

Contribution of Intermediate Progenitor Cells to Cortical Histogenesis

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The mammalian cerebral cortex is the most cellularly complex structure in the animal kingdom. Almost all cortical neurons are produced during a limited embryonic period by cortical progenitor cells in a proliferative region that surrounds the ventricular system of the developing brain. The proliferative region comprises 2 distinct zones, the ventricular zone, which is a neuroepithelial layer directly adjacent to the ventricular lumen, and the subventricular zone, which is positioned superficial to the ventricular zone. Recent advances in molecular and cell biology have made possible the study of specific cell populations, and 2 cortical progenitor cell types, radial glial cells in the ventricular zone and intermediate progenitor cells in the subventricular zone, have been shown to generate neurons in the embryonic cerebral cortex. These findings have refined our understanding of cortical neurogenesis, with implications for understanding the causes of neurodevelopmental disorders and for their potential treatment.

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The mature brain is composed of 100 billion to 200 billion neurons and perhaps 10 times as many glial cells. Generation of the 1 trillion diverse, complex cells that regulate every aspect of behavior is accomplished in human beings during a brief span of just 3 to 4 months. This critical period of gestation is sensitive to interference from environmental, pathogenic, and genetic factors, and defects in proliferation at this stage of development can produce severe cortical malformations such as lissencephaly. We review the current state of understanding of cortical progenitor cells in the embryonic cerebral cortex.

The principal cell types in the brain, neurons and glia, are generated in the proliferative zones that surround the ventricles, after which they migrate into the overlying cortical mantle. Neuroanatomists in the late 19th century noted the presence of mitotic cells at the surface of the ventricular lumen in the embryonic cerebral cortex and inferred this to be the site of neurogenesis.¹ Wilhelm His called the surface mitoses *germinal cells*, but today they are known as *radial glial cells* that re-

side in the ventricular zone (VZ) during cortical development.² A second population of mitotic cells that divide away from the ventricular lumen was also identified in 19th-century studies.¹ The nonsurface progenitor cells were recognized as distinct from surface dividing progenitor cells on the basis of distinguishing morphological characteristics and were presumed to be offspring of germinal cells that had migrated away from the ventricle,¹ but their function in the embryonic brain remained elusive for more than 100 years. Nonsurface cells, or adventricular mitotic cells, have been called *extraventricular cells*,¹ *subependymal cells*,³ and *subventricular zone (SVZ) cells*, but for the purposes of this review we use the current term *intermediate progenitor (IP) cells* (**Figure**).⁴

ORIGINS OF CORTICAL PROGENITOR CELLS

After the neural tube closes, neuroepithelial cells begin expressing markers such as vimentin and nestin, marking the point at which radial glial cells appear in the cerebral cortex. Whether neuroepithelial cells represent truly unique neural stem cells that generate a distinct population of radial glial cells

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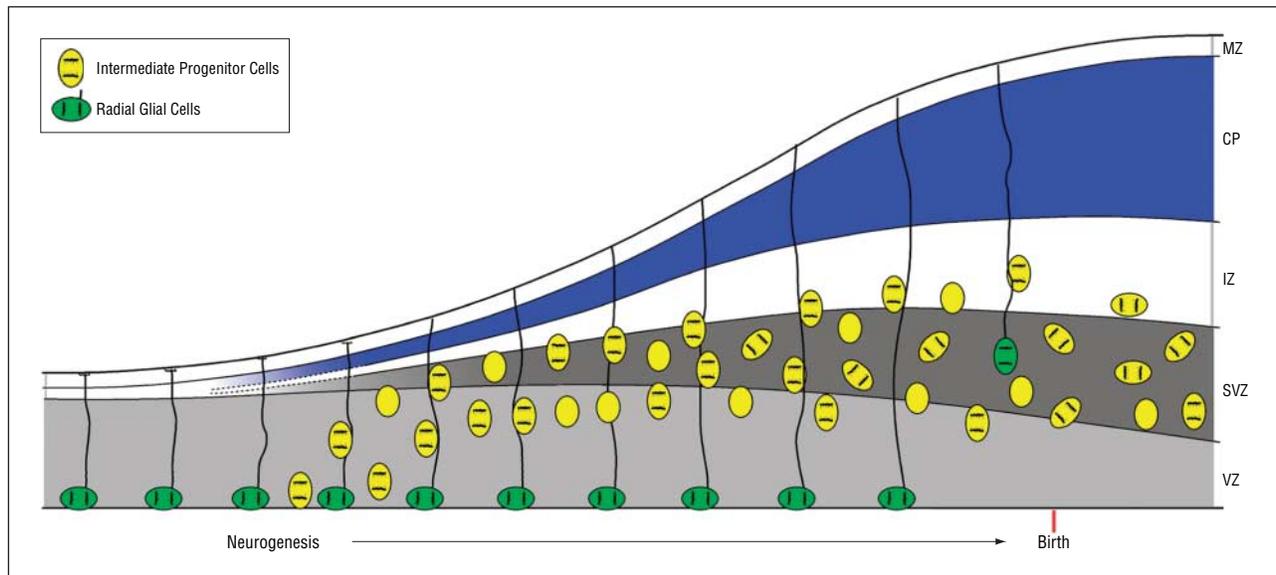


Figure. Scheme shows the location of radial glial cells and intermediate progenitor cells in the developing cerebral cortex. Radial glial cells (green) are located in the ventricular zone (VZ, light gray) and divide at the surface of the ventricle. Radial glial cells have key morphological features such as the ascending pial fiber. The cerebral cortex is formed almost entirely by radial glial cells in the VZ at early stages of cortical development. Intermediate progenitor cells (yellow) are generated by radial glial divisions in the VZ and migrate away from the ventricle to form the subventricular zone (SVZ, dark gray). Intermediate progenitor cells are concentrated in the SVZ and divide away from the ventricle; most do not contact the ventricular or pial surfaces of the developing brain. Both radial glial cells and intermediate progenitor cells produce neurons that migrate into the forming cortical plate (CP) (blue). After cortical neurogenesis is complete, the VZ shrinks to a single layer of ependymal cells, but intermediate progenitor cells in the SVZ persist after birth into adulthood. IZ indicates intermediate zone; MZ, marginal zone.

through division or whether neuroepithelial cells initiate the expression of new proteins at the onset of cortical neurogenesis remains to be determined. During early stages of cortical development, the cerebral cortex is composed almost entirely of proliferative radial glial cells that divide at the ventricular surface. At the onset of neurogenesis, radial glial cells begin generating both IP cells and cortical neurons, which migrate away from the ventricle. Neurons migrate to a superficial position to form the cortical mantle and IP cells migrate away from the ventricular surface and establish the SVZ as a distinct proliferative layer superficial to the VZ. The IP cells are mostly concentrated in the SVZ, but they are also distributed throughout the upper VZ and the lower intermediate zone.³⁻⁵ The SVZ is initially seeded by IP cells generated by radial glial cells, but at later stages of cortical development the SVZ progenitor cell pool may expand through symmetric proliferative IP cell divisions.⁴ In addition, progenitor cells from the ventral telencephalon may migrate into the dorsal cortex to contribute to the cortical SVZ progenitor pool. Thus, from the midstages of cortical neurogenesis onward, the size of the SVZ expands. In contrast, the VZ reaches its peak size in the midstages of neurogenesis, after which it begins to shrink. When cortical neurogenesis is complete, radial glial cells transform into astrocytes and exit the VZ, which thins to a single layer of ependymal cells in the postnatal cortex.² As a result, the proliferative IP cells become a progressively larger component of the cortical progenitor cell pool and represent the majority of mitotic progenitor cells by end stages of embryonic neurogenesis.¹ Furthermore, while only a single layer of VZ cells remain in postnatal animals, large numbers of mitotic IP cells are present in the SVZ in postnatal animals and persist into adulthood.

SPECIAL CHARACTERISTICS OF EMBRYONIC CORTICAL PROGENITOR CELLS

Radial glial cells and IP cells have distinguishing morphological features, behavioral characteristics, and protein expression patterns that set them apart. Radial glial cells are bipolar pseudostratified epithelial cells with a cell body located in the VZ, a single descending process that contacts the ventricular lumen, and a long thin ascending process with multiple endfeet that contributes to the glia limitans directly under the pia mater of the developing brain. Radial glia appear to maintain contact with the ventricular and pial surfaces throughout cortical neurogenesis and also make contact with blood vessels. Radial glial cells exhibit an up-and-down interkinetic nuclear movement during the cell cycle such that the nucleus moves away from the ventricle during G1 phase, enters S phase at the top of the VZ, reapproaches the ventricle during G2 phase, and passes through M phase at the ventricular surface. Radial glia express a variety of proteins and transcription factors such as vimentin, nestin, and *Pax6* that can be used to identify these cells. In addition, radial glia express many phosphorylated proteins during the M phase, such as phosphorylated vimentin, phosphorylated growth-associated protein-43 (human monoclonal antibody 2G12), and phosphorylated histone H3. These phosphorylated markers label the cell body of the mitotic radial glial cells and also a portion of the ascending pial fiber, which remains intact throughout mitosis.⁴ These morphological characteristics, combined with the known position of mitotic radial glial cells at the ventricular surface, allow for their rapid identification with M-phase markers.

Intermediate progenitor cells are multipolar cells primarily located in the SVZ that do not appear to maintain contact with either the ventricular or pial surfaces. These cells are not static but extend and retract multiple processes.⁴ It has not been confirmed whether they contact neighboring blood vessels, as do radial glial cells, but IP cells are not present in the cortex until after blood vessels have invaded the cortical wall.¹ In addition to distinct morphological features, IP cells also exhibit key behavioral differences from radial glial cells. The IP cells do not undergo interkinetic nuclear migration, as do radial glial cells, but pass through the phases of the cell cycle away from the ventricle.⁵ Intermediate progenitor cells express some of the same markers that are expressed by radial glial cells, particularly M-phase markers such as phosphorylated vimentin, phosphorylated growth-associated protein-43, and phosphorylated histone H3. Despite this similarity, most IP cells can be differentiated from radial glia on the basis of their abventricular position during mitosis though some can divide at or near the ventricular surface. In addition, several transcription factors have recently been described that specifically label IP cells, including *Svet1*⁶ and *Tbr2*.⁷

FUNCTIONS OF CORTICAL PROGENITOR CELLS

Although the location of mitotic cells in the embryonic cortex was noted in the late 19th century, the exact identity of neuronal progenitor cells remained obscure until recent advances in molecular biology made it possible to label and characterize specific populations of cells in the embryonic brain. Radial glial cells serve as migratory guides for young cortical neurons as they traverse the relatively long distance from the proliferative zones near the ventricle to the developing cortical plate.² Migrating neurons maintain a close relationship with the pial fibers of radial glial cells, mediated by adhesion molecules, and migrate radially into the overlying cortex.² Recent experiments have used technical advances to label progenitor cells with fluorescent reporter genes that permit detailed lineage studies and have demonstrated that radial glial cells also function as neuronal progenitor cells.⁸ Thus, radial glia serve to generate and guide the migration of their own daughter cells, highlighting the critical relationship between cortical neurons and their parent radial glial cells. In addition to generating neurons, recent experiments have shown that radial glial cells also generate IP cells,⁴ as had been inferred in early studies of the nervous system.¹ Therefore, radial glial cells produce neurons both directly and indirectly through IP cells and guide their radial migration to the cortical plate. These new findings show that radial glial cells are crucially involved in cytogenetic and histogenetic processes during gestation and, because they can self-renew and produce neurons, astrocytes, and oligodendrocytes, may be classified as neural stem cells.

Although IP cells had been identified and characterized previously,^{1,3,5} their contribution to the histogenesis of the embryonic cerebral cortex remained elusive. Birth-dating experiments suggested that IP cells produce neurons in the postnatal SVZ, but because new neurons were not found in the overlying cerebral cortex, it was pre-

sumed that the newborn neurons degenerated within the SVZ.³ Thus, IP cells were commonly thought to be glial progenitor cells, largely because glial cells are generated in the postnatal SVZ.^{3,9} However, Lois and Alvarez-Buylla¹⁰ later demonstrated that progenitor cells in the postnatal and adult SVZ generate neurons that migrate to the olfactory bulb. Recent studies have shown that IP cells in the embryonic SVZ also generate cortical neurons,^{4,6} and may generate all excitatory neurons that are destined for the upper cortical layers.⁶ Thus, IP cells in the SVZ provide an important contribution to cortical neurogenesis both during embryonic stages of development and in adulthood.

FUTURE DIRECTIONS

These recent findings have refined our understanding of cortical neurogenesis, but many important questions remain unanswered. For example, the lineage relationship between cortical neurons that are generated in the embryonic SVZ and glial cells that are generated in the postnatal SVZ remains to be established. Evidence indicates that separate lineages of neuronal and glial progenitor cells exist side by side in the VZ during embryonic stages of cortical development.² However, cortical neurogenesis is completed before the onset of gliogenesis in the early postnatal period, which raises questions about the function of glial-restricted progenitor cells in the embryonic cortex. Cortical astrocytes and excitatory cortical neurons are both found within the *Emx1* gene expressing lineage.¹¹ In addition, retroviral lineage studies have shown that cortical neurons and astrocytes are found together in some cortical clones.¹¹ These data, together with the known time line of neurogenesis and gliogenesis in the developing cortex, suggest that populations of cortical progenitor cells progress through specific stages of cell production, generating cortical neurons first and astrocytes later.

Whether a lineage relationship exists between neurons generated in the SVZ during embryonic stages of cortical development, postnatal stages of development, and in adulthood has not been determined. Are the olfactory interneurons that are generated in the adult SVZ lineally related to the excitatory pyramidal neurons generated in the embryonic SVZ? Cortical interneurons are generated by progenitor cells that reside in the medial ganglionic eminence of the ventral telencephalon and migrate tangentially into the cerebral cortex via several routes, including the SVZ.⁸ Is it possible that the medial ganglionic eminence sends both interneurons and progenitor cells to the SVZ of the embryonic cerebral cortex? The relationship between cortical interneurons generated in the medial ganglionic eminence of embryonic cortex and olfactory interneurons generated in the SVZ of postnatal cortex should be determined. Examining lineage relationships among progenitor cells in the embryonic and adult SVZ may yield important clues about the factors that regulate the proliferative behavior of these progenitor cell types. Furthermore, if it is determined that adult SVZ progenitor cells are descended from embryonic IP cells, the number of cell types produced by SVZ progenitor cells would expand to include both excitatory and inhibitory neurons, as well as astrocytes. This would suggest that SVZ progenitor cells possess a stem cell-like potential to generate multiple cell types in the cerebral cortex and

that this potential persists into adulthood. The embryonic SVZ is host to several important cell types in the developing cortex, such as IP cells, tangentially migrating interneurons, olfactory bulb neuronal progenitor cells, and glial progenitor cells. The confluence of important developmental processes in the SVZ suggests that this region of the developing brain is an important developmental niche containing important regulatory signals.

Another intriguing question that remains to be addressed is why radial glial cells in the VZ undergo interkinetic nuclear migration and IP cells in the SVZ do not. It has been suggested that the peculiar behavior of radial glial cells may be the by-product of spatial constraints at the ventricular surface.³ In this model, the movement of G1-phase radial glial cells away from the ventricle is simply an accommodation that makes room for M-phase radial glial cell bodies at the ventricular surface that swell to nearly twice their normal size during mitosis. The thickness of the VZ is correlated with the number of radial glial progenitor cells present in that layer, but it could also be correlated with the length of the radial glial cell cycle. Takahashi et al⁵ found that the VZ cell cycle becomes progressively longer as cortical neurogenesis proceeds but that the length of the SVZ cell cycle does not change. During early stages of neurogenesis, the thickness of the VZ expands to include an increased number of radial glial cells but the surface area at the ventricle remains relatively unchanged. Thus, as the VZ cell population swells, radial glial cells may have to wait a progressively longer time for their turn to undergo division at the ventricular surface, which could contribute to the longer cell cycle times observed by Takahashi et al.⁵ If spatial considerations in the VZ influence mitosis, one might expect cell cycle times to become shorter as the VZ becomes thinner at the end of neurogenesis, a finding that has been reported in primate neocortex.¹²

Do the different behaviors exhibited by radial glial cells and IP cells influence cortical development? Studies have shown that IP cells and radial glial cells adopt different modes of division, with IP cells more likely to undergo symmetric division and radial glial cells more likely to undergo asymmetric division.⁴ Furthermore, IP cells seem to be more restricted in their potential to generate different cell types than are radial glial cells.⁴ The distinct morphological features and behavioral differences exhibited by radial glial cells in the VZ and IP cells in the SVZ suggest that environmental factors unique to each of these distinct niches may provide access to different sets of signaling factors.

CLINICAL CONSIDERATIONS

Defects in proliferation produce cortical malformations that range from subtle, ectopic clusters of cortical neurons to the profound cortical disorganization observed in lissencephaly or anencephaly. The proliferation of cortical progenitor cells is tightly regulated and most likely relies on numerous signaling factors. The identification of cortical progenitor cells in the developing cortex is a critical step toward understanding how their proliferative behavior is regulated. Future studies should help identify specific factors that regulate proliferation and also identify signals that might prematurely shut down or terminate progenitor cell proliferation. Such studies could identify genetic or envi-

ronmental risk factors linked to defects in cortical proliferation. Understanding the lineage relationships between cortical progenitor cells and their potential to generate different cortical cell types may also increase our understanding of the etiology of neurodevelopmental disorders by pinpointing cell-signaling pathways associated with specific progenitor cell populations. Elucidating the full potential of cortical progenitor cells in the embryo and the adult and understanding the factors that guide the production of specific cell types will also be essential for potential treatments of neurodegenerative diseases through cell replacement strategies.

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