beta*), *Il-6 signal transducer* (*Il6st*), and *Jak1*, which are major components of the JAK-STAT pathway, resulting in the inhibition of astrogliogenesis during the neurogenic stage. miR-9 KD in neurogenic NSCs increased astrocyte differentiation both in vitro and in vivo, whereas its overexpression decreased it. Therefore, as well as miR-17/106 and miR-153, miR-9 is also an important regulator for the fate specification of NSCs.

Furthermore, many other miRNAs have been identified as being specifically expressed in the mammalian brain. For example, Let-7 and miR-137 are highly expressed from the embryonic to the adult brain. In addition, each neural cell type differentially expresses distinct miRNAs: miR-124 is highly expressed in neurons, whereas miR-23, 26, 29, and 146a are predominantly expressed in astrocytes (Iyer et al., 2012; Smirnova et al., 2005). These cell type–specific expression patterns of miRNAs suggest their critical roles in regulating some function in each cell type, such as NSC proliferation and differentiation.

Among these miRNAs, miR-124 comprises 25%–48% of total miRNAs in the brain and has been extensively studied (Lagos-Quintana et al., 2002). miR-124, whose expression increases during brain development, promotes neuronal differentiation of NSCs (Fig. 1). miR-124 directly targets the small C-terminal domain phosphatase 1 (SCP1), an antineural factor expressed in nonneuronal tissues. SCP1 is recruited to repressor element 1–containing neural genes by repressor element 1 silencing transcription factor (REST), also known as neuron-restrictive silencer factor, and is involved in their repression (Visvanathan et al., 2007; Yeo et al., 2005). miR-124 suppresses SCP1 expression during CNS development, thereby inducing neurogenesis. In addition, miR-124 down-regulates the expression of the RNA-binding protein polypyrimidine tract binding protein 1, a global repressor of CNS-specific alternative pre-mRNA

splicing in nonneuronal cells (Makeyev et al., 2007). miR-124, thus, plays an important role in the differentiation of NSCs to mature neurons by increasing neuron-specific transcript levels.

Another brain-enriched miRNA, miR-137, plays an important role in regulating fate determination of NSCs (Sun et al., 2011b) (Fig. 1). Overexpression of miR-137 suppresses proliferation and accelerates neuronal differentiation of NSCs. In this regard, it has been suggested that miR-137 makes a negative feedback loop with the histone H3K4 demethylase lysine-specific demethylase 1 (LSD1) and the orphan nuclear receptor TLX, which is an essential regulator of NSC self-renewal. miR-137 targets LSD1 and inhibits its function. On the other hand, TLX represses the expression of miR-137 by recruiting LSD1 to the genomic region of miR-137. This feedback loop of miR-137 with LSD1 and TLX may regulate the balance between proliferation and differentiation of embryonic NSCs during neural development.

1.5. Epigenetic regulation in adult neurogenesis

Even after brain development is completed, a limited number of NSCs is maintained in the adult brain. These NSCs contribute to brain functions through daily production of neurons. Adult neurogenesis occurs in 2 restricted brain regions (Bond et al., 2015; Christian et al., 2014; Lledo et al., 2006; Ma et al., 2009a; Ming and Song, 2011). The subventricular zone (SVZ) of the lateral ventricle is one of the restricted regions in which neurogenesis persists. NSCs in the SVZ, named type B cells, proliferate infrequently and mostly display a quiescent state in the cell cycle. In the process of neurogenesis, they first give rise to transit-amplifying NPCs (type C cells), which then proliferate and differentiate into neuroblasts (type A cells). Neuroblasts migrate into the olfactory bulb (OB) along the rostral migratory stream and differentiate into mature granule and periglomerular neurons there (Fig. 2).

The subgranular zone (SGZ) of the adult hippocampal dentate gyrus (DG) also sustains NSCs into adulthood. Similar to the adult SVZ, relatively quiescent radial glia–like cells (type 1 cells) serve as NSCs in the SGZ. Type 1 cells give rise to transit-amplifying NPCs (type 2a/b cells), and they eventually differentiate into neurons through the neuroblast stage (type 3 cells) (Fig. 3). Newly generated neurons migrate into the granule cell layer along blood vessels and integrate in preexisting neural circuits (Sun et al., 2015). These newly generated neurons have been implicated in various hippocampal functions, such as hippocampus-dependent learning and memory.

For adult neurogenesis to occur, NSCs must go through several steps including maintenance and proliferation of Neural Stem/Precursor Cell (NS/PC) and neuronal commitment, migration, and maturation (Christian et al., 2014; Duan et al., 2008; Sun et al., 2011a) (Figs. 2, 3). A growing number of studies have shown that these processes in adult neurogenesis are governed by both cell extrinsic and intrinsic factors including extracellular signals from the stem cell niche, transcription factors, and epigenetic regulators (Bond et al., 2015; Fuentealba et al., 2012; Jobe et al., 2012; Ming and Song, 2011; Sun et al., 2011a). Moreover, to ensure neurogenesis in the adult brain, NSCs need to be maintained from embryonic to adult stages. Thus, the mechanism that sustains NSCs into adulthood is also pivotal for adult neurogenesis to occur. For instance, recent studies have elucidated the importance of histone modifications in regulating these processes to warrant neurogenesis in the adult brain (Lim et al., 2009; Molofsky et al., 2003). In this section, we introduce examples of epigenetic mechanisms contributing to the maintenance of adult neurogenesis.

1.6. Histone modifications in adult NS/PCs

The PcG and Trithorax (TrxG) complexes have been implicated in regulating adult neurogenesis. The PcG complex is responsible for gene repression, which is mediated by H3K27 methylation, whereas the TrxG complex catalyzes trimethylation of H3K4 (H3K4me3) in promoter-proximal nucleosomes to activate expression of their target genes (Blackledge et al., 2015; Ng and Gurdon, 2008; Ringrose and Paro, 2007). PRC1 consists of multiple subunits, and one of its components, B cell–specific Moloney murine leukemia virus integration site 1 (Bmi1), contributes to modulating the proliferation capacity of NSCs and maintaining them to adulthood (Fig. 2). Deletion of *Bmi1* attenuates the proliferation capacity of NSCs, resulting in the depletion of NSCs during development (Molofsky et al., 2003). This occurs through up-regulation of the cell cycle–dependent kinase inhibitor $p16^{Ink4a}$, whose deletion partially rescues the phenotype of *Bmi1* KO mice.

The TrxG complex component mixed-lineage leukemia 1 (Mll1) histone methyltransferase is required for neuronal differentiation in the SVZ (Lim et al., 2009) (Fig. 2). Deletion of *Mll1* in NSCs caused no defects in NSC proliferation, survival, and glial differentiation but severely impaired neuronal differentiation. Mll1 is responsible for H3K4me3, and its recruitment to gene promoters has been associated with gene activation. Mll1 directly targets distal-less homeobox 2 (Dlx2), a homeodomain-containing transcription factor important for neurogenesis in the OB. When *Dlx2* expression is active, its promoter shows high level of H3K4me3. However, in the absence of Mll1, the *Dlx2* promoter enters the bivalent state, i.e., a posed state for transcription harboring both repressive (H3K27me3) and active (H3K4me3) histone modifications. These findings indicate that Mll1-mediated resolution of the poised state (H3K4me3 + H3K27me3) to the active state (H3K4me3 only) of the *Dlx2* promoter is crucial for neuronal differentiation of NSCs.

One model to explain how bivalency is resolved by Mll1 is the cooperation of Mll1 with histone demethylase against H3K27me3 to activate target gene expression. A Jumonji domain containing 3 (Jmjd3; also called KDM6B), which is an H3K27me3-specific demethylase belonging to the family of JmjC domain–containing proteins, also acts as a critical activator of neuronal differentiation of adult SVZ NSCs (Fig. 2). Jmjd3 activates expression of a number of neuronal genes, including *doublecortin*, *Nkx2.2*, *Dlx2*, and *Dlx5* (Jepsen et al., 2007; Park et al., 2014). Upon neuronal differentiation, Jmjd3 is enriched in both the promoter and enhancer of *Dlx2*. This is coupled with decreased H3K27me3 level and increased *Dlx2* expression. Interestingly, this enrichment of Jmjd3 is impaired by deletion of *Mll1*, suggesting that Mll1 is required for the recruitment of Jmjd3 to the gene expression regulatory region. These findings highlight the importance of crosstalk between histone methylation enzymes in adult neurogenesis.

Adult neurogenesis is known to be regulated by many physiological and pathologic conditions. One of the pathologic conditions, kainic acid–induced seizure, leads to aberrant neurogenesis in the adult hippocampus (Jessberger et al., 2005, 2007a, 2007b; Matsuda et al., 2015). The HDAC inhibitor valproic acid, an antiepileptic agent that is widely used in human clinical treatment, suppresses kainic acid–induced aberrant proliferation of NS/PCs in the adult DG, contributing to the alleviation of cognitive impairment in hippocampus-dependent learning (Jessberger et al., 2007a,b). In addition, valproic acid also functions to induce neuronal differentiation of adult hippocampal NSCs by up-regulating the proneural gene *NeuroD*, whereas it inhibits astrocyte and oligodendrocyte differentiation (Hsieh et al., 2004). In the HDAC family, HDAC2 plays a critical role in neuronal maturation in both adult hippocampal DG and SVZ (Figs. 2, 3). It has been shown that specific ablation of HDAC2 in NSCs impairs neuronal differentiation and induces abnormal maturation of newborn neurons and cell death in both SGZ and SVZ, although the proliferation rate of transit-amplifying NPCs is increased by HDAC2 deletion. This combination of increased proliferation and defective neuron generation in HDAC2-deficient mice may result from the prolonged expression of stem cell–specific genes during neuronal differentiation. Sox2, an important transcription factor for proliferation and stemness

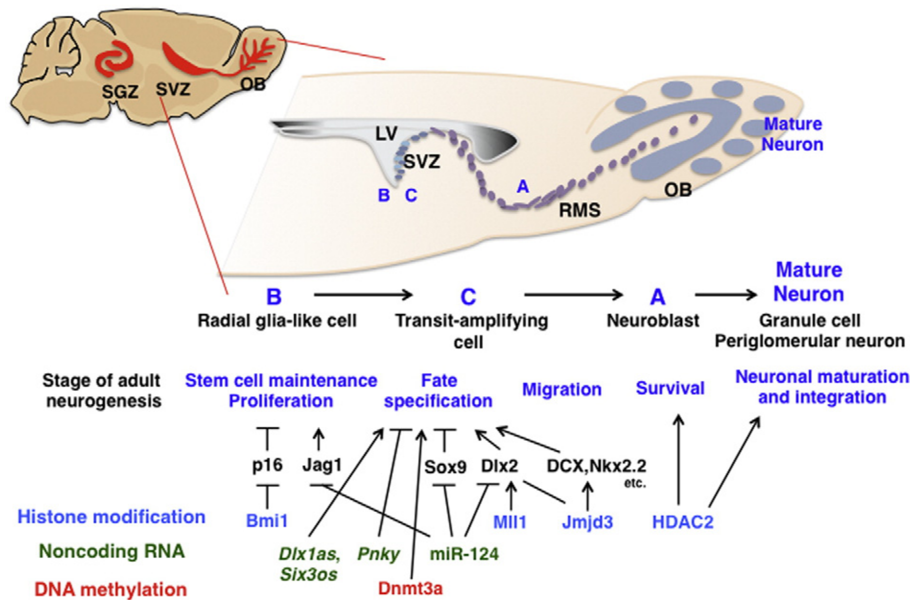

Fig. 2. Schematic representation of adult neurogenesis and factors relevant to epigenetic regulation in the SVZ and OB. Adult neurogenesis in the SVZ is composed of 5 stages: (1) stem cell maintenance and proliferation, (2) fate specification, (3) migration, (4) survival, and (5) neuronal maturation and integration. Briefly, radial glia-like cells (type B) become activated and produce transit-amplifying NPCs (type C), which frequently proliferate and generate neuroblasts (type A). Newly generated neuroblasts migrate along the rostral migratory stream and differentiate into immature neurons in the OB. Finally, synaptic integration and maturation of granule cells and periglomerular neurons are induced in the OB. Representative epigenetic factors that are relevant in regulating each stage of adult SVZ neurogenesis are also shown. Abbreviations: LV, lateral ventricle; OB, olfactory bulb; RMS, rostral migratory stream. →, promotion; ⊣, inhibition.

of NSCs, is not normally expressed in neuroblasts, which are already committed to differentiate into neurons. However, Sox2 expression is sustained even in the neuroblasts in the DGs of HDAC2-deficient mice, suggesting that HDAC2 is essential to terminate Sox2 expression as NS/PCs differentiate into neurons and that Sox2 expression should be repressed in neuroblasts and neurons to ensure proper neuronal differentiation and maturation (Jawerka et al., 2010).

Sox2 limits the activity of the PRC2 complex to suppress excessive acquisition of H3K27me3 at the regulatory regions of proneural and neurogenic genes, such as *Neurog2*, *NeuroD1*, and *brain-derived*

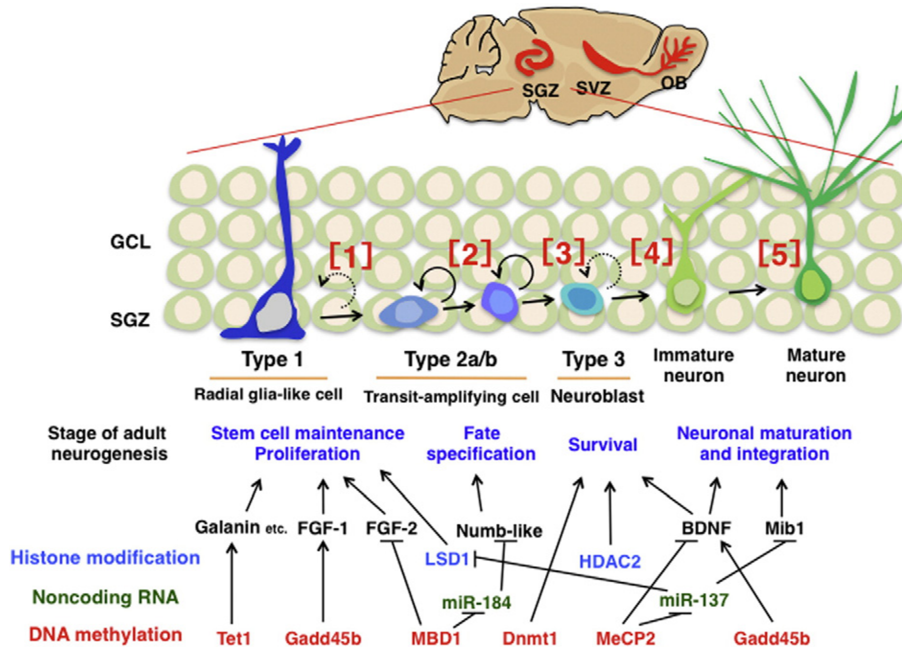

Fig. 3. Schematic diagram of the current view of lineage relationships and epigenetic regulation during adult hippocampal neurogenesis. Adult hippocampal neurogenesis mainly proceeds through 4 stages: (1) stem cell maintenance and proliferation, (2) fate specification, (3) survival, and (4) neuronal maturation and integration into preexisting circuits. The numbers on the figure indicate the detailed developmental steps of adult hippocampal neurogenesis: [1] activation of quiescent radial glia-like cells (type 1) in the SGZ, [2] proliferation of precursor and intermediate progenitors (type 2a, 2b, transit-amplifying cells), [3] generation of neuroblasts (type 3), [4] differentiation into immature neurons, and [5] maturation of immature neurons. Epigenetic factors, including DNA methylation, histone modification, and noncoding RNAs, regulate adult hippocampal neurogenesis by means of crosstalk between each pathway. Solid (frequent) and dashed (slow or rare) semicircular arrows indicate the cell division frequency of each cell type. Abbreviations: GCL, granule cell layer; SGZ, subgranular zone. →, promotion; ⊣, inhibition.

neurotrophic factor (*Bdnf*) (Amador-Arjona et al., 2015). These genes have bivalency in their promoters, whereby their expression is poised to be activated once NSCs initiate neuronal differentiation in response to the stimuli of neurogenic cues. Sox2 interacts with these gene promoters and precludes the recruitment of the PRC2 component Ezh2. In the absence of Sox2, H3K27me3, but not H3K4me3, increases in the poised proneural and neurogenic gene promoters, correlating with the increased accumulation of Ezh2. Moreover, Wnt signaling, a neurogenic cue that induces neurogenesis in adult as well as embryonic stages, activates the expression of poised proneural and neurogenic genes. Sox2-deficient NSCs, however, fail to express these genes upon Wnt stimulation. Specific ablation of *Sox2* in adult hippocampal NSCs impairs both proneural and neurogenic gene expression, resulting in decreased neurogenesis, accompanied by increased cell death and functionally aberrant newborn neurons. These findings suggest the important role of Sox2 in maintaining permissive epigenetic states of neurogenesis-related genes in the adult hippocampal NSCs, allowing these genes to respond to neurogenic cues immediately.

1.7. DNA methylation in adult neurogenesis

The role of DNA methylation in adult neurogenesis is still unclear compared with other epigenetic regulation characters such as histone modification or noncoding RNAs. Expression of Dnmt1 and of the de novo DNA methyltranseferase Dnmt3a, but not 3b, is observed in the adult brain. Dnmt1 is particularly highly expressed in proliferative NS/PCs compared with quiescent NSCs and postmitotic neurons and astrocytes in the adult hippocampus. Although deletion of *Dnmt1* in adult NSCs does not affect their proliferation or differentiation, it decreases the survival of newly generated neurons in the adult hippocampal DG (Noguchi et al., 2015). Interestingly, *Dnmt1* KO in postmitotic neurons does not affect their survival, suggesting that DNA methylation maintained by Dnmt1 in the NSC stage plays an important role for the survival of newly generated neurons only after NSCs differentiate into neurons in the adult hippocampus (Fig. 3). On the contrary, Dnmt3a is reportedly associated with adult neurogenesis (Wu et al., 2010) (Fig. 2). Deletion of *Dnmt3a* decreased neuronal differentiation of NSCs in the SVZ, resulting in a reduction of neuroblasts migrating into the OB. Dnmt3a targets nonpromoter regions of neurogenic genes and induces their expression. DNA methylation and Dnmt3a occupancy in nonpromoter regions are negatively correlated with elevation of H3K27me3 and enrichment of the PRC2 components Ezh2 and suppressor of zeste 12. This suggests that Dnmt3a-dependent methylation promotes neuronal differentiation by inhibiting PRC2-mediated repression of gene expression.

In addition to DNA methyltransferase, Tet1, an important factor for active demethylation via oxidation of 5mC, also plays an important role in regulating adult hippocampal neurogenesis (Zhang et al., 2013) (Fig. 3). *Tet1* deficiency impairs the proliferation of adult NSCs and causes DNA hypermethylation of genes involved in proliferation and neuronal protection, including *Galanin*, *Ng2*, and *Neirglobin* (*Ngb*), resulting in decreased neurogenesis. Moreover, *Tet1* KO mice display impaired spatial learning and memory, suggesting that Tet1-mediated DNA demethylation is important for cognition.

Active DNA demethylation occurs not only in NS/PCs but also in the cells providing the niche for NS/PCs, which contributes to regulating the expression of neurogenic cues. A member of the growth arrest and DNA damage 45 (Gadd45) gene family, Gadd45b, is induced in mature hippocampal neurons by neuronal activity, such as electroconvulsive treatment and voluntary running. Gadd45b expression in mature hippocampal neurons is dependent on depolarization-induced calcium influx and calmodulin kinase activity (Greer and Greenberg, 2008; Ma et al., 2009b). Gadd45b has been implicated in the DNA excision repair–based DNA demethylation mechanism and induces active DNA demethylation in vertebrates. In the adult mouse hippocampus, Gadd45b promotes activity-induced DNA demethylation within the regulatory region IX of *Bdnf* and brain-specific promoter B of *fibroblast growth factor 1* (*Fgf1*). These 2 genes play crucial roles in adult neurogenesis as well as neuronal survival and maturation. Thus, Gadd45-dependent DNA demethylation and expression of secreted factors, such as BDNF and FGF1, influence the neurogenic niche, and these proteins serve as key regulators of homeostasis in adult neurogenesis (Fig. 3).

DNA methylation normally leads to repression of gene expression through the recruitment of methylated DNA-binding protein family members such as methyl-CpG-binding protein 1 (MBD1) and methyl-CpG-binding protein 2 (MeCP2). These 2 factors are also involved in controlling the expression of neurogenic cues. NSC-specific *MBD1* KO decreases adult neurogenesis and impairs spatial learning. MBD1 directly interacts with the promoter region of *Fgf2*, an essential growth factor for NSCs. In the absence of *MBD1*, the *Fgf2* promoter region becomes hypomethylated, resulting in increased expression of *Fgf2* in NSCs and further leading to neuronal differentiation arrest (Li et al., 2008a) (Fig. 3). *MeCP2*, the causal gene of Rett syndrome, is highly expressed in postmitotic neurons and functions as a major regulator for neuronal gene expression in the CNS (Amir et al., 1999; Lyst and Bird, 2015; Shahbazian et al., 2002). *MeCP2* KO mice display no deficiencies in early postnatal neurogenesis, but neuronal maturation of newly generated neurons is impaired in the adult hippocampal DG (Smrt et al., 2007) (Fig. 3). One of the best-known targets of MeCP2 is *Bdnf*, which regulates several aspects of neurogenesis, including proliferation, differentiation of NSCs, and development and survival of newborn neurons (Li et al., 2008b; Murray and Holmes, 2011). MeCP2 physically occupies the hypermethylated *Bdnf* promoter and suppresses its expression. This interaction between MeCP2 and the *Bdnf* promoter is disrupted by the induction of DNA demethylation in response to neuronal activity, which increases *Bdnf* transcription. In addition, neuronal activity–induced calcium influx causes posttranscriptional modification of MeCP2, such as phosphorylation at Ser421, which decreases the affinity of MeCP2 for the *Bdnf* promoter and facilitates its transcription (Chen et al., 2003; Zhou et al., 2006b). MeCP2 Ser421 phosphorylation is induced in response to neuronal stimulation and plays a significant role in synapse development and behavior (Cohen et al., 2011).

1.8. Noncoding RNAs in adult neurogenesis

The roles of miRNA in NSCs and in adult neurogenesis have been extensively studied as well. Accumulating evidence indicates that miRNA plays a substantial role in fine tuning the progression of adult neurogenesis (Figs. 2, 3).

In the SVZ of the adult mammalian brain, brain-specific miR-124 is highly expressed during the transition from transit-amplifying NPCs to neuroblasts and immature neurons (Cheng et al., 2009). miR-124 promotes the differentiation of the NPCs into neuroblasts and controls the timing of lineage progression. miR-124 has several known direct targets in the SVZ including *Jag1*, *Dlx2*, and *Sox9*. Because these genes are known to contribute to distinct steps in adult neurogenesis, such as self-renewal and neuronal differentiation of NSCs, it is conceivable that miR-124 affects multiple aspects of adult neurogenesis in the SVZ (Cheng et al., 2009; Doetsch et al., 2002; Nyfeler et al., 2005) (Fig. 2).

Another representative miRNA that plays an important role in NSC proliferation and differentiation is miR-137, which is a direct target of Sox2 and MeCP2 in adult SGZ (Szulwach et al., 2010). Overexpression of miR-137 promotes proliferation of NSCs in the hippocampal DG, whereas its repression enhances the differentiation of NSCs into both neurons and astrocytes. One of the targets of miR-137 is *Lsd1*, which is known to suppress the proliferation of NSCs in the embryonic brain and adult hippocampus (Sun et al., 2010) (Fig. 3). In addition, miR-137 also regulates neuronal maturation, dendritic elaboration, and spine development by suppressing mind bomb 1 expression

(Smrt et al., 2010) (Fig. 3). Mind bomb 1 is a ubiquitin ligase that is important for neurodevelopment, and its overexpression partially rescues the neuronal maturation impairments caused by miR-137 overexpression. MBD1 targets miR-184 and represses its expression in adult NSCs (Liu et al., 2010). miR-184 targets mRNA for *Numb-like* (*Numbl*), an inhibitor of Notch signaling, for maintenance of adult NSCs. High levels of miR-184 expression promote proliferation at the expense of differentiation of adult hippocampal NSCs. Thus, MBD1, miR-184 and Numbl form a regulatory network to maintain the balance between proliferation and differentiation of NSCs in the adult hippocampal DG (Fig. 3).

Recently, the roles of lncRNA in adult neurogenesis have just begun to be studied. The lncRNAs *Dlx1as* and *Six3os* are expressed in NSCs in the SVZ and play key roles in the glial-neuronal lineage specification of adult NSCs (Ramos et al., 2013) (Fig. 2). *Dlx1as* is transcribed from the *Dlx1/2* gene cluster, whereas *Six3os* is transcribed from a site proximal to the *Six3* homeobox gene (Dinger et al., 2008; Liu et al., 1997). Loss-of-function analysis in the SVZ revealed that both *Dlx1as* and *Six3os* affect fate determination of adult NSCs into neurons, but *Six3os* has an additional function in regulating NSC differentiation into oligodendrocytes. Another example of functional lncRNA is the *rhabdomyosarcoma 2-associated transcript* (*RMST*), which is specifically expressed in the brain and whose expression is repressed by REST/neuron-restrictive silencer factor. *RMST* directly interacts with Sox2 and tethers it to the promoter regions of neurogenic transcription factors such as *Ascl1*, *Dlx1*, and *Neurog2* to induce their expression and the neuronal differentiation of NSCs (Ng et al., 2012, 2013). *Pinky* (*Pnky*) has been identified by means of RNA-seq as a neural-specific lncRNA, expressed in both embryonic and adult NSCs (Ramos et al., 2015). *Pnky* interacts with the splicing regulator polypyrimidine tract binding protein 1 and suppresses neuronal differentiation of NSCs by inhibiting the precise expression and alternative splicing of key genes related to neuronal differentiation (Fig. 2).

2. Closing remarks and perspective

In the last 2 decades, the existence of NSCs even in human brain has been clearly proven, and NSC research is currently progressing with dramatic speed and nurturing the hope that NSCs can be used for the clinical treatment of various neural disorders and diseases. For example, transplantation of NSCs into the lesion site of injured spinal cord has been shown to have great potential to recover locomotion ability in patients affected with spinal cord injury (Abematsu et al., 2010; Fujimoto et al., 2012; Iwanami et al., 2005; Ogawa et al., 2002). In addition, implanting cells that are genetically engineered to become dopaminergic neurons, one of the neuronal subtypes specifically lost in Parkinson's disease, into the brain of Parkinson's disease model animals has been undertaken to ask if such a strategy could be a clinical treatment for Parkinson's disease (Kim et al., 2011; Liu et al., 2012). For these and other neuronal disorders, establishing a treatment to recover abilities of the CNS that are lost in diseases and injury is now becoming one of the most encouraging and worthwhile challenges in NSC research. In terms of producing desirable cells from NSCs for such treatments, a deep understanding of the mechanisms regulating NSC proliferation and differentiation is essential. Development of an in vitro culture system for NSCs triggered research to verify the signaling molecules that enable them to proliferate and differentiate into specific cell types in the CNS. These approaches have already identified many neurogenic and gliogenic molecules, but they have also uncovered the existence of intrinsic mechanisms in NSCs that influence their responsiveness to these cues. As we have discussed in this review, epigenetic regulation constitutes these intrinsic mechanisms, and recent researches have shown its significant contribution to brain development and adult neurogenesis. Nevertheless, there are still many questions to be addressed regarding the mechanisms by which epigenetic modifications regulate the maintenance and differentiation of NSCs. The current revolution of next-generation sequencing technology allows us to analyze genome-wide expression patterns and epigenetic modifications in each cell type. These analyses have demonstrated that different types of cells have unique patterns of epigenetic modifications and that these patterns are established as stem cells differentiate into each lineage (Jepsen et al., 2007; Park et al., 2014; Ramos et al., 2015; Wapinski et al., 2013; Wu et al., 2010). Although these studies have revealed significant epigenetic modifications regulating differentiation of NSCs, we do not yet know how these unique patterns are established in specific gene promoters or indeed how much they are involved in stem cell differentiation.

We introduced above several examples of epigenetic modification changes in specific gene promoters that are induced by transcription factors via blocking and recruiting epigenetic modification enzymes to their cognate loci. However, the affinity of transcription factors for the loci themselves is also influenced by epigenetic modifications (Takizawa et al., 2001; Wapinski et al., 2013). Thus, it is plausible that the unique epigenetic modification patterns established in each gene locus or cell type are governed by the interplay between multiple transcription factors and epigenetic modification enzymes. Recently, advanced genome-editing systems, such as the TALEN and CRISPR-Cas9 systems, have been developed (Doudna and Charpentier, 2014; Hsu et al., 2014; Vasileva et al., 2015). We believe that, by using these systems, we will be able to alter the epigenetic states of specific sites in the genome, without changing DNA sequences to manipulate the cell's behavior (Kearns et al., 2015; Mendenhall et al., 2013).

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