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## MicroRNAs in Cerebral Ischemia-Induced Neurogenesis

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### Abstract

Cerebral ischemia induces neurogenesis including proliferation and differentiation of neural progenitor cells and migration of newly generated neuroblasts. microRNAs (miRNAs) are small non-coding RNAs that decrease gene expression through mRNA destabilization and/or translational repression. Emerging data indicate that miRNAs have a role in mediating processes of proliferation and differentiation of adult neural progenitor cells. This article reviews recent findings on miRNA profile changes in neural progenitor cells after cerebral infarction and the contributions of miRNAs to their ischemia-induced proliferation and differentiation. We highlight interactions between the miR-124 and the miR17-92 cluster and the Notch and Sonic hedgehog signaling pathways in mediating stroke-induced neurogenesis.

### Keywords

miRNAs; Neural progenitor cells; Neurogenesis; Stroke

## INTRODUCTION

Neural stem cells in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone of the dentate gyrus generate new neurons throughout the life of adult rodents (1-4). This neurogenesis has major impacts on neurological function (5-11). Focal cerebral ischemia/infarction is a major cause of disability (12-17). Preclinical and clinical studies demonstrate that stroke promotes neurogenesis in the adult brain (18-35), and in an experimental stroke model, blockage of newly generated neuroblasts exacerbates spontaneous neurological recovery (6). These findings suggest the possibility that amplifying neurogenesis may improve neurological outcomes following cerebral ischemia/infarction in humans (5-7, 10, 11).

### microRNAs

microRNAs (miRNAs), a family of short noncoding RNA molecules of 20 to 25 nucleotides, play important roles in neural stem cells during brain development and are involved in physiologic function and in disease processes by decreasing gene expression through mRNA destabilization and/or translational repression (36). In mice, inhibition of

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miRNA biogenesis in neural stem cells during development by ablation of Dicer (an endoribonuclease that cleaves double-stranded RNA and pre-miRNA into short double-stranded RNA) results in reduction of neural stem cells, abnormal neuronal differentiation, and a thin cortical wall of the brain (37, 38). miRNAs also regulate neural stem cell function in the adult brain (39-46). The biological function of miRNAs in neurogenesis has recently been reviewed (39,47-51). Emerging data indicate that adult neural stem cells express miRNAs and that cerebral infarction substantially alters miRNA profiles in neural stem cells. In this review, we briefly describe neurogenesis and its potential involvement in ischemic brain repair and functional outcome, and then review evidence that miRNAs mediate processes cerebral infarct-induced neurogenesis.

### Neurogenesis in Cerebral Ischemia

The SVZ of the lateral ventricle of adult rodents contains at least 3 types of cells: 1) neural stem cells, a subpopulation of glial fibrillary acidic protein-positive cells expressing nestin; 2) actively proliferating intermediate neural progenitor cells that express a *Ascl1*, a basic helix-loop-helix transcription factor; and 3) neuroblasts, which express doublecortin and polysialylated neural adhesion cell molecule (1-4). Under non-ischemic conditions, neural stem cells in the SVZ generate neuroblasts that travel the rostral migratory stream to the olfactory bulb where they differentiate into granule and periglomerular neurons (52-54). More than 30,000 neuroblasts are generated daily in the rodent SVZ (55, 56). Neuroblasts generated in the subgranular zone differentiate into dentate granule cells and integrate into the preexisting neuronal network (57).

Cerebral infarction increases neural stem cell proliferation that results in early expansion of the neural progenitor pool in the SVZ (58-60). Neural progenitor cells preferentially differentiate into neuroblasts (58-60); neuroblasts then migrate out of the SVZ to the ischemic cortex and striatum (Fig. 1) (61, 62). Studies in transgenic mice with inducible Cre recombinase under control of the *Ascl1* or *Nestin* promoter indicate that newly arrived neuroblasts in the ischemic boundary regions exhibit phenotypes and electrophysiological characteristics of mature neurons (18, 19, 22, 23, 25, 33). This suggests that neuroblasts mature into resident neurons in the ischemic brain but that the effect of neuroblasts on the ischemic brain may extend beyond the replacement of damaged neurons. For example, neurogenesis is coupled with angiogenesis in the ischemic brain, i.e. ischemic neural progenitor cells promote endothelial cells to form capillary-like tubes (angiogenesis) by secreting vascular endothelial growth factor in vitro (63). Suppressing angiogenesis either with endostatin or with a neutralizing antibody against the angiopoietin receptor, *Tie2*, substantially attenuates migration of neuroblasts newly born in the SVZ to the ischemic region (11). Using transgenic mice that express herpes simplex virus thymidine kinase under the control of the promoter for doublecortin, Wang et al demonstrated that ablation of doublecortin-expressing neuroblasts substantially exacerbated ischemic damage and worsened neurological outcome (6). These data indicate that cerebral infarct-induced neurogenesis may affect stroke recovery.

### miRNAs in Neural Progenitor Cells Following Cerebral Ischemia

miRNAs play important roles in the regulation of adult neurogenesis (64). For example, miR-124, the most abundant neuronal miRNA, is expressed by neural progenitor cells in the SVZ of adult rodent brains (40, 42). Attenuation of endogenous miR-124 in neural progenitor cells can abolish neuronal differentiation, whereas overexpression of miR-124 promoted neuronal differentiation in the mouse brain (40, 42). The SRY-box transcription factor *Sox9* is a physiological target of miR124 (42). Another example is miR-9, which is also expressed by adult SVZ neural progenitor cells (65). miR-9 suppresses the expression

of the orphan nuclear receptor TLX to negatively regulate neural stem cell proliferation and accelerate neural differentiation (65).

The biological functions of miRNAs in cerebral ischemia-induced neurogenesis have not been extensively studied. Using an miRNA array that detects mature miRNAs, Liu et al showed that cultured neural progenitor cells harvested from the SVZ of animals subjected to cerebral ischemia markedly altered miRNA expression profiles compared to neural progenitor cells from non-ischemic SVZ (64). Bioinformatics analysis of 18 and 21 miRNAs downregulated and upregulated by cerebral ischemia, respectively, revealed that these miRNAs selectively affect several signaling pathways including transforming growth factor  $\beta$ , Wnt, and Sonic hedgehog (Shh) signals, which are known to regulate neural stem cell function (66).

### The Shh Signaling Pathway and miR17-92 Cluster in Ischemia-induced Neurogenesis

Shh is a member of the family of the hedgehog proteins known to exert important regulatory functions in patterning and growth in a large number of tissues during embryogenesis (67-69); it has an important role in regulating neural progenitor cell proliferation and differentiation in the brain (68, 69). Canonically, Shh binds to the transmembrane receptor protein, patched (ptc), which, in the absence of Shh, exerts an inhibitory effect on the 7 transmembrane receptor smoothened (Smo). Binding of Shh to ptc blocks the inhibitory effect of ptc on Smo (70-72). Once activated, Smo induces a complex series of intracellular reactions that target the Gli family of transcription factors (70, 72). Exogenous Shh increases neurogenesis in the SVZ, whereas inactivation of Shh signals depletes neural stem cells and intermediate neural progenitor cells in the SVZ (67, 70). The Shh pathway also mediates stroke-induced neurogenesis (73).

The miR17-92 cluster comprises a cluster of 6 miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1) on chromosome 13 that is transcribed as a single polycistronic unit (74). During development, the miR17-92 cluster regulates neural progenitor cell proliferation and oligodendrogenesis (75, 76). Using a conditional knockout of the miR17-92 cluster in 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase)-expressing oligodendrocytes, Budde et al showed that ablation of the miR17-92 cluster substantially reduced oligodendrocyte progenitor cell proliferation and survival in post-natal mice (76). The clinical relevance of the miR17-92 cluster has recently been suggested in patients with the autosomal dominant Feingold syndrome in which there is a germline deletion of the miR17-92 cluster that is associated with microcephaly and skeletal abnormalities (77). The miR17-92 cluster is upregulated miRNA in SVZ neural progenitor cells after cerebral ischemia (64, 78). Overexpression of the miR17-92 cluster enhances stroke-induced progenitor cell proliferation, whereas attenuation of endogenous miR-18a and miR-19a suppresses this neural progenitor cell proliferation. Cerebral ischemia substantially reduces individual members of 2 miR17-92 cluster analogs (i.e. miR106a-363 and miR106b-25 [78]), but the biological function of these clusters in adult neurogenesis has not been studied. The positive effect of the miR17-92 cluster on neurogenesis may be partially attributed to suppression of phosphatase and tensin homolog deleted on chromosome 10 (i.e. PTEN), a protein that negatively regulates embryonic neural stem cell proliferation and survival (79-85).

Emerging data also indicate that the Shh pathway is closely associated with expression of the miR17-92 cluster, i.e. the Shh signaling pathway interacts with the miR-17-92 cluster to contribute to neural progenitor cell proliferation (75, 86). In cultured neural progenitor cells, attenuation of endogenous Shh and addition of exogenous Shh downregulate and upregulate the miR17-92 cluster expression, respectively (78). Intraventricular administration of exogenous Shh to animals with cerebral ischemia further upregulated miR17-92 cluster

expression in SVZ neural progenitor cells, whereas blockage of the Shh pathway suppressed ischemia-upregulated miR17-92 cluster expression and ischemia-increased neural progenitor cell proliferation (78).

The Shh pathway likely regulates miR17-92 cluster expression in SVZ neural progenitor cells in ischemic brain via Myc, one of the most potent oncogenic agents (87). Myc is a downstream target of Shh and the miR17-92 cluster is a direct transcriptional target of c-Myc (88, 89). Cerebral ischemia enhances the binding of c-Myc to the promoter region of the miR17-92 cluster in neural progenitor cells (78). Thus, the miR17-92 cluster likely mediates processes of Shh-induced neural progenitor cell proliferation (Fig. 2A).

### **The Notch Signaling Pathway and miR-124 in Cerebral Ischemia-induced Neurogenesis**

Notch receptors are transmembrane proteins activated by Delta and Jagged ligands (90, 91). On activation, the Notch internal cellular domain is cleaved by presenilin-1 and the  $\gamma$ -secretase enzyme complex is translocated into the nucleus, leading to activation of transcription factors (91). The Notch signaling pathway plays a pivotal role in maintaining the embryonic neural stem cell pool and promoting gliogenesis (92). In the ischemic brain, activation of the Notch pathway increases neural progenitor cell proliferation, whereas blockage of the pathway abolishes stroke-increased progenitor cell proliferation (93). In non-ischemic brain, miR-124a in neural progenitor cells regulates neuronal differentiation by targeting SOX9 (42). Cerebral ischemia substantially reduces miR-124a expression in SVZ neural progenitor cells, which is inversely associated with upregulation of Jagged-1 (JAG1), one of the target genes of miR-124a (64). Introduction of miR-124a dramatically inhibits stroke-increased neural progenitor cell proliferation and promotes the neuronal differentiation of the progenitor cells by targeting JAG1, but not SOX9 (64). These data suggest that miR-124a mediates adult neurogenesis either by targeting SOX9 or the Notch signaling pathway under non-ischemic and ischemic conditions (Fig. 2B).

### **Other Signaling Pathways and miRNAs**

Other signaling pathways such as Wnt and bone morphogenic protein (BMP) also regulate neurogenesis in the adult brain (94, 95). Overexpression of BMP7 in ependymal cells inhibits neural progenitor cell proliferation and neuroblast production, demonstrating that BMPs potently inhibit neurogenesis (94). On the other hand, the Wnt pathway promotes neurogenesis in the dentate gyrus (95). Cerebral ischemia changes expression of Wnt and BMP family genes in SVZ neural progenitor cells of adult rodents (96, 97). Let-7b and miR-9 regulate adult neurogenesis by controlling the balance between the proliferation and differentiation of neural stem cells through the TLX nuclear receptor, which forms a feedback regulatory loop in the mouse (65, 98). Cerebral ischemia substantially downregulates Let-7 and miR-964. Wnt/ $\beta$ -catenin signaling represses Let 7 in tumor cells, while miR-92 regulates BMP signals (99, 100). However, it remains to be determined whether the coupling of stroke-altered miRNAs with the signaling pathways mediates proliferation and differentiation of neural progenitor cells in stroke-induced neurogenesis.

### **Future Perspectives**

Individual signaling pathways likely affect a cluster of miRNAs that subsequently trigger or repress other pathways. The intertwined interaction of miRNAs and signaling pathways in regulating neural stem cell functions has provided new insights into how miRNAs function during adult neurogenesis under normal and ischemic conditions. Ultimately, further identification of miRNA function in neural stem cells in the adult brain may provide a novel avenue for development of miRNA-based therapies for treating diseases of the CNS. Additional studies are warranted to examine whether the preclinical findings in rodents described in this review can be translated into clinical applications in humans.

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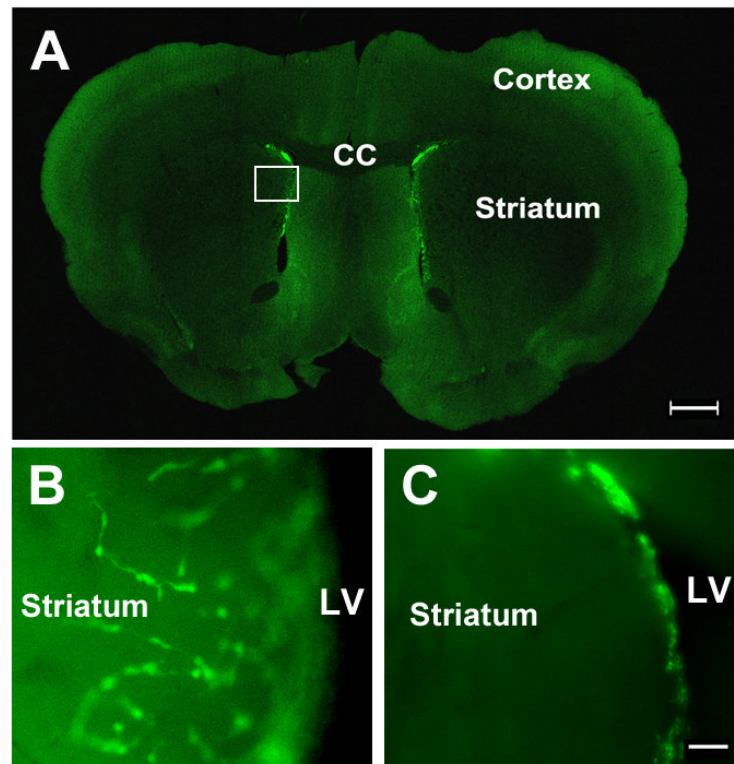
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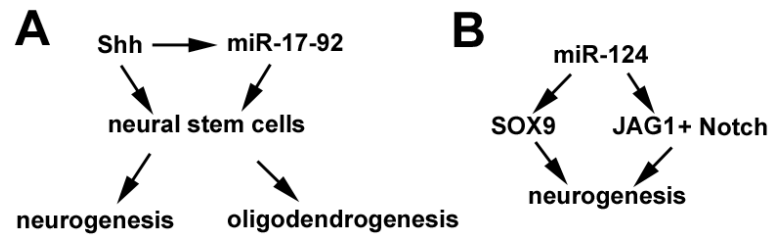
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**Figure 1.** Time-lapse microscopic images of coronal brain slices obtained from doublecortin–green fluorescent protein (DCX–eGFP) transgenic mice. **(A)** Coronal section showing DCX–eGFP–expressing neuroblasts (green) in the subventricular zone (SVZ) of the lateral ventricle. **(B)** DCX–eGFP neuroblasts (green) emigrated laterally from the SVZ to the ischemic striatum in an ischemic brain slice. **(C)** DCX–eGFP neuroblasts (green) are localized in the SVZ in a non-ischemic brain slice. The boxed area in panel **A** indicates the location of panel **B**. CC, corpus callosum; LV, lateral ventricle. Bars: **A** = 1 mm; **C** = 50  $\mu$ m.



**Figure 2.**

(A) Schematic representation of miR-17-9 cluster and the sonic hedgehog (Shh) signaling pathways in mediating processes of neurogenesis and oligodendrogenesis. (B) The miR-124 and Notch signaling pathways involved in neurogenesis.