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Glioma migration: clues from the biology of neural progenitor cells and embryonic CNS cell migration

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Summary

Neural stem cells have recently come to the forefront in neurobiology because of the possibilities for CNS repair by transplantation. Further understanding of the biology of these cells is critical for making their use in CNS repair possible. It is likely that these discoveries will also have spin-offs for neuro-oncology as primary brain tumors may arise from a CNS progenitor cell. An understanding of the normal migratory ability of these cells is also likely to have a very important impact on the knowledge of brain tumor invasion.

Introduction

Until recently, the brain was thought to be incapable of self-renewal. All neurons in the brain were thought to be born during embryogenesis or shortly after. In the past decade, evidence now strongly supports the existence of neural stem cells in the postnatal mammalian brain. Neural stem cells have capacity for self-renewal, proliferation, and generation of differentiated progeny that make up the primary cell types of the brain, neurons, astrocytes, and oligodendrocytes. These stem cells are thought to exist through adulthood in the subventricular zone along the entire neuraxis. These cells have been extensively studied in vertebrates such as fish, amphibians, songbirds, and rodents; but they have also been recently isolated from humans. In addition to proliferation for the generation of cell numbers of the brain parenchyma during embryonic development, the progenitor cells of the developing brain undergo migration over very long distances.

The properties of a neural stem cell or a progenitor cell, namely proliferation, self-renewal, and migration, are reminiscent of qualities of a neoplastic neural or glial cell. The recent most exciting advances in cancer research have come from recognizing that developmental signaling pathways are perturbed in cancer cells [1]. Indeed, probably the most significant understanding of the molecular pathogenesis of CNS tumors in the past decade has come from the discovery that medulloblastomas arise from a disturbance in the sonic hedgehog (shh) developmental signaling pathway. Heterozygous knockouts of the patched 1 gene, a component of the sonic hedgehog receptor, develop medulloblastoma [2]. Mutations in patched 1 occur in 10–20% of sporadic medulloblastomas [3,4]. Subsequently, studies of shh signaling in the developing cerebellum exquisitely explained how this could come about. Shh, produced by Purkinje cells, acts on external granule cells of the immature cerebellum to increase their proliferation and prevent their terminal differentiation [5]. Loss of one patched allele in the knockout mice presumably leads to increased Shh signaling in the external granule cell. Such oncological discoveries made in the context of aberrant developmental biological processes are leading to further intense study by cancer researchers of the normal developmental biology of tissues associated with hematologic and solid malignancies. The focus of this review will be to briefly discuss the properties of neural stem cells and to review some aspects of normal CNS migration as well as briefly discuss molecules involved in the migration of developing CNS cells. It is conceivable that the stem cell-like properties of brain tumor cells may also include the capacity to migrate, and a better understanding of the biology of the motility and migration of progenitor cells in the CNS may help us to better understand the invasive or migratory nature of brain tumor cells.

Neural stem cells

Our present conceptual understanding of stem cells and their relationship to tissue development have been derived mainly from studies of hematopoietic development. A stem cell has the ability to proliferate, undergo self-renewal, generate differentiated progeny, and repair the tissue in which it resides [6,7]. There remains considerable controversy about the actual criteria for definition of a stem cell in different tissues [8,9], although self-renewal and the ability to generate differentiated progeny are generally agreed upon [8]. Furthermore, an absolutely clear distinction between a 'stem cell' and a 'progenitor cell' (a cell with more restricted lineage potential) so far remains elusive, especially in the nervous system.

Despite arguments over a precise definition of a stem cell, the existence of a stem cell in the CNS is now widely accepted, although the identity of *the* exact stem cell has been elusive [8–13]. There is some general agreement, however, that the CNS stem cell is most abundantly found in the brain regions immediately adjacent to the ventricle (the subventricular zone (SVZ), see Figure 1) [11–20]. A clear distinction between the CNS 'stem cells' and 'progenitor cells' is incomplete [9], partly because there are fewer useful markers to identify CNS stem and progenitor cells compared to the hematopoietic system. Currently, the best marker for uncommitted CNS cells *in vitro* and *in vivo* is expression of the nestin intermediate filament protein.

The brain develops from a population of proliferating primitive neuroepithelial cells that line the ventricular zone (VZ) of the developing brain and neural tube. These pluripotential neuroepithelial cells (CNS stem cells) give rise to both neuronal and glial cell types (astrocytes and oligodendrocytes). CNS stem cells are identified by expression of the intermediate filament protein nestin [21–23]. Nestin is expressed during the earliest steps of neural plate induction in embryogenesis [24] and before the onset of neurogenesis [25]. Nestin is most widely expressed at the peak of neuroepithelial proliferation, embryonic day 10.5 in the mouse, and is present in only a small number of cells by birth [21]. Terminal differentiation of neurons and astrocytes *in vivo* and *in vitro* leads to down-regulation of nestin and expression of distinct lineage-specific markers [26]. Consequently, nestin expression is retained in only a small population of cells in the mouse brain by postnatal day 1 as most stem cells in the ventricular zone of the developing brain have differentiated. Nestin expression persists postnatally in external granule cells

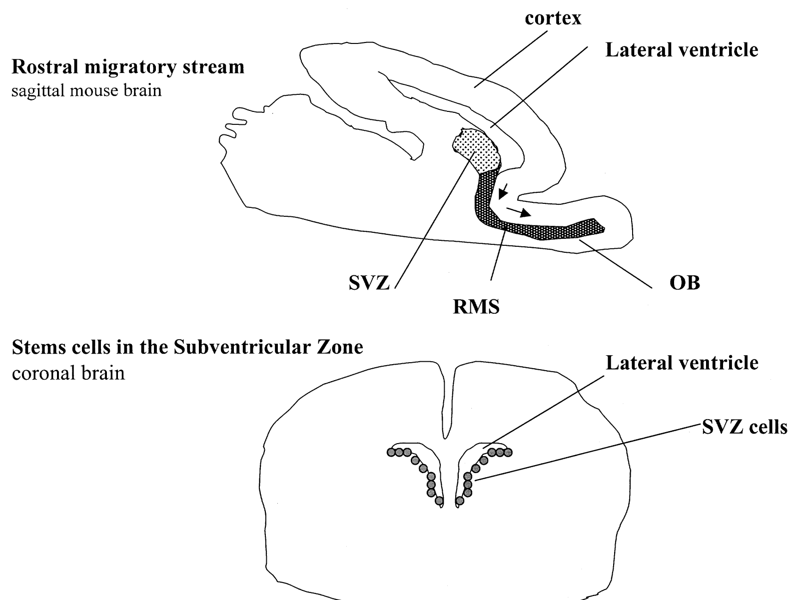

Figure 1. The upper panel depicts a parasagittal section of a mouse brain demonstrating the rostral migratory stream (RMS), where neural precursors which emerge from the proliferative subventricular zone (SVZ) migrate to the olfactory bulb (OB). The lower panel depicts a coronal section of the brain with the location of the SVZ.

of the cerebellum, which proliferate for 2 weeks after birth in the mouse [27] and in the adult mouse brain in cells adjacent to the ventricles (the SVZ) [12,16]. This region has long been identified by labeling studies to demonstrate a proliferative capacity into adulthood in mammalian species from mouse to humans and these properties persist throughout life [28–36].

Cells with properties of stem cells have been identified in the embryonic and adult mammalian brain which can be propagated *in vitro* under specific growth factor conditions [11,12,14,16,19,26,37–40]. Like their embryonic counterparts, the adult-derived CNS stem cells are capable of proliferation, self-renewal, and differentiation into neurons, astrocytes, and oligodendrocytes. In rodents, CNS stem cells are thought to be most abundant in the SVZ of the lateral ventricle in the forebrain [17], but can also be isolated from the SVZ of the entire neuraxis. Cells with *in vitro* stem cell properties can be still isolated from 24-month-old (aged) mice [41]. Proliferative hippocampal progenitors *in vivo* seem to be a dynamic population and are capable of responding to mental activity [42,43], hormonal changes [44,45], stress [46], exogenous administration of growth factors [47], and even exercise [48,49]. The exact identity of the adult mammalian CNS stem cell has not yet been pinpointed and may be an ependymal cell, a SVZ astrocyte, a radial glial cell, or a different cell in the SVZ [11–13,50–53]. It is not clear whether *in vivo* it is an undifferentiated or a differentiated cell that is the stem cell. A differentiated cell may be able to trans-differentiate into other cell types. Most studies of neural stem cells have focused on studies of rodent brains, but there is good evidence to suggest that these cells also exist in primates and humans. Recent studies have described isolation of cells with stem cell properties *in vitro* from the post-mortem human adult brain or from neurosurgical brain specimens [54–57]. In these human studies, the progenitor cells were most easily isolated from younger subjects and from the region adjacent to the ventricle or from the hippocampus.

Stem cells are also capable of migration in the postnatal CNS. In neonatal and adult rodents, cells from the SVZ migrate along a defined pathway called the rostral migratory stream (RMS, see Figure 1) [19,58–62]. These cells migrate up to 8 mm from the SVZ to reach the olfactory bulb where they differentiate into neurons [12,60,63]. Many studies of migration of CNS cells in the mammalian brain have involved an analysis of the migration in this pathway. Insults to the brain can modify the migration of SVZ cells. Interestingly, in response to a spinal cord injury, nestin positive cells are seen to migrate away from the spinal cord central canal towards the site of injury, suggesting that CNS stem cells can be induced to proliferate as well as migrate in response to a stimulus [64,65]. Infusion of growth factors into the cerebral ventricular system has also stimulated migration of progenitor cells from the SVZ [66,67].

CNS migration in embryogenesis

We cannot presume that a neoplastic brain cell is migratory (or 'invasive') simply because it represents a developmentally immature cell endowed with high migratory ability. The normal immature neural cell is migratory during development because the embryonic brain environment supports migration. The regulatory control of migration of immature neural cells is under exquisite and complex control in development and is responsible for the proper formation of the laminated cerebral cortex. However, it is not difficult to conceive how a transformed progenitor cell could exhibit some behavior reminiscent of the normal cell, with a tendency to migrate. In the following section, I will briefly discuss normal migration of neural progenitors in normal development followed by an introduction to some of the newly discovered molecular pathways involved. Most of the studies of migration have focused on neuronal precursors or early immature but post-mitotic neurons and substantially less is known about the migration of glial precursors to their final locations. To my knowledge, none of the pathways to be described have yet been implicated in neural tumor invasion. A number of these pathways are more closely studied in axonal pathfinding but they may have a role in neural migration.

Most knowledge of the histiogenesis of the brain comes from analyses of the development of the brain in simpler vertebrates such as mice and rats, and some analyses from primate studies. The developmental mechanisms in these organisms also operate in humans. The human brain develops over a much longer period and additional mechanisms may be involved in the formation of regions of brain that are particularly structurally or functionally more elaborate in humans.

The brain forms from a pseudostratified columnar epithelium that initially comprises the neural tube. There is a cell cycle dependent position of the cell nuclei in these proliferating elongated neuroepithelial

cells, those nuclei at a distance from the ventricular surface are undergoing DNA synthesis and those close to the ventricle undergo mitosis [30]. Two processes of cell division occur in the embryonic neuroepithelium, based on the orientation of the mitotic spindle: symmetric division and asymmetric division [68]. Symmetric division involves cell cleavage along a vertical axis, orthogonal to the ventricular lining, leading to two daughter cells that remain close to the ventricular surface. In this position the cells have identical phenotypes and fates and form an expanding population of precursor cells. Asymmetric cell division leads to the formation of two daughter cells that have distinct fates. It involves a horizontal cleavage plane parallel to the ventricular surface so that one daughter cell remains adjacent to the ventricular surface as a precursor cell, and the other cell, now separated by a distance from the ventricular surface, undergoes differentiation into a neuron or glial cell. In the developing CNS it is thought that neurons generally emerge from the proliferative neuroepithelium first followed by the birth of glial cells. These cells then migrate to form the tissues of the cerebral hemispheres [30].

There are four embryonic zones in the developing CNS: VZ, SVZ, intermediate zone, and marginal zone [30,69,70]. The VZ consists of the proliferating pseudostratified columnar neuroepithelial cells. The marginal zone, located outside of the VZ (peripherally), initially consists of the processes of the VZ cells. The marginal zone later is comprised of ingrowing axons and dendrites. The intermediate zone forms between the marginal zone and VZ and consists of immature neurons which send axons radially and peripherally to the marginal zone or sends axons tangentially to other CNS regions. The first neurons to emerge from the VZ migrate peripherally and come to lie beneath the pia, forming the cortical preplate [71]. Further emergence of neurons for the cerebral cortex leads to splitting of the preplate into two, a subplate which later becomes the deep layer (layer 6) of the cortex and a sparser population of cells superficially forming layer 1 of the cortex. The first neurons in the marginal zone are known as Cajal–Retzius cells. These cells produce factors that establish the radial glial cells which provide the scaffolding for migration of immature neurons to the cortex [72]. The final zone to be formed, the subventricular zone, forms at the junction of the ventricular and intermediate zones and consists of small round cells capable of giving rise to all cell types of the CNS. The VZ disappears by late embryogenesis and the SVZ persists through adulthood.

Billions of neurons emerging from the VZ travel up to centimeters to reach their final destinations in the cortex, where they form the six layers of cortex. It is thought that CNS patterning precedes neuronal migration in development in the establishment of the anterior–posterior and dorsal–ventral axes of the CNS. The patterning of the CNS along these two axes, identified by regional expression of specific transcription factors, lays down the template for organization of the cortex. Migration of neurons to these regionally specified areas establishes the cell populations required for cortex formation. It is believed that extensive neuronal arborization or synapse formation with the migrated neurons shuts down the process for further migration [58].

Radial migration

Cell migration to form the cerebral cortex involves radial and tangential patterns (with 80% estimated to be radial) [58,73–76]. Radial migration involves cellular locomotion along the cellular processes of a specialized radial glial scaffold, formed by the processes of radial glial cells (RGCs) that span the entire thickness of the developing CNS. It is thought that to a large degree, positional information for the organization for the cerebral cortex is already contained within the immature neuroepithelium. Evidence supports this hypothesis through the discoveries of early and regionally specific expression of a variety of markers (mainly transcription factors) at an early stage in neural tube formation. Subsequent radial migration of cells from the neuroepithelium preserves the positional information in the emerging cerebral cortex. A temporal sequence of birth of newly differentiated neurons from the VZ with subsequent migration along radial glial cells leads to laminar organization of the cerebral cortex. The laminar organization involves an inside-out pattern, with earliest neurons that exit the cell cycle in the VZ arriving in deeper layers compared to later emerging immature neurons [30]. RGCs are thought to be only present in embryogenesis, traditionally thought to later give rise to astrocytes [30,77–79]. In the cerebellum, two forms of radial migration occur. After cells of the deep cerebellar nuclei and Purkinje cells migrate along RGCs to reach their positions, Bergmann glial cells form and then direct migration of external cerebellar granule cells into the internal granule layer, completing formation of the mature cerebellum [58,80]. A form of radial ‘migration’ in the cerebral cortex distinct from

cell locomotion may also occur. This form involves translocation of the nucleus or cell body (nucleokinesis) of a neuron with an elongated process contacting the pial surface [81]. The leading process becomes progressively shorter as the cell soma moves toward the pial surface.

Tangential migration

Not all cortical neurons arrive in their final locations due to radial migration from the VZ. A substantial number of neurons migrate tangentially in the VZ, SVZ, intermediate zone, or cortex before coming to their final locations [73,75]. These neurons migrate orthogonal to radial glial fibers. In contrast to the VZ protomap associated with radial migration, with intrinsic determination of position, these tangentially migrating neurons are thought to respond to local environmental clues to specify their ultimate fate or location. The radial hypothesis suggests that cell fate is determined before migration, but the discovery of tangential migration suggests that possibly random migration occurs in progenitor cells (committed or uncommitted) prior to final determination of fate. Lineage analysis of developing mouse brains suggests that there may be subpopulations of cells in the primitive neuroepithelium that migrate tangentially (such as those later expressing GABA) and others migrate radially (such as those later expressing glutamate) [82]. Earlier differentiated neurons may use a radial pattern of migration and later neurons may migrate more tangentially. Tangential migration occurs in the olfactory system as progenitors arising in the SVZ migrate in the RMS to the olfactory bulb where terminal differentiation occurs into granule and periglomerular interneurons [61,83,84]. This process involves migration of immature neuroblasts along a chain without glial guidance. Specialized longitudinal migration also occurs in the cerebellum, as cerebellar granule cell precursors migrate from the rhombic lip to the external granule layer of the cerebellum [58]. Tangential migration has also been reported (in addition to Bergmann glial guided radial migration) in postmitotic external granule cells as they migrate across the cerebellar molecular layer to the internal granule layer [85].

Molecular biology of CNS migration

The molecular biology of radial migration involves interaction between neuronal and glial cells by cell surface receptors and ligands. Astrotactin, a glycoprotein with extracellular EGF repeats and fibronectin III domains, has been shown to be required for proper radial migration of granule cells in the developing cerebellum [58,86,87]. The receptor for this protein is not known. Neuregulin (glial growth factor), expressed on granule cells, interacts with its receptor erbB4 on radial glial cells to also influence external granule cell migration [88,89]. Laminin, the ligand for $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrin receptors, has been shown to have punctate deposition along radial glial fibers, suggesting that an integrin mediated process may also play a role in radial neural precursor migration [90]. $\alpha 3$ integrin is also highly expressed in VZ cells and in migratory cells of the intermediate zone, and αv has been shown to be expressed by RGCs [91]. Migration is disrupted *in vitro* by anti-α-integrin antibodies. $\alpha 3$ integrin-deficient mice have abnormalities of neuronal migration and cortical lamination [91].

The molecular biology of tangential migration is distinct from radial migration and is not as well understood. As mentioned above, specialized tangential migration from the SVZ to the olfactory bulb occurs by glial independent like-cell to like-cell interactions with elongated aggregates of cells moving rapidly along in chains [61]. This pattern of migration has been referred to as chain migration and seems to be specific to SVZ precursors, as cerebral cortical cells and external granule cells are not capable of this migration in *in vitro* analysis [84]. This migration occurs in adult mice [60,61]. Integrins have also been shown to regulate this process, as incubating forebrain neural precursors in the presence of $\beta 1$ integrin antibodies inhibited migration [92]. Polysialated neural cell adhesion molecule (PSA-NCAM) has also been shown to be important for this migration *in vivo*. Mice deficient for this molecule show a reduced size of the olfactory bulb and decreased numbers of migrating precursors along the RMS [93,94], due to impaired but not absent chain migration [95].

Evidence suggests that some proteins involved in axonal guidance are also involved in CNS migration. A repulsive diffusible factor known as Slit has also been identified in the midline forebrain septum, which steers immature neurons into the RMS to the olfactory bulb [96,97]. Slit's most important function probably relates to regulation of axonal guidance and it acts through binding to a receptor known as Roundabout (Robo). Netrin 1, a laminin-related protein that acts as a chemorepellant or chemoattractant factor, has been shown to influence the migration of cells

from the rhombic lip of the developing cerebellum [98]. Netrin 1 binds to a number of different receptors including DCC (deleted in colon cancer), a neural cell adhesion molecule of the Ig superfamily. Another new family of receptor tyrosine kinases and ligands has been discovered that has importance for neural precursor migration and axonal pathfinding, ephrins and Eph receptors [99–101]. Eph receptors undergo clustering when activated by their membrane bound ephrin ligands. Ephrins fall into two distinct classes based on the method of attachment to the cell membrane: ephrin A ligands have a glycosylphosphatidylinositol (GPI) linkage and ephrin B ligands have a transmembrane domain and short cytoplasmic tail. Interestingly, ephrin–Eph interactions can generate bidirectional signals between adjacent cells, each component acting as both a receptor and ligand. In the nervous system these interactions involve repulsion or attraction. Cells or axons can be prevented or attracted to certain territories establishing boundaries and tissue organization. Disruption of normal ephrin–Eph interactions has been shown to disturb the proliferation and migration of SVZ precursors [102]. Ephrin–Eph interactions may be particularly important for topographical organization in the brain such as in the visual system. Oncogenic forms of ephrins and Eph receptors have not been found, but elevated expression has been found to correlate with metastases of breast carcinomas and melanomas [100]. Hepatocyte growth factor/scatter factor (HGF) and its receptor c-met, implicated in epithelial–mesenchymal interactions in development and thought to be involved in a multitude of developmental processes, may also be important for migration of interneurons in the developing CNS [103].

Migration of transplanted CNS stem cells

CNS stem cells that have been propagated in culture and then transplanted into the brains of recipients have demonstrated dramatic migration as well as tropism for areas of CNS pathology. These results suggest that transplanted CNS stem cells are capable of responding to local environmental clues and that stem cells can be used as cell replacement therapy or as vehicles for delivery of therapeutic molecules. Transplantation of embryonic human or rodent, and adult rodent CNS stem cells into the neurogenic regions of the adult rodent brain (hippocampus, SVZ, or lateral ventricle) leads to seamless incorporation with appropriate differentiation into all lineages and migration in expected directions [28,104–106]. Rat CNS stem cells or oligodendrocyte progenitors generated *in vitro* from multipotential stem cells integrate and myelinate following transplantation into the spinal cords of myelin-deficient rodents [107,108]. When embryonic human CNS cells are infused into the ventricles of embryonic rodents, there is widespread incorporation into all regions of the brain, highlighting the importance of embryonic environment in determining the *in vivo* behavior of the stem cells [109]. Interestingly, transplantation of neural stem cells into brains with pathologic abnormalities leads to migration of the stem cells to the site of pathology. Transplantation of neural stem cells into the mouse cerebral hemisphere contralateral to the hemisphere containing an implanted glioblastoma, leads to migration of those transplanted cells over long distance to the tumor [110]. The transplanted cells seemed to 'seek out' infiltrating tumor cells. These results particularly suggest that neural stem cells may be vehicles for brain tumor therapy and highlight the potential migratory properties of these cells. In the absence of pathology, the cells have much more restricted migratory ability.

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