

The Progenitor Cells of the Embryonic Telencephalon and the Neonatal Anterior Subventricular Zone Differentially Regulate their Cell Cycle

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Abstract

For the last 10 years our laboratory has been studying the proliferation, migration and differentiation of neuronal progenitor cells located in the anterior part of the postnatal forebrain subventricular zone (SVZa). SVZa-derived cells possess a number of proliferative characteristics that distinguish them from the other progenitor cells in the central nervous system. This review summarizes our recent findings, in which we compared the pattern of cell cycle inhibitory proteins expressed by the neonatal SVZa to that of telencephalic ventricular zone cells.

Introduction

Most neurons of the mammalian forebrain arise prenatally from progenitor cells within the ventricular zone (VZ) and subventricular zone (SVZ) that line the lateral ventricles. The immature neurons generated at the ventricular surface migrate through the overlying intermediate zone (IZ) to reach their destinations in the cortical plate (CP). Once the immature neurons of the VZ cease proliferation and begin to differentiate and migrate, they remain forever postmitotic.

Not all neural progenitor cells of the central nervous system (CNS) obey the order of ‘proliferation arrest prior to migration and differentiation’. The neuronal progenitor cells, which concurrently undergo division while they migrate, even though they express a neuronal phenotype, are located within a distinct region of the anterior part of the postnatal forebrain subventricular zone [the SVZa (Luskin, 1993; Menezes *et al.*, 1995)]. SVZa-derived cells migrate along a highly restricted pathway, the rostral migratory stream (RMS), to reach the subependymal zone in the middle of the olfactory bulb. Subsequently, SVZa-derived progenitor cells migrate radially to their final destinations in the granule cell and glomerular layers, where they become postmitotic interneurons. Thus, unlike the progenitor cells of the embryonic telencephalic VZ, SVZa-derived cells initiate differentiation without becoming postmitotic.

The decision to proliferate or become postmitotic is made in the G₁ phase of the cell cycle, where intrinsic and extrinsic signals come together to act on different groups of proteins that inhibit or facilitate cell cycle progression (Sherr, 1994). Transition from G₁ to S phase is negatively regulated by two families of cyclin-dependent kinase inhibitors (CKIs): the

CIP/KIP family (including p21^{CIP1}, p27^{KIP1} and p57^{KIP2}) and the INK4 family (including p15^{INK4b}, p16^{INK4a}, p18^{INK4c} and p19^{INK4d}) (Elledge and Harper, 1994). Among these CKIs, p18^{INK4c}, p19^{INK4d} and p27^{KIP1} are expressed in the CNS during development (Zindy *et al.*, 1997; van Lookeren Campagne and Gill, 1998) and may play pivotal roles governing neurogenesis.

As indicated above, SVZa-derived cells can undergo cell division despite the initiation of differentiation and migration, unlike the progenitor cells of the embryonic telencephalic VZ. This unique property of the SVZa cells prompted us to examine whether they regulate their cell cycle in a different manner from other CNS progenitors. To determine whether the differential regulation of the G₁–S progression can account for the unusual proliferative behavior of SVZa progenitor cells, we have compared the pattern of p19^{INK4d} expression by the cells of the neonatal SVZa to that of telencephalic VZ cells.

p19^{INK4d} is expressed by postmitotic immature neurons of the telencephalic VZ

VZ progenitors of the developing cerebral cortex withdraw from the cell cycle at the ventricular surface, and the newly generated postmitotic neurons initiate differentiation and migrate to their final destinations in the CP (Bayer *et al.*, 1991). Our staining of the developing cerebral cortex with an antibody to p19^{INK4d} revealed that the newly generated neurons at the ventricular surface express p19^{INK4d}, while the progenitor cells that are in the S phase at the basal border of the VZ are devoid of p19^{INK4d} immunoreactivity (Coskun and Luskin, 2001). This suggests that p19^{INK4d}

plays a role in regulating the timely withdrawal of VZ progenitors from the cell cycle.

Based on the $p19^{\text{INK4d}}$ expression pattern, the VZ can be divided into a $p19^{\text{INK4d}}(-)$ upper (VZu) and $p19^{\text{INK4d}}(+)$ lower (VZl) subdivision. Furthermore, our experiments demonstrated that $p19^{\text{INK4d}}$ expression is down-regulated

while the cells ascend through VZu and reactivated upon entering the IZ. Migrating cortical neurons in the IZ start to express $p19^{\text{INK4d}}$ in a punctate manner in the apical domain of the cell at the cytoplasmic–nuclear border, while the neurons of the CP express $p19^{\text{INK4d}}$ in a more diffuse pattern (i.e. crescent shaped) (Figure 1C).

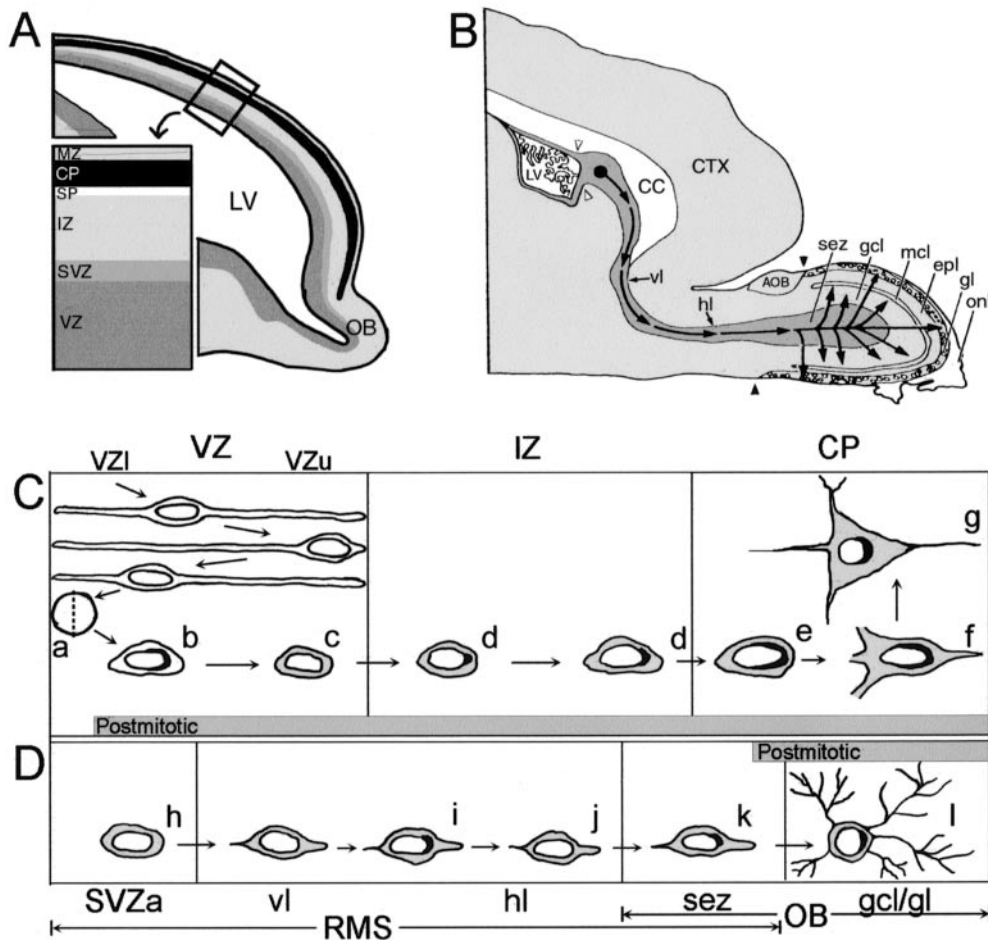


Figure 1 Summary diagram depicting the subcellular changes of $p19^{\text{INK4d}}$ expression by VZ cells compared with SVZa cells as they migrate to their final destinations. **(A)** Sagittal view of the embryonic telencephalon illustrating the laminar subdivisions of the developing cerebral cortex. Immature neurons of the VZ migrate through the layers of the telencephalon to reach the CP, where they complete their differentiation. **(B)** Sagittal view of the neonatal forebrain illustrating the migratory path of the SVZa-derived cells. The neurons that arise in the SVZa (solid black circle) migrate along the RMS to reach the olfactory bulb, where they differentiate into interneurons. The set of open arrowheads demarcates the border between the neurogenic SVZa and gliogenic posterior portion of the SVZ. The set of closed arrowheads indicates the caudal border of the main olfactory bulb. **(C)** Expression of $p19^{\text{INK4d}}$ during the generation of a typical cortical neuron of the embryonic telencephalon. After VZ progenitor cells divide and withdraw from the mitotic cycle at the ventricular surface (a), the postmitotic progeny starts expressing $p19^{\text{INK4d}}$ (black) at the cytoplasmic–nuclear border in the VZl (b). $p19^{\text{INK4d}}$ expression is down-regulated as the migrating neurons pass through the VZu (c) and is re-expressed in a punctate manner upon entering the IZ (d). Furthermore, within the CP, the $p19^{\text{INK4d}}$ expression persists in the differentiating neurons (e–g) in a more diffuse manner, with a perinuclear crescent-shaped pattern. Gray shading represents expression of neuron-specific markers (Menezes and Luskin, 1994). **(D)** Expression of $p19^{\text{INK4d}}$ during the generation of a typical interneuron of the neonatal olfactory bulb. Unlike the cells of the telencephalic VZ, the SVZa progenitor cells and their progeny are immunoreactive for the neuron-specific antibody TuJ1 (gray shading) at virtually all times in the RMS (h–l). The SVZa progenitor cells exhibit only negligible amounts of $p19^{\text{INK4d}}$ in the SVZa (h). However, when in the proximal portion of the RMS, the TuJ1(+) SVZa-derived cells start to express $p19^{\text{INK4d}}$ at their apical pole while *en route* to the olfactory bulb (i). Subsequently, SVZa-derived cells down-regulate their expression of $p19^{\text{INK4d}}$ and re-enter the cell cycle to undergo another round of cell division in the RMS (j). When in the olfactory bulb, the SVZa-derived cells terminally exit the cell cycle and migrate into one of the overlying cellular layers of the olfactory bulb. The $p19^{\text{INK4d}}$ persists in the postmitotic olfactory bulb interneurons within the gcl and gl (k and l). AOB, accessory olfactory bulb; CC, corpus callosum; CP, cortical plate; CTX, cerebral cortex; epl, external plexiform layer; gcl, granule cell layer; gl, glomerular layer; hl, horizontal limb of the RMS; IZ, intermediate zone; LV, lateral ventricle; mcl, mitral cell layer; MZ, marginal zone; OB, olfactory bulb; RMS, rostral migratory stream; onl, olfactory nerve layer; sez, subependymal zone; SP, subplate; SVZa, anterior part of the neonatal subventricular zone; vl, vertical limb of RMS; VZ, ventricular zone; VZl, lower portion of the ventricular zone; VZu, upper portion of the ventricular zone.

The suggestion has been put forward that CKIs not only coordinate the cell cycle exit of the progenitor cells, but also maintain the postmitotic cells in a quiescent state (Zindy *et al.*, 1999). In agreement with this notion, our data revealed that p19^{INK4d} expression persists in the differentiating postmitotic neurons of the cerebral cortex. This persistent expression of p19^{INK4d} in quiescent cells suggests that p19^{INK4d} prevents postmitotic cells from re-entering the proliferative cycle; the neurons in p19^{INK4d}-p27^{KIP1} double knock out mice undergo extra rounds of cell division in regions where only quiescent neurons reside in wild type animals (Zindy *et al.*, 1999). Collectively, our results revealed that in the developing telencephalon, p19^{INK4d} is expressed mainly by the postmitotic immature neurons and has a characteristic perinuclear distribution that is correlated with a cell's laminar position and state of differentiation.

SVZa-derived cells down-regulate p19^{INK4d} expression in the RMS and re-enter the cell cycle

In order to determine whether the pattern of p19^{INK4d} expression by SVZa neuronal progenitor cells can account for their unusual proliferative behavior, we analyzed the spatiotemporal expression pattern of p19^{INK4d} by the cells of the rodent RMS. Our data revealed that SVZa-derived cells exhibit an anterior^{high}-posterior^{low} gradient of p19^{INK4d} expression along the RMS (Coskun and Luskin, 2001). Detection of progressively more p19^{INK4d}-immunoreactive cells in the RMS as the olfactory bulb is approached indicates that few cells withdraw from the cell cycle in the SVZa and increasingly more do so as they reach the bulb.

To reconcile the observation that cells of the SVZa/RMS undergo division as they migrate and that SVZa-derived cells in the proximal portion of the RMS initiate p19^{INK4d} expression (usually indicative of a postmitotic cell), we investigated the hypothesis that SVZa cells may down-regulate the expression of p19^{INK4d} and re-enter the cell cycle to undergo another round of cell division before reaching the subependymal zone. To test this hypothesis, we administered the proliferation marker bromodeoxyuridine (BrdU) to rat pups at 3 and 9 h before their perfusion and examined the expression of p19^{INK4d} by the BrdU(+) cells along the RMS. We observed that at 3 h following BrdU administration, very few SVZa-derived cells along the RMS co-localize BrdU and p19^{INK4d}. However, at 9 h following BrdU administration, a significant fraction of the BrdU(+) cells were immunoreactive for both BrdU and p19^{INK4d}. Taken together, these findings indicate that SVZa-derived cells in the RMS successively down-regulate their p19^{INK4d} expression prior to undergoing division, which may enable them to repeatedly exit and re-enter the cell cycle. Consistent with the idea that p19^{INK4d} expression persists in postmitotic cells to maintain them in a quiescent state, postmitotic

neurons at their final destinations in the olfactory bulb were also p19^{INK4d}-immunoreactive.

Conclusions and future directions

In conclusion, based on the p19^{INK4d} immunoreactivity and cell cycle kinetics, SVZa-derived cells appear to successively exit and re-enter the cell cycle, despite expressing a neuronal phenotype. This is in contrast to telencephalic VZ cells, in which p19^{INK4d} expression is initiated even before they undergo differentiation. Since the SVZa-derived cells in the RMS appear to repeatedly down-regulate p19^{INK4d} expression, this might indicate that SVZa cells continue to undergo multiple rounds of de-differentiation and division. Therefore, the unique proliferation characteristics of the SVZa-derived cells can, to some extent, be attributed to the dynamic regulation of p19^{INK4d} expression.

It had been suggested that different members of the CIP/KIP and INK4 families might cooperate to ensure timely cell cycle exit of proliferating cells (Thullberg *et al.*, 2000). In order to determine the cooperative interactions between the CKIs in the regulation of SVZa cell proliferation and differentiation, our future experiments will determine which other CKIs are expressed by SVZa cells in addition to p19^{INK4d}. We have also initiated studies to determine the extracellular signaling molecules that act on CKIs to regulate the proliferation of SVZa-derived cells (Coskun *et al.*, 2001).

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