Published in final edited form as:
J Cell Physiol. 2009 October ; 221(1): 5–9. doi:10.1002/jcp.21862.

Neural Stem Cells in the Developing and Adult Brains

Qiuhao Qu and **Yanhong Shi**
Department of Neurosciences, Center for Gene Expression and Drug Discovery, Beckman Research Institute of City of Hope, 1500 E. Duarte Rd, Duarte, CA 91010.

Abstract

Neural stem cells exist in the mammalian developing and adult nervous system. Recently, tremendous interest in the potential of neural stem cells for the treatment of neurodegenerative diseases and brain injuries has substantially promoted research on neural stem cell self-renewal and differentiation. Multiple cell-intrinsic regulators coordinate with the microenvironment through various signaling pathways to regulate neural stem cell maintenance, self-renewal and fate determination. This review focuses on essential intracellular regulators that control neural stem cell maintenance and self-renewal in both embryonic brains and adult nervous system. These factors include the orphan nuclear receptor TLX, the high-mobility-group DNA binding protein Sox2, the basic helix-loop-helix transcription factor Hes, the tumor suppressor gene Pten, the membrane-associated protein Numb and its cytoplasmic homolog Numblike. The aim of this review is to summarize our current understanding of neural stem cell regulation through these important stem cell regulators.

Keywords

neural stem cells; self-renewal; differentiation; TLX (NR2E1); Sox2; Hes; Pten; Numb

1. Introduction

Identifying regulators that control neural stem cell self-renewal and differentiation is essential for the development of neural stem cell-based cell replacement therapies for neurodegenerative diseases and brain injuries. Stem cells have the ability to self-renew and differentiate into multiple lineages. Regulation of neural stem cell self-renewal and differentiation is mediated through the combination of cell intrinsic factors and extracellular signaling molecules. The cell-intrinsic regulators coordinate with extrinsic signals to control the balance between neural stem cell self-renewal and differentiation. These regulators include the nuclear hormone receptor TLX (tailless), the high-mobility-group transcription factor Sox2 (Sex determining region of Y chromosome-related high mobility group box 2), the basic helix-loop-helix transcriptional repressor Hes (hairy and enhancer of split), the tumor suppressor phosphotase Pten (phosphatase and tensin homolog deleted on chromosome 10), and the *Drosophila* membrane-associated protein Numb homologs, Numb and Numblike. In this review, the effect of these recently characterized intracellular regulators on neural stem cell maintenance, self-renewal and differentiation is discussed.

2. TLX

Batteries of transcription factors and other cell-intrinsic regulators have been proposed to control neural stem cell maintenance, self-renewal, and differentiation (Fig. 1).TLX is a member of the nuclear receptor superfamily that was initially identified as a homolog of the

Correspondence should be addressed to Y.S. yshi@coh.org.

Drosophila tailless (Yu et al., 1994) and plays an important role in vertebrate brain functions (Chiang and Evans, 1997;Monaghan et al., 1997;Land and Monaghan, 2003;Stenman et al., 2003;Roy et al., 2004;Shi et al., 2004;Christie et al., 2006;Li et al., 2008). TLX knockout mice are viable and appear normal at birth. However, mature TLX knockout mice have significantly reduced cerebral hemispheres (Chiang and Evans, 1997;Monaghan et al., 1997) and severe retinopathies (Yu et al., 2000;Miyawaki et al., 2004;Uemura et al., 2006;Zhang et al., 2006). Behaviorally, adult TLX mutants exhibit increased aggressiveness, decreased copulation, progressively violent behavior, late onset epilepsy and reduced learning abilities (Chiang and Evans, 1997;Monaghan et al., 1997;Roy et al., 2002;Young et al., 2002).

TLX expression in the mouse starts at embryonic day 8 (E8), peaks at E13.5, and declines from E16 through neonate. The expression of TLX increases after birth, with high levels in adult brains (Monaghan et al., 1995). During development, TLX is expressed specifically in periventricular neural stem cells. Considerable thinning of neocortex was detected in embryonic TLX-null brains. Both reduced labeling of neural progenitor marker nestin and decreased cell proliferation were observed in the germinal zone of embryonic TLX-null brains. Cell cycle analysis revealed prolonged cell cycles and increased cell cycle exit in embryonic brains of TLX-null mice. Increased expression of a cyclin-dependent kinase inhibitor p21 and decreased expression of cyclin D1 provide a molecular basis for the deficiency of cell cycle progression in embryonic brains of TLX-null mice. Interestingly, transient knockdown of TLX by *in utero* electroporation led to precocious cell cycle exit and differentiation of neural stem cells. These results indicate that TLX plays an important role in neural stem cells of the developing brain.

What are the primary cellular functions exerted by TLX in neural development? TLX could be required in early neural progenitor cells to regulate proliferative divisions and prevent neurogenesis in the absence of differentiation cues. In support of this idea, we have shown that both systematic knockout and transient siRNA knockdown of TLX led to increased cell cycle exit and reduced cell proliferation. On the other hand, TLX could also act by regulating the types of cell divisions that progenitor cells make in embryonic brains. Early progenitor cells expand by symmetric divisions, establishing the surface area and number of radial units in the cortex (Rakic, 1988), while later progenitors undergo asymmetric divisions, establishing the number of neurons per radial unit and the depth of the cerebral cortex. Therefore the length of the cell cycle and the duration of each phase of cell division are critical in controlling the size of the cerebral cortex (Rakic, 1995; Takahashi et al., 1996; Caviness et al., 2003). We have shown that neural progenitor cells in embryonic brains of TLX-mutant mice go through fewer cell cycles than that in their wild type littermates, perhaps by prematurely switching from symmetric divisions to asymmetric divisions. TLX has also been shown to be required to regulate the timing of neurogenesis in the cortex (Roy et al., 2004) and to control patterning of lateral telencephalic progenitor domains during development (Stenman et al., 2003). Characterizing the role of TLX in the developing brain provides a means to elucidate the molecular and cellular mechanisms underlying embryonic neural stem cell proliferation and differentiation.

At the adult stage, TLX is expressed in neural stem cells in the subventricular zones and hippocamal dentate gyrus, the two adult neurogenesis areas. We have shown that TLX is an essential regulator of neural stem cell maintenance and self-renewal in adult brains (Shi et al., 2004). TLX maintains adult neural stem cells in an undifferentiated, self-renewable state. 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 brains of adult TLX-mutant mice failed to proliferate. Reintroducing TLX into TLX-null cells rescued their ability to proliferate and self-renew (Shi et al., 2004). TLX maintains neural stem cells in an undifferentiated and self-renewable state by complexing with histone deacetylases

(HDACs) to repress TLX downstream target genes, p21 and Pten (Sun et al., 2007). Either inhibition of HDAC activity, knockdown of HDAC expression, or disruption of TLX and HDAC interaction led to marked induction of p21 and Pten gene expression and dramatically reduced neural stem cell proliferation (Sun et al., 2007). Furthermore, TLX forms a negative regulatory loop with microRNA miR-9. miR-9 suppresses TLX expression in neural stem cells, while TLX represses miR-9 pri-miRNA expression. This feedback loop provides a novel strategy to control the balance between neural stem cell proliferation and differentiation (Zhao et al., 2009). Furthermore, the TLX-positive neural stem cells in the dentate gyrus play an important role in spatial learning and memory (Zhang et al., 2008), while the TLX-positive cells in the subventricular zones were identified to be the slowly dividing type B neural stem cells (Liu et al., 2008). TLX could be a key regulator that is modulated by histone modifying enzymes and microRNAs and acts by controlling the expression of a network of target genes to establish the undifferentiated and self-renewable state of neural stem cells (Fig. 2).

3. Sox2

The Sox2 transcription factor is also expressed at high levels in neural stem and progenitor cells in both embryonic and adult brains (Episkopou, 2005). Sox2 is one of the earliest transcription factors expressed in the developing brain and is a member of the extended Sox family that contains a characteristic high-mobility-group domain (Gubbay et al., 1990; Laudet et al., 1993). Along with Sox1 and Sox3, two other members of the Sox family, Sox2 has been shown to maintain neural stem cell identity in the developing brain. Constitutive expression of Sox2 inhibited neuronal differentiation and maintained neural progenitor characteristics, whereas inhibition of Sox2 led to precocious neuronal differentiation (Bylund et al., 2003; Graham et al., 2003). Sox2 mutant mice are embryonic lethal, indicating that Sox2 plays an essential role in early development (Avilion et al., 2003). Conditional disruption of Sox2 in neural stem and progenitor cells by crossing Sox2 floxed mice with nestin-Cre mice revealed important roles of Sox2 in the developing brain. These conditional mutant animals died soon after birth. Deletion of Sox2 in neural progenitor cells led to reduced neural progenitor populations and enlarged lateral ventricles, suggesting that Sox2 is important for maintaining neural stem and progenitor cells in the developing brain (Miyagi et al., 2008).

Sox2 is also expressed in neural stem cells in the adult brain (Ferri et al., 2004). The function of Sox2 in the adult brain was addressed by generating an enhancer deletion of the Sox2 gene that reduces its expression in the brain (a regulatory mutant allele). Adult mice with one Sox2 regulatory mutant allele and one Sox2 null allele had lower Sox2 expression and exhibited substantial reduction in proliferating neural precursors, suggesting an important role of Sox2 in neural stem cell maintenance or proliferation in adult brains (Ferri et al., 2004; Episkopou, 2005).

Overexpression of Sox2 in neural progenitors resulted in upregulation of Notch1 and its downstream effector, Hes5 (Bani-Yaghoub et al., 2006). On the other hand, Sox2 expression is regulated by sonic hedgehog (Shh) signaling pathway through its downstream effector, Gli2. Ovexpression of Sox2 rescued Hes expression in truncated Gli2-expressing cells. A mechanism has been proposed that Sox2 maintains the undifferentiated state of neural stem cells through the Gli2-Sox2-Hes5 signal cascade (Takanaga et al., 2008).

4. Hes

Mammalian neural development is controlled by multiple basic helix-loop-helix (bHLH) transcription factors. Hes genes are repressor-type bHLH genes that are homologous to the *Drosophila* hairy and enhancer of split and play an essential role in brain development (Kageyama et al., 2008). Among seven members of the Hes family, Hes1 and Hes5 are essential effectors of Notch signaling. Both Hes1 and Hes5 are highly expressed by neural stem cells

(Akazawa et al., 1992; Sasai et al., 1992; Allen and Lobe, 1999), the expression of which is up-regulated by Notch activation (Jarriault et al., 1995; Ohtsuka et al., 1999). Hes3, another member of the Hes family, is also expressed by neural precursor cells in the ventricular zone of the developing nervous system (Lobe, 1997; Allen and Lobe, 1999).

Overexpression of Hes1, Hes3, or Hes5 inhibited neuronal differentiation and maintained neural stem cells in the embryonic brain (Ishibashi et al., 1994; Hirata et al., 2000; Ohtsuka et al., 2001). In contrast, accelerated neurogenesis was observed in Hes1 knockout mice. However, the defects are relatively mild, presumably due to elevated expression of Hes5 (Ishibashi et al., 1995). Neural progenitors underwent severer premature differentiation in Hes1 and Hes5 double knockout mice (Ishibashi et al., 1995; Ohtsuka et al., 1999; Cau et al., 2000; Hatakeyama et al., 2004). But there are still neural stem cells in the developing nervous system, due to potential compensatory effect of Hes3. Triple knockout of Hes1, Hes3, and Hes5 led to extensively accelerated neuronal differentiation and a wide range of defects in brain formation, suggesting that Hes genes are essential for the maintenance of neural stem cells in the developing brain (Hatakeyama et al., 2004). Hes genes regulate neural stem cell self-renewal by repressing premature onset of the activator type bHLH genes, such as Mash1, Math, and Neurogenin, all of which promote neuronal differentiation of neural stem cells (Kageyama et al., 2005).

5. Pten

The Pten tumor suppressor gene is a phosphatase frequently mutated in human cancers and plays an important role in brain development (Li et al., 1997; Backman et al., 2001; Groszer et al., 2001; Kwon et al., 2001). Conventional Pten-null mice died early during embryogenesis, indicating an essential role of Pten in early development (Di Cristofano et al., 1998; Stambolic et al., 1998; Suzuki et al., 1998). Conditional knockout of Pten in neural stem/progenitor cells by crossing $Pten^{loxp/loxp}$ mice with nestin-Cre mice revealed that Pten is indispensable for proper control of neural stem cell proliferation and self-renewal in the developing brain (Groszer et al., 2001). These Pten conditional mutant mice have enlarged brains resulted from increased neural stem cell proliferation, decreased cell death, and enlarged cell size. The Pten-deficient neural stem/progenitor cells have a greater proliferation potential, due in part to a shortened cell cycle (Groszer et al., 2001). Loss of Pten in neural stem/progenitor cells also confers increased self-renewal capacity by modulating G0-G1 cell cycle entry (Groszer et al., 2006). Pten regulates cell cycle progression by suppressing cell cycle-related genes, such as cyclin B1, cyclin B2, cyclin D1, and cyclin E1 (Groszer et al., 2006). Furthermore, double knockout of p53 and Pten led to compromised differentiation potential and sustained self-renewal of neural stem cells by means of elevated Myc expression (Zheng et al., 2008). In summary, Pten is critical in keeping neural stem cells in check in the developing brain.

In addition to its function in neural development, Pten also regulates neural stem cells in the adult brain. Comparing neural stem cells derived from the subventricular zone of wild type and Pten heterozygote mice revealed that Pten is important for regulation of adult neural stem cell motility and apoptosis (Li et al., 2002). Loss of one copy of Pten led to increased migration of subventricular zone cells to the olfactory bulb, reduced H_2O_2-induced apoptosis, and substantial changes in gene expression of neural progenitor cells (Li et al., 2002; Li et al., 2003). Moreover, conditional deletion of Pten in a subpopulation of adult neural stem cells in the subventricular zone resulted in enhanced neural stem cell self-renewal. These Pten-deleted mice have increased olfactory bulb mass and enhanced olfactory function (Gregorian et al., 2009). Therefore, Pten is also an important player in adult neural stem cell self-renewal and neurogenesis.

6. Numb and Numblike

Mouse Numb and Numblike play redundant, but critical roles in maintaining neural progenitor cells during brain development by allowing their progenies to choose progenitor over neuronal fates (Zhong et al., 1997). Numb is asymmetrically distributed during cortical progenitor cell divisions and has an important role in generating asymmetric cell division and diverse cell fates during mouse cortical development (Zhong et al., 1996; Zhong et al., 1997; Shen et al., 2002). Numb suppresses Notch signaling by physical interaction with the intracellular domain of Notch 1. Mouse Numblike has extensive sequence similarity to Numb. While Numblike fails to enable neural precursors to divide asymmetrically, it allows cells to adopt the fate determined by Numb (Zhong et al., 1997).

In mice with double knockout of Numb and Numblike in the nervous system, early neurons emerge at the expense of progenitor cells, leading to a nearly complete depletion of dividing cells shortly after the onset of neurogenesis (Petersen et al., 2002). This finding indicated that the main function of Numb and Numblike in mouse neurogenesis is to maintain progenitor cells during the initial progenitor versus neuronal fate decision. This is also supported by gain-of-function studies in chick showing an increase in progenitor pools upon over-expression of Numb (Wakamatsu et al., 1999). As occurs during early neurogenesis, the loss of Numb and Numblike also causes premature progenitor cell depletion and consequently, malformation of the neocortex and hippocampus, after initial waves of neurogenesis (Petersen et al., 2004).

Deletion of Numb and Numblike in the postnatal subventricular zone using a tamoxifen-inducible Cre transgene (nestin-creER^{tm}) resulted in severe damage to brain lateral ventricle integrity and identified a role for Numb and Numblike in regulating ependymal wall integrity and subventricular zone neuroblast survival (Kuo et al., 2006). A new role was revealed for Numb recently in the maintenance of cadherin-mediated cell adhesion and polarity of radial glila cells and consequently, the integrity of the ventricular zone and cerebral cortex (Rasin et al., 2007). The additional functions for Numb in regulating ventricular zone homeostasis and neural progenitor cell adhesion and polarity indicate that Numb has diverse roles in neural development.

7. Conclusion

An emerging regulatory network controlling stem cell self-renewal and differentiation is defined by integration of multiple cell-intransic regulators with cell-extransic signals from stem cell niches. These diverse players are coordinated to regulate the maintenance, self-renewal, and differentiation of neural stem cells in both embryonic and adult brains. Unraveling how these regulatory cascades function to regulate neural stem cell maintenance and self-renewal is essential to better understand neural stem cell biology. It will facilitate the development of new and targeted therapies using neural stem cells for a host of neurological disorders, including neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, and brain injuries.

Acknowledgments

We apologize to colleagues whose work could not be cited due to space limitations. We thank members of the Shi Laboratory for their critical inputs and suggestions. This work is supported by Whitehall Foundation, James S. McDonnell Foundation, and NIH NINDS R01 NS059546 (to Y.S.).

References

Akazawa C, Sasai Y, Nakanishi S, Kageyama R. Molecular characterization of a rat negative regulator with a basic helix-loop-helix structure predominantly expressed in the developing nervous system. J Biol Chem 1992;267(30):21879–21885. [PubMed: 1400497]

Allen T, Lobe CG. A comparison of Notch, Hes and Grg expression during murine embryonic and post-natal development. Cell Mol Biol (Noisy-le-grand) 1999;45(5):687–708. [PubMed: 10512199]

Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev 2003;17(1):126–140. [PubMed: 12514105]

Backman SA, Stambolic V, Suzuki A, Haight J, Elia A, Pretorius J, Tsao MS, Shannon P, Bolon B, Ivy GO, Mak TW. Deletion of Pten in mouse brain causes seizures, ataxia and defects in soma size resembling Lhermitte-Duclos disease. Nat Genet 2001;29(4):396–403. [PubMed: 11726926]

Bani-Yaghoub M, Tremblay RG, Lei JX, Zhang D, Zurakowski B, Sandhu JK, Smith B, Ribecco-Lutkiewicz M, Kennedy J, Walker PR, Sikorska M. Role of Sox2 in the development of the mouse neocortex. Dev Biol 2006;295(1):52–66. [PubMed: 16631155]

Bylund M, Andersson E, Novitch BG, Muhr J. Vertebrate neurogenesis is counteracted by Sox1-3 activity. Nat Neurosci 2003;6(11):1162–1168. [PubMed: 14517545]

Cau E, Gradwohl G, Casarosa S, Kageyama R, Guillemot F. Hes genes regulate sequential stages of neurogenesis in the olfactory epithelium. Development 2000;127(11):2323–2332. [PubMed: 10804175]

Caviness VS Jr, Goto T, Tarui T, Takahashi T, Bhide PG, Nowakowski RS. Cell output, cell cycle duration and neuronal specification: a model of integrated mechanisms of the neocortical proliferative process. Cereb Cortex 2003;13(6):592–598. [PubMed: 12764033]

Chiang, MY.; Evans, RM. Reverse Genetic Analysis of Nuclear Receptors, RXRγ, RARβ, and TLX in Mice. Dissertation. La Jolla, CA: Univ of California San Diego; 1997.

Christie BR, Li AM, Redila VA, Booth H, Wong BK, Eadie BD, Ernst C, Simpson EM. Deletion of the nuclear receptor Nr2e1 impairs synaptic plasticity and dendritic structure in the mouse dentate gyrus. Neuroscience 2006;137(3):1031–1037. [PubMed: 16289828]

Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. Pten is essential for embryonic development and tumour suppression. Nat Genet 1998;19(4):348–355. [PubMed: 9697695]

Episkopou V. SOX2 functions in adult neural stem cells. Trends Neurosci 2005;28(5):219–221. [PubMed: 15866195]

Ferri AL, Cavallaro M, Braida D, Di Cristofano A, Canta A, Vezzani A, Ottolenghi S, Pandolfi PP, Sala M, DeBiasi S, Nicolis SK. Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. Development 2004;131(15):3805–3819. [PubMed: 15240551]

Graham V, Khudyakov J, Ellis P, Pevny L. SOX2 functions to maintain neural progenitor identity. Neuron 2003;39(5):749–765. [PubMed: 12948443]

Gregorian C, Nakashima J, Le Belle J, Ohab J, Kim R, Liu A, Smith KB, Groszer M, Garcia AD, Sofroniew MV, Carmichael ST, Kornblum HI, Liu X, Wu H. Pten deletion in adult neural stem/progenitor cells enhances constitutive neurogenesis. Journal of Neuroscience 2009;29(6):1874–1886. [PubMed: 19211894]

Groszer M, Erickson R, Scripture-Adams DD, Dougherty JD, Le Belle J, Zack JA, Geschwind DH, Liu X, Kornblum HI, Wu H. PTEN negatively regulates neural stem cell self-renewal by modulating G0–G1 cell cycle entry. Proc Natl Acad Sci U S A 2006;103(1):111–116. [PubMed: 16373498]

Groszer M, Erickson R, Scripture-Adams DD, Lesche R, Trumpp A, Zack JA, Kornblum HI, Liu X, Wu H. Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. Science 2001;294(5549):2186–2189. [PubMed: 11691952]

Gubbay J, Collignon J, Koopman P, Capel B, Economou A, Munsterberg A, Vivian N, Goodfellow P, Lovell-Badge R. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature 1990;346(6281):245–250. [PubMed: 2374589]

Hatakeyama J, Bessho Y, Katoh K, Ookawara S, Fujioka M, Guillemot F, Kageyama R. Hes genes regulate size, shape and histogenesis of the nervous system by control of the timing of neural stem cell differentiation. Development 2004;131(22):5539–5550. [PubMed: 15496443]

Hirata H, Ohtsuka T, Bessho Y, Kageyama R. Generation of structurally and functionally distinct factors from the basic helix-loop-helix gene Hes3 by alternative first exons. J Biol Chem 2000;275(25): 19083–19089. [PubMed: 10858455]

Ishibashi M, Ang SL, Shiota K, Nakanishi S, Kageyama R, Guillemot F. Targeted disruption of mammalian hairy and Enhancer of split homolog-1 (HES-1) leads to up-regulation of neural helix-loop-helix factors, premature neurogenesis, and severe neural tube defects. Genes Dev 1995;9(24): 3136–3148. [PubMed: 8543157]

Ishibashi M, Moriyoshi K, Sasai Y, Shiota K, Nakanishi S, Kageyama R. Persistent expression of helix-loop-helix factor HES-1 prevents mammalian neural differentiation in the central nervous system. Embo J 1994;13(8):1799–1805. [PubMed: 7909512]

Jarriault S, Brou C, Logeat F, Schroeter EH, Kopan R, Israel A. Signalling downstream of activated mammalian Notch. Nature 1995;377(6547):355–358. [PubMed: 7566092]

Kageyama R, Ohtsuka T, Hatakeyama J, Ohsawa R. Roles of bHLH genes in neural stem cell differentiation. Exp Cell Res 2005;306(2):343–348. [PubMed: 15925590]

Kageyama R, Ohtsuka T, Kobayashi T. Roles of Hes genes in neural development. Development, growth & differentiation 2008;50:S97–S103.

Kuo CT, Mirzadeh Z, Soriano-Navarro M, Rasin M, Wang D, Shen J, Sestan N, Garcia-Verdugo J, Alvarez-Buylla A, Jan LY, Jan YN. Postnatal deletion of Numb/Numblike reveals repair and remodeling capacity in the subventricular neurogenic niche. Cell 2006;127(6):1253–1264. [PubMed: 17174898]

Kwon CH, Zhu X, Zhang J, Knoop LL, Tharp R, Smeyne RJ, Eberhart CG, Burger PC, Baker SJ. Pten regulates neuronal soma size: a mouse model of Lhermitte-Duclos disease. Nat Genet 2001;29(4): 404–411. [PubMed: 11726927]

Land PW, Monaghan AP. Expression of the transcription factor, tailless, is required for formation of superficial cortical layers. Cereb Cortex 2003;13(9):921–931. [PubMed: 12902391]

Laudet V, Stehelin D, Clevers H. Ancestry and diversity of the HMG box superfamily. Nucleic Acids Res 1993;21(10):2493–2501. [PubMed: 8506143]

Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275(5308):1943–1947. [PubMed: 9072974]

Li L, He F, Litofsky NS, Recht LD, Ross AH. Profiling of genes expressed by PTEN haploinsufficient neural precursor cells. Mol Cell Neurosci 2003;24(4):1051–1061. [PubMed: 14697668]

Li L, Liu F, Salmonsen RA, Turner TK, Litofsky NS, Di Cristofano A, Pandolfi PP, Jones SN, Recht LD, Ross AH. PTEN in neural precursor cells: regulation of migration, apoptosis, and proliferation. Mol Cell Neurosci 2002;20(1):21–29. [PubMed: 12056837]

Li W, Sun G, Yang S, Qu Q, Nakashima K, Shi Y. Nuclear Receptor TLX Regulates Cell Cycle Progression in Neural Stem Cells of the Developing Brain. Mol Endocrinol 2008;22(1):56–64. [PubMed: 17901127]

Liu HK, Belz T, Bock D, Takacs A, Wu H, Lichter P, Chai M, Schutz G. The nuclear receptor tailless is required for neurogenesis in the adult subventricular zone. Genes Dev 2008;22(18):2473–2478. [PubMed: 18794344]

Lobe CG. Expression of the helix-loop-helix factor, Hes3, during embryo development suggests a role in early midbrain-hindbrain patterning. Mech Dev 1997;62(2):227–237. [PubMed: 9152013]

Miyagi S, Masui S, Niwa H, Saito T, Shimazaki T, Okano H, Nishimoto M, Muramatsu M, Iwama A, Okuda A. Consequence of the loss of Sox2 in the developing brain of the mouse. FEBS Lett 2008;582 (18):2811–2815. [PubMed: 18638478]

Miyawaki T, Uemura A, Dezawa M, Yu RT, Ide C, Nishikawa S, Honda Y, Tanabe Y, Tanabe T. Tlx, an orphan nuclear receptor, regulates cell numbers and astrocyte development in the developing retina. J Neurosci 2004;24(37):8124–8134. [PubMed: 15371513]

Monaghan AP, Bock D, Gass P, Schwager A, Wolfer DP, Lipp HP, Schutz G. Defective limbic system in mice lacking the tailless gene. Nature 1997;390(6659):515–517. [PubMed: 9394001]

Monaghan AP, Grau E, Bock D, Schutz G. The mouse homolog of the orphan nuclear receptor tailless is expressed in the developing forebrain. Development 1995;121(3):839–853. [PubMed: 7720587]

Ohtsuka T, Ishibashi M, Gradwohl G, Nakanishi S, Guillemot F, Kageyama R. Hes1 and Hes5 as notch effectors in mammalian neuronal differentiation. Embo J 1999;18(8):2196–2207. [PubMed: 10205173]

Ohtsuka T, Sakamoto M, Guillemot F, Kageyama R. Roles of the basic helix-loop-helix genes Hes1 and Hes5 in expansion of neural stem cells of the developing brain. J Biol Chem 2001;276(32):30467–30474. [PubMed: 11399758]

Petersen PH, Zou K, Hwang JK, Jan YN, Zhong W. Progenitor cell maintenance requires numb and numblike during mouse neurogenesis. Nature 2002;419(6910):929–934. [PubMed: 12410312]

Petersen PH, Zou K, Krauss S, Zhong W. Continuing role for mouse Numb and Numbl in maintaining progenitor cells during cortical neurogenesis. Nat Neurosci 2004;7(8):803–811. [PubMed: 15273690]

Rakic P. Specification of cerebral cortical areas. Science 1988;241(4862):170–176. [PubMed: 3291116]

Rakic P. A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. Trends Neurosci 1995;18(9):383–388. [PubMed: 7482803]

Rasin MR, Gazula VR, Breunig JJ, Kwan KY, Johnson MB, Liu-Chen S, Li HS, Jan LY, Jan YN, Rakic P, Sestan N. Numb and Numbl are required for maintenance of cadherin-based adhesion and polarity of neural progenitors. Nat Neurosci 2007;10(7):819–827. [PubMed: 17589506]

Roy K, Kuznicki K, Wu Q, Sun Z, Bock D, Schutz G, Vranich N, Monaghan AP. The Tlx gene regulates the timing of neurogenesis in the cortex. J Neurosci 2004;24(38):8333–8345. [PubMed: 15385616]

Roy K, Thiels E, Monaghan AP. Loss of the tailless gene affects forebrain development and emotional behavior. Physiol Behav 2002;77(4–5):595–600. [PubMed: 12527005]

Sasai Y, Kageyama R, Tagawa Y, Shigemoto R, Nakanishi S. Two mammalian helix-loop-helix factors structurally related to Drosophila hairy and Enhancer of split. Genes Dev 1992;6(12B):2620–2634. [PubMed: 1340473]

Shen Q, Zhong W, Jan YN, Temple S. Asymmetric Numb distribution is critical for asymmetric cell division of mouse cerebral cortical stem cells and neuroblasts. Development 2002;129(20):4843–4853. [PubMed: 12361975]

Shi Y, Chichung Lie D, Taupin P, Nakashima K, Ray J, Yu RT, Gage FH, Evans RM. Expression and function of orphan nuclear receptor TLX in adult neural stem cells. Nature 2004;427(6969):78–83. [PubMed: 14702088]

Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP, Mak TW. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 1998;95(1):29–39. [PubMed: 9778245]

Stenman JM, Wang B, Campbell K. Tlx controls proliferation and patterning of lateral telencephalic progenitor domains. J Neurosci 2003;23(33):10568–10576. [PubMed: 14627641]

Sun G, Yu RT, Evans RM, Shi Y. Orphan nuclear receptor TLX recruits histone deacetylases to repress transcription and regulate neural stem cell proliferation. Proc Natl Acad Sci U S A 2007;104(39):15282–15287. [PubMed: 17873065]

Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, Ho A, Wakeham A, Itie A, Khoo W, Fukumoto M, Mak TW. High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. Curr Biol 1998;8(21):1169–1178. [PubMed: 9799734]

Takahashi T, Nowakowski RS, Caviness VS Jr. The leaving or Q fraction of the murine cerebral proliferative epithelium: a general model of neocortical neuronogenesis. J Neurosci 1996;16(19):6183–6196. [PubMed: 8815900]

Takanaga H, Tsuchida-Straeten N, Nishide K, Watanabe A, Aburatani H, Kondo T. Gli2 Is A Novel Regulator of Sox2 Expression In Telencephalic Neuroepithelial Cells. Stem Cells. 2008

Uemura A, Kusuhara S, Wiegand SJ, Yu RT, Nishikawa S. Tlx acts as a proangiogenic switch by regulating extracellular assembly of fibronectin matrices in retinal astrocytes. J Clin Invest 2006;116(2):369–377. [PubMed: 16424942]

Wakamatsu Y, Maynard TM, Jones SU, Weston JA. NUMB localizes in the basal cortex of mitotic avian neuroepithelial cells and modulates neuronal differentiation by binding to NOTCH-1. Neuron 1999;23(1):71–81. [PubMed: 10402194]

Young KA, Berry ML, Mahaffey CL, Saionz JR, Hawes NL, Chang B, Zheng QY, Smith RS, Bronson RT, Nelson RJ, Simpson EM. Fierce: a new mouse deletion of Nr2e1; violent behaviour and ocular abnormalities are background-dependent. Behav Brain Res 2002;132(2):145–158. [PubMed: 11997145]

Yu RT, Chiang MY, Tanabe T, Kobayashi M, Yasuda K, Evans RM, Umesono K. The orphan nuclear receptor Tlx regulates Pax2 and is essential for vision. Proc Natl Acad Sci U S A 2000;97(6):2621–2625. [PubMed: 10706625]

Yu RT, McKeown M, Evans RM, Umesono K. Relationship between Drosophila gap gene tailless and a vertebrate nuclear receptor Tlx. Nature 1994;370(6488):375–379. [PubMed: 8047143]

Zhang CL, Zou Y, He W, Gage FH, Evans RM. A role for adult TLX-positive neural stem cells in learning and behaviour. Nature 2008;451(7181):1004–1007. [PubMed: 18235445]

Zhang CL, Zou Y, Yu RT, Gage FH, Evans RM. Nuclear receptor TLX prevents retinal dystrophy and recruits the corepressor atrophin1. Genes Dev 2006;20(10):1308–1320. [PubMed: 16702404]

Zhao C, Sun G, Li S, Shi Y. A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination. Nat Struct Mol Biol 2009;16(4):365–371. [PubMed: 19330006]

Zheng H, Ying H, Yan H, Kimmelman AC, Hiller DJ, Chen AJ, Perry SR, Tonon G, Chu GC, Ding Z, Stommel JM, Dunn KL, Wiedemeyer R, You MJ, Brennan C, Wang YA, Ligon KL, Wong WH, Chin L, DePinho RA. p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. Nature 2008;455(7216):1129–1133. [PubMed: 18948956]

Zhong W, Feder JN, Jiang MM, Jan LY, Jan YN. Asymmetric localization of a mammalian numb homolog during mouse cortical neurogenesis. Neuron 1996;17(1):43–53. [PubMed: 8755477]

Zhong W, Jiang MM, Weinmaster G, Jan LY, Jan YN. Differential expression of mammalian Numb, Numblike and Notch1 suggests distinct roles during mouse cortical neurogenesis. Development 1997;124(10):1887–1897. [PubMed: 9169836]

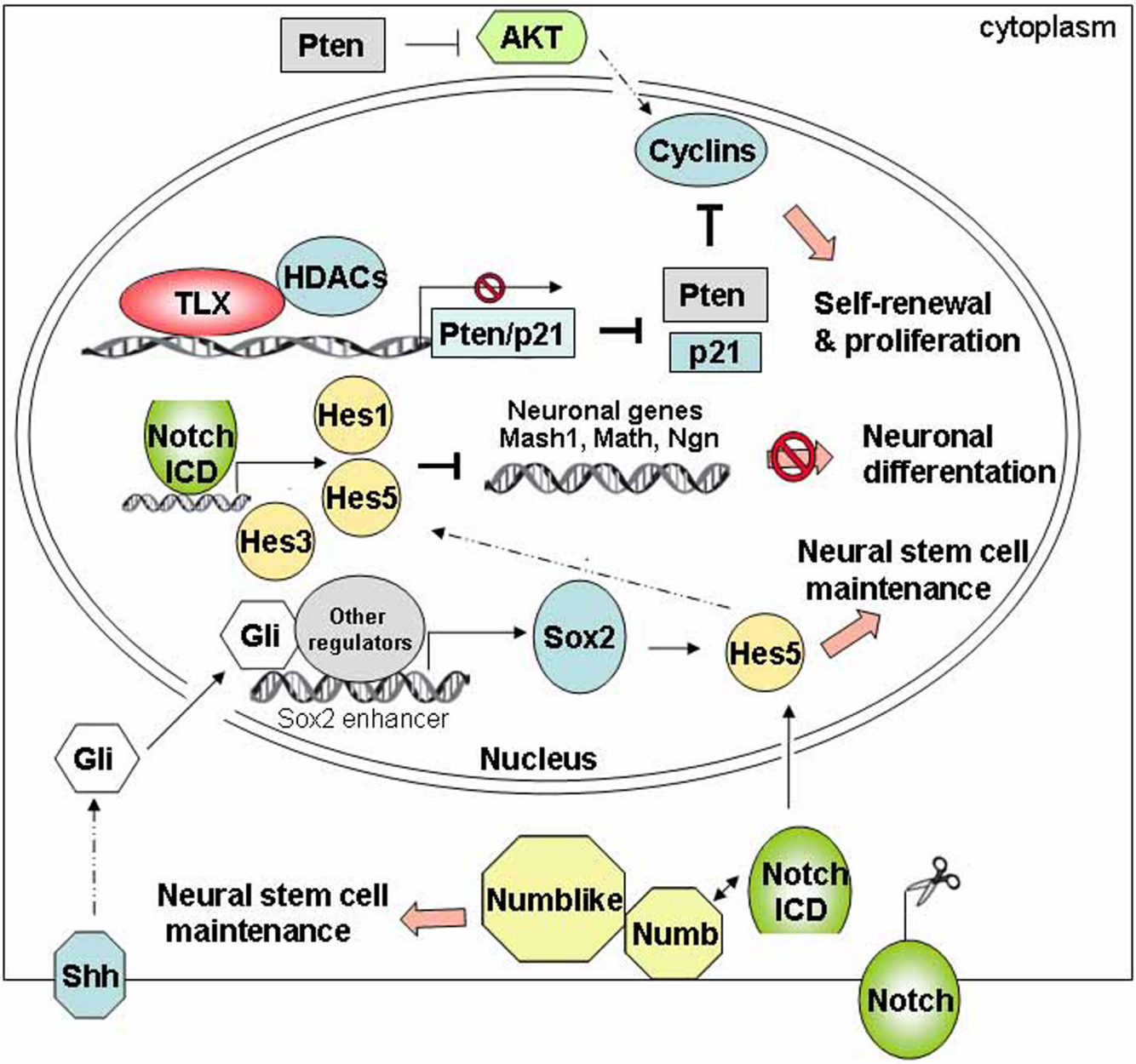

Fig. 1. Cell-intrinsic regulators in neural stem cell maintenance, self-renewal and differentiation

Several intracellular regulators function to maintain neural stem cells in the undifferentiated and self-renewable state. The nuclear receptor TLX is an essential neural stem cell regulator by negatively regulating its downstream target genes, such as the tumor suppressor Pten and the cyclin-dependent kinase inhibitor p21. Pten negatively regulates neural stem cell self-renewal by down-regulation of cell cycle-related genes, such as the cyclins. The bHLH transcriptional repressors Hes genes are downstream effectors of Notch signaling. They regulate neural stem cell maintenance and self-renewal by repressing proneuronal genes, such as Mash1, Math and Neurogenin (Ngn). Sox2 is also important for maintaining neural stem cells. Sox2 is activated by the sonic hedgehog (Shh) effector Gli2 and regulates the Notch downstream effector Hes5. The Gli-Sox2-Hes cascade represents one of the mechanisms for Sox2-mediated neural stem cell regulation. The membrane-associated protein Numb also

targets Notch signaling to regulate neural stem cell maintenance, self-renewal and differentiation, along with its cytoplasmic homolog, Numblike.

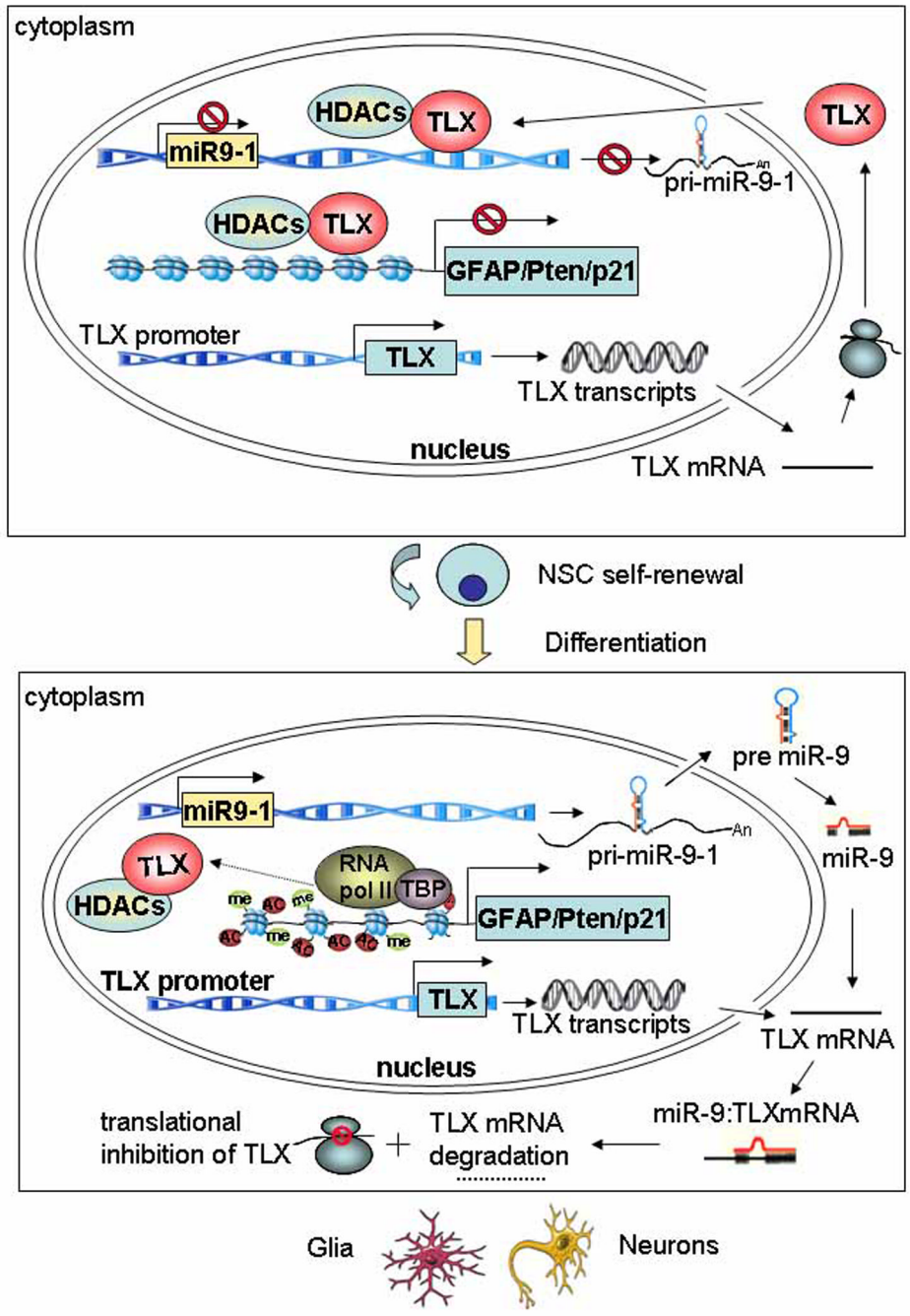

Fig. 2. TLX regulates neural stem cell self-renewal and proliferation

TLX controls neural stem cell self-renewal and differentiation through its downstream target genes, such as p21, Pten, and GFAP. TLX recruits histone deacetylases (HDACs) to the promoters of p21 and Pten genes to repress their expression, which in turn promotes neural stem cell proliferation. The TLX-HDAC complex also binds to microRNA miR-9 precursor miR-9-1 to repress its expression in neural stem cells. Upon receiving differentiation cues, miR-9 expression is up-regulated. miR-9 binds to the 3' untranslated region (UTR) of TLX mRNA to mediate TLX mRNA degradation and/or translational inhibition. Reduced expression of TLX leads to dissociation of the TLX-HDAC complex from its target gene

promoters and induced expression of these genes. Accordingly, neural stem cells are differentiated into mature neurons or glia.