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## Neural Stem Cell Self-renewal

**Yanhong Shi, Guoqiang Sun, Chunnian Zhao, and Richard Stewart**

*Neuroscience Division, Center of Gene Expression and Drug Discovery, Beckman Research Institute of City of Hope, 1500 E. Duarte Rd, Duarte, CA 91010. Email: yshi@coh.org*

### Abstract

Two fundamental properties of stem cells are their ability to self-renew and to differentiate. Self-renewal is an integration of proliferation control with the maintenance of an undifferentiated state. Stem cell self-renewal is regulated by the dynamic interplay between transcription factors, epigenetic control, microRNA (miRNA) regulators, and cell-extrinsic signals from the microenvironment in which stem cells reside. Recent progress in defining specific roles for cell-intrinsic factors and extrinsic factors in regulating stem cell self-renewal starts to unfold the multilayered regulatory networks. This review focuses on cell-intrinsic regulators, including orphan nuclear receptor TLX, polycomb transcriptional repressor Bmi1, high-mobility-group DNA binding protein Sox2, basic helix-loop-helix Hes genes, histone modifying enzymes and chromatin remodeling proteins, and small RNA modulators, as well as cell-extrinsic signaling molecules, such as Wnt, Notch, Sonic hedgehog (Shh), TGF $\alpha$ , EGF, and FGF. Unraveling the mechanisms by which neural stem cells renew themselves will provide insights into both basic neurosciences and clinical applications of stem cell-based cell replacement therapies for neurodegenerative diseases.

### Keywords

neural stem cells; self-renewal; transcriptional regulators; epigenetic control; microRNA; nuclear receptors; histone deacetylases; Wnt

## 1. Introduction

One of the most important issues in stem cell biology is to uncover molecular mechanisms underlying stem cell self-renewal. Self-renewal is essential for stem cells because it is required for all stem cells to perpetuate themselves. Neural stem cells are a subset of undifferentiated precursors that retain the ability to proliferate and self-renew, and have the capacity to give rise to both neuronal and glial lineages [1-4]. Although the functional properties of neural stem cells have been studied extensively, we have just begun to understand how self-renewal of neural stem cells is regulated. A complete understanding of neural stem cells requires the identification of molecules that determine the self-renewal character of these cells. This review touches on some of the recently characterized pathways that are involved in regulating this process.

## 2. Transcription Regulators

Batteries of transcription factors have been proposed to control neural stem cell self-renewal (Fig. 1). Orphan nuclear receptor TLX is an essential transcriptional regulator of neural stem cell maintenance and self-renewal in the adult brain [5]. Although TLX knockout mice are viable and appear normal at birth, the TLX gene has been shown to be required for the formation of superficial cortical layers and the zinc-containing cortical circuits in embryonic brains [6, 7], to regulate the timing of neurogenesis in the cortex [8], and to control patterning of lateral telencephalic progenitor domains during development [9]. Mature TLX knockout mice have significantly reduced cerebral hemispheres [10,11] and severe retinopathies [12-15].

Behaviorally, adult TLX mutants exhibit increased aggressiveness, decreased copulation, progressively violent behavior, late onset epilepsy and reduced learning abilities [10,11,16, 17].

We have shown that TLX is an essential regulator of neural stem cell self-renewal [5]. TLX maintains adult neural stem cells in an undifferentiated and self-renewable state (Fig. 1). The TLX-expressing cells isolated from adult TLX-heterozygote brains can proliferate, self-renew and differentiate into all neural cell types *in vitro*. By contrast, TLX-null cells isolated from the brains of adult TLX-mutant mice fail to proliferate. Reintroducing TLX into TLX-null cells rescues their ability to proliferate and self-renew [5]. *In vivo*, TLX mutant mice show a loss of cell proliferation and reduced neural precursors in the neurogenic areas of adult brains. TLX represses the expression of astrocyte markers, such as GFAP, and tumor suppressor gene, pten, in neural stem cells, suggesting that transcriptional repression is crucial in maintaining the undifferentiated state of neural stem cells [5,14]. Similarly, the *Drosophila* tailless acts as a dedicated repressor in the early *Drosophila* embryo to support normal embryonic development and establish accurate patterns of gene expression [18]. TLX could be a key regulator that acts by controlling the expression of a network of target genes to establish the undifferentiated and self-renewable state of neural stem cells. Elucidation of the network regulated by TLX in producing these outcomes would be a significant advance in understanding neural stem cell self-renewal and neurogenesis.

Recently, other nuclear receptors such as estrogen receptors (ER), thyroid hormone receptors (TR), and peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), have also been shown to regulate neural stem cell proliferation and differentiation [19-24]. Significant neuronal loss has been detected in adult brains of ER $\beta$  knockout mice, suggesting an important role of ER $\beta$  in neuronal maintenance in the central nervous system [25]. Knockout of TR $\alpha$  inhibits progression of neural stem cells through cell cycles, suggesting a critical role of TR $\alpha$  in neurogenesis of mammalian adult brains [26]. Neural stem cells prepared from heterozygous PPAR $\gamma$ -deficient mouse brains have significantly reduced cell growth, which is also seen in PPAR $\gamma$  short hairpin RNA silenced or dominant negative PPAR $\gamma$ -treated neural stem cells, suggesting that PPAR $\gamma$  plays a role in neural stem cell proliferation control [27]. N-CoR, a nuclear receptor co-repressor, also plays a role in neural stem cell self-renewal. Deficiency in N-CoR leads to reduced neural stem cell self-renewal and premature differentiation into astrocytes [28].

Bmi1 is a polycomb family transcriptional repressor that has been shown to be required for post-natal maintenance of neural stem cells in the peripheral and central nervous system [29]. Deficiency in Bmi1 leads to progressive postnatal growth retardation and neurological defects [30]. Bmi1-null mice exhibit a post-natal self-renewal defect that leads to the depletion of stem cells by early adulthood [29]. One way in which Bmi1 promotes the maintenance of adult stem cells is by repressing the cyclin-dependent kinase inhibitors, p16<sup>Ink4a</sup> and p19<sup>Arf</sup> [31,32].

The Sox family of high-mobility-group (HMG) DNA binding proteins plays a role in maintaining the undifferentiated state of neural stem cells in a context-dependent manner. In vertebrates, SoxB1 factors (Sox1, Sox2, and Sox3) are widely expressed in proliferating neural stem/progenitor cells, throughout development and adulthood [33-35]. SoxB1 factors have been shown to play a role in maintaining the undifferentiated state of embryonic neural progenitors [36]. Overexpression of Sox2 and/or Sox3 inhibits neuronal differentiation of neural progenitors and causes them to retain undifferentiated properties. In contrast, expression of a dominant negative form of Sox2 and/or Sox3 results in premature exit of neural progenitors from cell cycle and onset of neuronal differentiation [37,38]. In addition to its function in early brain development [37-40], Sox2 is also necessary for the maintenance of neural stem cells in adult neurogenic areas [41,42]. Regulatory mutations of Sox2 cause neurodegeneration and

impaired adult neurogenesis [41]. In parallel, Sox10, a member of the Sox E subfamily, maintains the multipotency of neural crest stem cells in the peripheral nervous system [43].

Multiple basic helix-loop-helix (bHLH) genes also play a critical role in regulation of neural stem cell maintenance and differentiation [44-46]. Hes genes, homologs of *Drosophila* hairy and enhancer of split, are repressor-type bHLH genes. Among seven members of the Hes family, Hes1 and Hes5 are essential effectors of Notch signaling, the expression of which is upregulated by Notch activation [47,48]. Hes1 and Hes5 are highly expressed by neural stem cells [49-51], mis-expression of which inhibits neuronal differentiation and maintains neural stem cells in the embryonic brain [52-54]. In contrast, neural progenitors underwent premature neuronal differentiation in Hes1 and Hes5 double knockout mice [48,55-57], suggesting that Hes1 and Hes5 are essential for the maintenance and self-renewal of neural stem cells. Hes genes regulate neural stem cell self-renewal by repressing premature onset of the activator type bHLH genes such as Mash1, Math, and Neurogenin [46]. Hes-related bHLH genes, Hesr1 and Hesr2 are also expressed by neural stem cells in the embryonic brains and act as Notch signaling effectors. Hesrs regulate neural stem cell maintenance and self-renewal too, possibly through cooperative action with Hes [46]. Together, these data suggest that intrinsic transcription factors can work together to coordinate neural stem cell maintenance and self-renewal.

### 3. Epigenetic Control

Stem cell self-renewal and differentiation are the result of transcription control in concert with chromatin remodeling and epigenetic modifications. During central nervous system development in vertebrates, neural stem cell fate are strictly controlled under regional and temporal manners [58], accompanied by precise epigenetic control, including covalent histone modification and DNA methylation of CpG dinucleotides.

Histone modification includes histone acetylation, methylation, phosphorylation ubiquitylation, sumoylation, and ADP-ribosylation [59]. Histone acetylation, which is regulated by histone acetylases (HATs) and histone deacetylases (HDACs) (Fig. 2), has been best studied. HDACs can deacetylate the conserved acetylated lysine residues in histone tail, resulting in local condensation in chromatin and block access of transcriptional factors to their target genes. Mammalian HDACs can be classified based on their structure and homology to yeast HDACs. Class I HDACs (HDAC1, 2, 3, 8, and 11) are ubiquitously expressed, whereas Class II HDACs (HDAC4, 5, 7, and 9) display tissue-specific expression. Class II HDACs contain an amino-terminal extension that interacts with other transcriptional cofactors and confers responsiveness to extracellular signals. We have shown that HDAC-mediated transcriptional repression is essential for the maintenance and self-renewal of neural stem cells (Sun et al. unpublished results). In addition to self-renewal, HDACs also regulate neural cell differentiation. Treatment of adult neural stem cells with HDAC inhibitors induced neuronal differentiation [60], due in part to upregulating REST (RE1 silencing transcription factor, or NRSF)-regulated neuronal-specific genes. REST is a key transcriptional regulator of many neuronal genes through binding to a conserved 21 bp RE1 binding site [61,62]. In non-neuronal cells, REST interacts with its co-factors, including Co-REST, N-CoR, and mSin3A, which then recruit HDAC complexes to repress neuronal gene expression through epigenetic regulation [61,63,64].

More recently, histone methylation has gained attention as an epigenetic marker [65,66]. Unlike histone acetylation, which only occurs on lysine (K) residues and is generally related with active transcription, methylation was detected on both lysine and arginine (R) residues and is linked to both transcriptional activation and repression [66]. For instance, histone H3 K9 methylation is associated with transcriptional silencing (Fig. 2). In contrast, methylation of histone H3 K4 and arginine residues of H3 and H4 leads to transcriptional activation [67].

Recent identification of LSD1 as a histone demethylase [68] indicates that similar to histone acetylation, histone methylation is also a dynamic process subject to regulation by both methyl transferases and demethylases. The degree of lysine methylation (mono-, di-, or trimethyl histones), as well as the residues modified, is tightly associated with neural cell differentiation [69]. For example, histones H3 trimethyl K9 and H4 monomethyl K20 were detected in proliferating neural stem cells, whereas histone H4 trimethyl K20 was enriched in differentiating neurons (Fig. 2).

The epigenetic state can also be regulated at the DNA level by DNA methylation. The most prominent form of DNA methylation in mammals is the symmetric methylation of cytosine in the 5' position in CpG dinucleotides. DNA methylation and its related chromatin remodeling play critical roles in regulating gene transcription in response to neuronal activity [70]. For example, DNA methylation can repress astrocytic GFAP expression by preventing the binding of a transcription factor, signal transducer and activator of transcription 3 (STAT3), to the GFAP gene promoter at early stages of brain development [71]. The gene silencing effect of DNA methylation is mediated by a family of methyl-cytosine-binding proteins, including MeCP2, which is abundantly expressed in the central nervous system [72,73]. MeCP2 is a member of a group of methylated-CpG binding proteins and is expressed at high levels in the postnatal brain. Mutations in the *Mecp2* gene have been linked to a neurodevelopmental disorder, Rett syndrome [74], suggesting that MeCP2 may play a role in regulating neuronal function. MECP2 has been shown to be critical for embryonic neurogenesis for *Xenopus* [75]. Recent studies demonstrated that *Mecp2* is involved in the maturation and maintenance of neurons at late stages of neuronal differentiation but not critical for embryonic neurogenesis in mammalian brains [76], consistent with the postnatal onset of symptoms in Rett Syndrome patients. Methyl-CpG binding protein MBD1 is also crucial for normal neural stem cell and brain functions. *MBD1*<sup>-/-</sup> neural stem cells exhibited reduced neuronal differentiation and increased genomic instability [77]. DNA methyltransferases are also expressed in the central nervous system and have a role in neurogenesis [78,79]. DNA methyltransferase 1 (*Dnmt1*) deficiency mice displayed decreased neurogenesis. Conditional knockout of *Dnmt1* in neural progenitor cells results in DNA hypomethylation and precocious astroglial differentiation [80]. Multiple layers of epigenetic modifications, therefore, regulate key transitions in the regulation of neural stem cell self-renewal and their differentiation.

#### 4. miRNA Regulators

Many different classes of small non-coding RNAs are present in the brain, with diverse roles including RNA modification and chromatin remodeling [81]. Small double-stranded modulatory RNAs have been proposed to regulate the generation of neurons from adult neural stem cells by binding to REST [82]. miRNA is another recently identified large family of small non-coding RNAs, which are likely key post-transcriptional players in stem cell self-renewal and differentiation (Fig. 3). miRNAs are short 20-22 nucleotide RNA molecules that are expressed in a tissue-specific and developmentally-regulated manner and function as negative regulators of gene expression in a variety of eukaryotes. miRNAs are involved in numerous cellular processes including development, proliferation, and differentiation [83,84].

miRNA genes belong to class II genes, which are transcribed by RNA polymerase II. A majority of miRNA loci are found in intronic regions of protein-coding or non-coding transcription units; others are found in exonic regions of non-coding transcription units [85]. The primary transcripts of miRNAs (normally > 1kb), called pri-miRNAs, are processed in the nucleus by RNase III endonuclease, Droscha, into 60 to 75 nucleotide hairpin-like precursors (pre-miRNAs). Pre-miRNAs are subsequently exported to the cytoplasm by exportin-5, a member of the Ran-dependent nuclear transport receptor family. In the cytoplasm, the hairpin precursors are cleaved into mature miRNAs by Dicer, a cytoplasmic RNase III-type protein. Mature

miRNAs are then transferred to Argonaute proteins in the RNA-induced silencing complex (RISC), which direct the miRNAs to their target transcripts [86]. By base pairing with the target mRNA, miRNA functions as a guide molecule in post-transcriptional gene silencing, leading to mRNA cleavage or translational repression (Fig. 3).

miRNAs are especially attractive candidates for regulating stem cell self-renewal and cell fate decisions because of their ability to simultaneously regulate many target genes. Studies based on expression patterns, predicted targets, and overexpression analyses suggest that miRNAs are key regulators in stem cell biology. Distinct sets of miRNAs have been shown to be specifically expressed in embryonic stem cells [87,88]. Loss of *Dicer1* causes embryonic lethality and loss of stem cell populations [89,90]. Argonaute family members, key components of RISC complexes, are required for maintaining germline stem cells in various species [91]. These observations together support a role for miRNAs in stem cell self-renewal.

Among miRNAs identified, around 70% of them are found to be expressed in mammalian brains [92,93], suggesting possible roles of these miRNAs in neural function [94-97]. Indeed, miRNAs have been shown to regulate the development of chemosensory system of *C. elegans* [98] and brain morphogenesis of zebrafish [99]. Study of a set of highly expressed neural miRNA during mammalian brain development revealed significant differences in the onset and magnitude of induction for individual miRNAs and marked lineage specificity of the miRNAs [100]. During differentiation, progenitor cells express families of miRNAs sequentially, resulting in expression of lineage-specific genes. The most highly expressed miRNAs in adult brain, miR-124 and miR-128, were preferentially expressed in neurons; while miR-23 was restricted to astrocytes; miR-26 and miR-29 have stronger expression in astrocytes than neurons; whereas miR-9 and miR-125 were fairly evenly distributed [100].

Overexpression of miR-124, miR-128, and miR-9 in neural precursors decreased astrocyte differentiation. In contrast, inhibition of miR-9 expression alone or in combination with miR-124 led to reduced neurogenesis [101]. The regulation mediated by miR-9 and miR-124 is at least partly through modulating the STAT3 signaling pathway [101]. Let-7 family members are also highly represented in libraries of brain miRNAs, although not restricted to the nervous system [102]. Let-7 family members are highly expressed in nervous tissues of zebrafish [92] and in the developing mouse brains [103]. Both transcriptional activation and increased precursor processing activity led to significant induction of mature forms of let-7 family members during neural differentiation, suggesting a role of let-7 in neural cell specification [103].

Target prediction of miRNA indicates that more than one-third of animal genes may be regulated by miRNAs [104]. Each miRNA may suppress multiple targets, and one mRNA can be targeted by many miRNAs [84]. So far, some of mRNA targets have been validated and shown to control over a broad spectrum of cellular process [83]. MiR-124, the most abundant miRNA in adult mammalian brains [102], has its binding motif in more than 1100 genes as predicted computationally [104]. Introduction of miR-124 into HeLa cells down-regulated more than 100 genes [105] and promoted a neuronal-like mRNA profile [106]. Recently laminin gamma 1 and integrin beta1 were identified as targets of miR-124 in chicken neural tube, both of which are highly expressed in neural precursor cells and repressed upon neuronal differentiation [107]. In addition, miR-124 binds to 3'UTR of small C-terminal domain phosphatase 1 (SCP1), a phosphatase that plays a role in neural development, to suppress its expression, further supporting a role of miR-124 in neurogenesis [108]. *Senseless*, a binary switch during sensory organ precursor selection in flies [109], has been identified as a target gene of miR-9 [110]. Loss of miR-9 in flies induced extra sense organs, whereas overexpression of miR-9 led to massive loss of sensory organ precursors [110]. Hunchback, a gene regulates the temporal identity of neuroblasts [111], is regulated by let-7 miRNA as a target gene [112, 113]. Identification of comprehensive miRNA targets in the neural system remains an

important and non-trivial task that will help us to better understand the regulation of neural stem cell self-renewal and differentiation.

## 5. Cell-extrinsic signaling

Stem cell self-renewal and differentiation is also regulated by the specialized microenvironment, or niche, in which these cells reside [114-117]. Direct physical interactions between stem cells and their niches are critical for maintaining stem cell characters. Signaling molecules in the niche are composed of soluble factors, membrane bound molecules, and extracellular matrix, including Wnt, Notch, and Sonic hedgehog (Shh) [114]. Receptor tyrosine kinase (RTK) signaling has also been implicated in regulation of neural stem cell proliferation and self-renewal.

Genetic studies have implicated the Wnt/ $\beta$ -catenin pathway in neural stem cell self-renewal. In mice that express a stabilized  $\beta$ -catenin, the central nervous system is greatly enlarged. Progenitors exit the cell cycle less frequently and continue to proliferate in the brain [118]. In contrast, ablation of  $\beta$ -catenin results in a marked decrease of the overall size of the nervous system [119]. Mice with null alleles of LRP6, a required co-receptor for Wnt signaling, also showed reduced dentate granule cell production and failed expansion of a defined dentate granule precursor cell pool [120]. These studies indicate that the Wnt/ $\beta$ -catenin signaling plays an important role in the proliferation and self-renewal of neural precursors, presumably through its downstream target genes, such as cyclin D1 [121]. On the other hand, Wnt proteins have been shown to promote neuronal differentiation in neural stem cell culture and in adult hippocampus [122-124], suggesting that signaling by the canonical Wnt pathway has multiple functions in stem cells. Indeed, recently it has been shown that Wnt3a and Wnt5a could both increase proliferation and stimulate neuronal differentiation of neural progenitor cells isolated from postnatal mouse brains [125]. Thus, Wnt pathways are critical regulators of both neural stem cell self-renewal and neurogenesis.

Notch signaling is activated when a Notch receptor on one cell interacts with Notch ligands, such as Delta, on an adjacent cell. This interaction triggers proteolytic release of the Notch intracellular domain and its translocation to the nucleus, where it binds to transcriptional regulator CSL and induces downstream effector expression [126]. The best known Notch effectors are members of the Hes families, including Hes1 and Hes5 [47,48,127]. Studies using mouse models of the Notch pathway led to a prevailing view that Notch maintains the self-renewable state of neural stem cells [128]. One of the first Notch pathway genes to be knocked-out was Notch1 [129,130]. Consistent with the view that Notch activity is needed for stem cell maintenance, increased neuronal differentiation was detected in Notch1 mutant brains. Conditional deletion of Notch1 in the neural progenitor pool was also found to result in precocious neuronal differentiation and earlier neural progenitor pool depletion [131,132]. In addition to the receptor mutations, many Notch ligand mutations have been examined. One such study found that deleting Delta-like 1 (Dll1) led to a decrease in the radial glia progenitor marker RC2 and an increase in neuronal marker expression in embryonic brains, supporting the traditional view that Notch signaling inhibits neuronal differentiation in the developing central nervous system [128]. Notch pathway components are also expressed in postnatal and adult mouse brains, both in germinal zones and in neurons [133]. Notch signaling can be context-dependent. In addition to maintaining stem cell state in germinal regions, Notch can also promote terminal glial differentiation [134].

Shh is an important morphogen in development. In Shh null mice, the telencephalon is greatly dysmorphic and much reduced in size. Both dorsoventral patterning and general brain proliferation are significantly affected [135,136]. Shh is also a potent mitogen for neural progenitor cells of adult brains [137]. Over-expression of Shh near the dentate gyrus increases

proliferation and neurogenesis of hippocampal subgranular zone cells [137]. *In vitro*, Shh maintains proliferation of adult hippocampal neuronal progenitors [137] and increases subventricular zone cell proliferation [138]. The hedgehog signaling is mediated by the zinc finger-containing transcription factor Gli1, a direct transcriptional target of Shh signaling [139,140], acting to both promote proliferation and maintain populations of neural progenitors in postnatal brains [141].

Epidermal growth factor (EGF), transforming growth factor  $\alpha$  (TGF $\alpha$ ), and fibroblast growth factor (FGF) are all extracellular ligands of RTKs and play critical roles in the proliferation of neural stem cells [142-145]. EGF and TGF $\alpha$  bind preferentially to the EGFR [146,147], a member of the RTK family. EGFR is expressed in neurogenic regions such as the adult subventricular zone (SVZ) [148-150]. Targeted disruption of EGFR causes forebrain cortical dysgenesis at late embryonic and postnatal ages [151-153]. TGF $\alpha$  is considered the predominant endogenous EGFR ligand [150], the expression of which is temporally and spatially coordinated with EGFR expression. TGF $\alpha$  null mice exhibit decreased proliferation within the SVZ [154]. *In vivo* administration of EGF and TGF $\alpha$  via intraventricular infusion increased neural stem cell proliferation in the adult brain. *In vitro* experiments have also shown that SVZ-derived progenitor cells can be expanded by EGF and FGF administration [143]. FGF acts primarily through FGF receptors (FGFR), members of the RTK family also (Johnson and Williams 1993). Targeted deletion of FGFR1 causes defects in cell proliferation and embryonic lethality [155-157]. FGF2, the first member of the FGF family, is expressed in the dorsolateral cortical neuroepithelium of the forebrain and acts primarily through FGFR1. FGF2 null mice show significant reduction in cortical progenitor cell proliferation before neurogenesis begins [158]. Intraventricular delivery of FGF2 increased cell proliferation within the adult SVZ [159,160]. Cyclin D2 has been recently suggested to be an effector of the FGF signaling [161], which promotes early G1 cell cycle progression, a function relevant to neural stem cell proliferation.

Neural stem cells in the brain are likely to be influenced by a convergence of extracellular signals, such as Wnt, Notch, and Shh, from many neighboring cell types, including astrocytes, neuroblasts, ependymal cells, and endothelial cells [117,162,163]. These extrinsic factors may interact with intrinsic regulators by various signaling cascades, including Wnt/ $\beta$ -catenin-cyclin D1 [121], Notch-Hes1/5 [47,48,127], and Shh-Gli1 [139,140]. Understanding more about these signaling pathways will help reveal how the niches influence neural stem cell self-renewal and tissue development [164].

## 6. Conclusion

An emerging regulatory network controlling stem cell self-renewal and differentiation is defined by integration of cell-intrinsic regulators, including transcription factors, epigenetic controls, and small RNA regulators, with cell-extrinsic signals from stem cell niches. These mechanisms are coordinated to regulate the development, maintenance, self-renewal, and differentiation of stem cells. Unraveling how individual signaling cascades integrate into the global regulatory networks will be essential to better understand stem cell biology. It will also facilitate the development of new and targeted therapies using neural stem cells for a host of neurological disorders, including brain injuries, brain tumors, and neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's disease.

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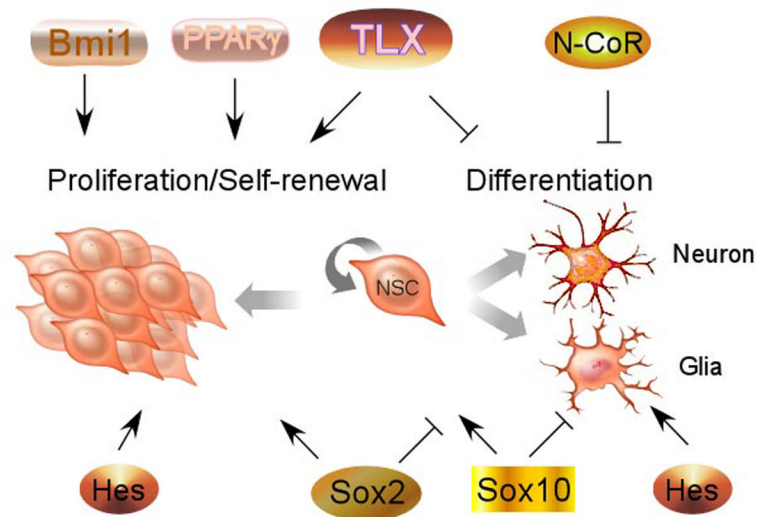
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## Biography

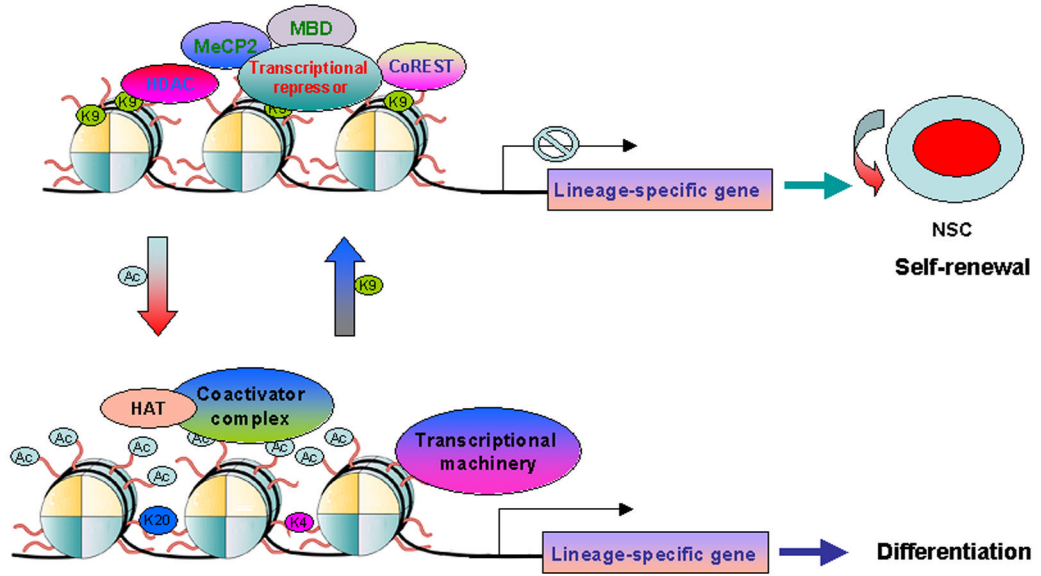
Dr. Yanhong Shi is an assistant professor of Neurosciences in Beckman Research Institute of City of Hope, Duarte, CA. Dr. Shi's research focuses on neural stem cells in the adult brain—specifically, she is interested in characterizing the molecular cascades that program these cells to be self-renewable. Dr. Shi received her PhD degree from Northwestern University, Evanston, IL, followed by a postdoctoral training in the Salk Institute, La Jolla, CA, where she gained valuable experiences in the gene expression laboratory and laboratory of Genetics under the guidance of Drs. Ronald M. Evans and Fred Gage. Dr. Shi is the recipient of a number of prestigious awards and has published her work extensively in respected scientific journals,

including *Nature*, *Genes and Development*, and *Proceedings of the National Academy of Sciences of the United States of America*.

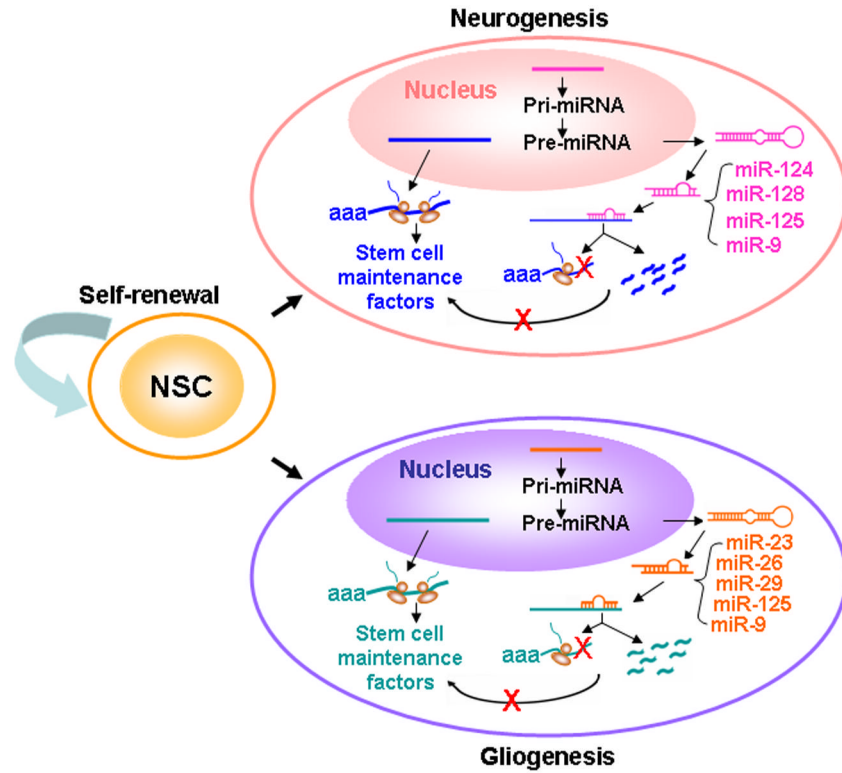




**Fig. 1.** Transcriptional regulators of neural stem cell self-renewal. Transcription factors that are expressed in neural stem cells can potentiate cell proliferation and repress cell differentiation to maintain the undifferentiated and self-renewable state of neural stem cells. Due to space limitations, not all related transcription factors are listed here.



**Fig. 2.** Epigenetic control of neural stem cell self-renewal. Maintenance of neural stem cells is normally associated with the repressive status of chromatin, which is represented by deacetylation of histones and methylation of histone H3 lysine 9 (K9). The repression is presumably resulted from recruitment of transcriptional corepressor complexes, including HDACs, MecP2, MBD, CoREST, to transcription factors at the promoter of target genes. Histone acetylation and methylation of histone H3 lysine 4 (K4) and lysine 20 (K20) are, on the other hand, involved in relaxation of chromatin structures and activation of lineage-specific gene expression, which in turn leads to differentiation of neural stem cells into mature neuron, astrocyte or oligodendrocyte. NSC stands for neural stem cells; Ac stands for histone acetylation.



**Fig. 3.**

A model for miRNA action in neural stem cells. Molecules that are expressed in neural stem cells to maintain their self-renewable state are collectively referred to as stem cell maintenance factors. miRNAs are initially transcribed in the nucleus as pri-miRNAs. The pri-miRNAs are processed into pre-miRNAs (hairpin) and transferred to the cytosol, where it is processed into 22-24 nucleotide mature miRNAs. Upon differentiation, miRNAs, such as miR-124 and miR-128, are highly expressed in the neuronal lineage, whereas miR-23, miR-26, and miR-29 are upregulated in glia. MiR-9 and miR-125 are expressed in both neurons and glia. These miRNAs form incomplete base pairing with their target mRNAs and direct the cleavage of these mRNAs or inhibit their translation, thus preventing the expression of the stem cell maintenance factors and enabling rapid neural differentiation.