Genetics of Childhood Disorders: XXXVIII. Stem Cell Research, Part 2: Reconstructing the Brain

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An unexpected form of plasticity in the adult brain is the constant generation of new neurons throughout adult life. The addition of thousands of new neurons each day may be a means of sculpting brain circuitry. This turnover has important implications not only for brain function but also for brain repair and pathological conditions such as tumors. This review will cover neurogenesis and the identity of stem cells in the adult brain.

The adult brain contains two main types of cells, neurons and macroglia. Macroglia comprise oligodendrocytes and astrocytes. Oligodendrocytes are the cells that form myelin sheaths around the axons of neurons and allow the rapid conduction of electrical impulses. Astrocytes are mysterious cells that have multiple functions, including maintaining homeostasis, absorbing neurotransmitters released by neurons, and providing trophic support. Astrocytes are also induced to divide in response to lesions of the brain resulting in the formation of glial scars.

Almost all neurons and glia in the brain are derived from multipotent stem cells located in the germinal layers next to the ventricular cavities of the developing brain. As development proceeds, precursors become progressively more restricted

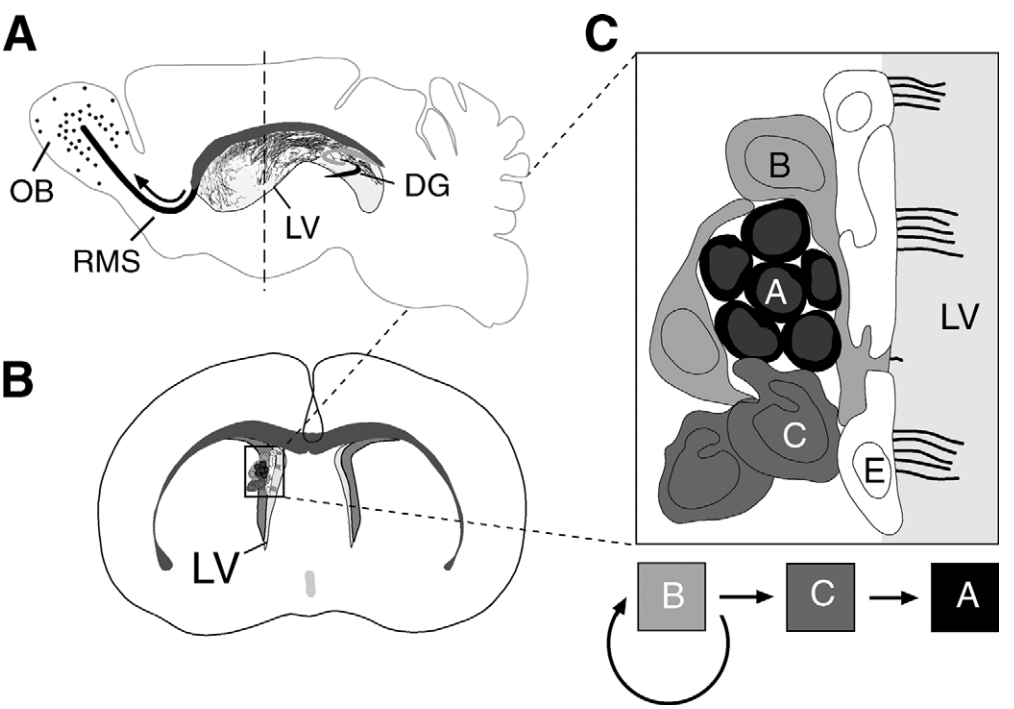

Fig. 1 (A) In rodents, two regions of the adult brain incorporate new neurons, the dentate gyrus (DG) within the hippocampus and the olfactory bulb (OB). This schematic longitudinal section of the adult mouse brain depicts the neurogenic regions and shows the lateral ventricle (LV) in its entirety. New neurons destined for the olfactory bulb are born many millimeters from their final destination in the SVZ, which lies along the length of the lateral ventricle. The newly generated olfactory bulb neurons migrate as chains through a network of pathways in the SVZ to converge on a restricted path, the rostral migratory stream (RMS), that leads into the olfactory bulb. In the olfactory bulb, they differentiate into two kinds of inhibitory interneurons. In contrast, newly generated neurons in the dentate gyrus are born locally in the subgranular layer. (B) Schematic cross-section of the adult mouse brain at the level of the dash in (A). The SVZ is hatched and is found next to the lateral ventricle. The region in the box is enlarged in C. (C) Four cell types are found in the SVZ: neuroblasts (A), SVZ astrocytes (B), rapidly dividing precursors (C), and multiciliated ependymal cells (E). The chains of neuroblasts migrate through glial tunnels formed by the processes of SVZ astrocytes. Focal clusters of rapidly dividing type C cells are found scattered along the network of chains. The multiciliated ependymal cells line the ventricle. The stem cells in the adult SVZ are SVZ astrocytes. SVZ astrocytes divide to give rise to rapidly dividing immature precursors (type C cells) that in turn generate the neurons that migrate to the olfactory bulb. (Modified from Doetsch and Alvarez-Buylla, 1996, and from Doetsch et al., 1997; copyright by the Society for Neuroscience.)

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into neuronal or glial lineages. After their birth, neurons use two kinds of migration to reach the appropriate layers of the brain. In radial migration, neurons migrate along the processes of radial glial cells, which span the width of the developing brain. In nonradial migration, cells disperse without the need for radial glia. Around the time of birth, radial glia lose their long processes and are transformed into astrocytes with their characteristic branched star-shaped morphology. Although neurogenesis is mostly restricted to embryonic development, glial cells continue to be generated throughout adulthood.

It has been known for about a century that the subventricular zone (SVZ), also called the subependymal zone, which lies next to the brain ventricle cavities of adult mammals, contains dividing cells. Various theories have raged about the fate of these dividing cells, with the most prominent being abortive cell death or the generation of glial cells. Thirty years ago, by labeling dividing cells with radioactive precursors, Joseph Altman first showed that dividing cells in the adult give rise to new neurons. However, the alternative hypotheses of cell death and gliogenesis dominated, and this idea was largely buried. Over the past decade, it has finally become widely accepted that new neurons are generated in two regions of the brains of adult mammals: the SVZ, which generates neurons destined for the olfactory bulb, and the dentate gyrus of the hippocampus. This required the advent of new methods for labeling dividing cells with permanent markers, such as retroviruses that integrate into the genome of a dividing cell and therefore tag all of its progeny, and microsurgery that allows focal labeling of cells in the brain.

Neurogenesis has been most convincingly demonstrated in adult rodents and birds, but the phenomenon has been documented in all vertebrates examined spanning the phylogenetic tree from lizards, fish, and cats to primates, including humans. It is interesting that neurons in different species integrate into different brain regions. These differences are likely due to the availability of distinct migration routes in each species. In adult birds and lizards, radial glia are maintained throughout adulthood and are used by newly generated neurons to migrate and settle throughout the forebrain. In contrast, in mammals, radial glia transform into astrocytes and this substrate for migration is lost. Consequently, the dispersal of newly generated neurons is more restricted in mammals. However, a recent report suggested that neurons are found throughout the neocortex of adult primates.

The SVZ is a thin layer of dividing cells that lies along the length of the lateral wall of the lateral ventricles (Fig. 1). It is a region dedicated to the production and trafficking of thousands of newly generated neurons each day. In adult mice, about 12,000 new cells reach the olfactory bulb daily, after migrating up to 8 mm from their origin in the SVZ (more than half the length of the forebrain). The extent of trafficking is dramatically revealed in whole-mount preparations that expose the entire surface of the ventricle (Fig. 1A). A network of newly generated neurons extends from the back of the ventricle to its anterior tip. New neurons within this network primarily stream forward to converge on a restricted path that leads into the olfactory bulb. The new neurons do not use radial glia or axonal guides, but migrate in association with one another in long chains. This novel form of translocation is called chain migration, and cells move rapidly through the brain by crawling over each other. The chains of neurons travel through glial tunnels formed by the processes of astrocytes. The role of these tunnels is unknown, but they may confine the newly generated neurons to their restricted path, or may provide them with, or protect them from, cues in the surrounding brain tissue. Once the new neurons reach the core of the olfactory bulb, they escape from the chains and migrate into two layers of the olfactory bulb, where they differentiate into granule and periglomerular neurons, two kinds of inhibitory interneurons. Many of the newly generated neurons are culled 2 weeks to 1 month after their arrival. It is not yet known whether those that survive replace other neurons in the olfactory bulb that have died, or whether they are being integrated into novel circuits as the need arises. The SVZ network of streaming neurons is present in all vertebrates examined so far, including primates.

In contrast to the extensive migration undertaken by olfactory bulb neurons, new neurons in the hippocampus are generated locally in the subgranular layer immediately adjacent to their final destination. They differentiate into granule neurons that project to the CA3 region of the hippocampus. The cell types in this region have not yet been characterized in detail. The newly generated granule neurons are also transitory cells and are eliminated about 2 weeks after their birth. Various factors have been proposed to regulate adult neurogenesis including stress, hormones, and physical exercise. The role of newly generated neurons and their potential involvement in learning and memory formation remain fascinating and unanswered questions.

The adult brain contains neural stem cells that have been proposed to be important for maintaining neurogenesis in vivo throughout adult life. These cells can be cultured in vitro and exhibit the two fundamental properties of stem cells. First, they demonstrate self-renewal, that is, they divide to give rise to another cell that is identical with themselves. Second, they are multipotential and can differentiate into neurons, astrocytes, and oligodendrocytes. These cells can be propagated over years in culture by different methods.

Which cells in the adult brain are the in vivo stem cells? Stem cells can be isolated from both the SVZ and the hippocampus, yet thus far only the stem cells from the SVZ have been identified. When the SVZ is dissociated to single cells, some divide in response to growth factors to generate floating balls of cells called neurospheres. These neurospheres can differentiate into both neurons and glia and can be passaged to generate new neurospheres, thereby satisfying the criteria of multipotentiality and self-renewal.

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The SVZ contains four main cell types: newly generated neurons, astrocytes, rapidly dividing precursors, and ependymal cells (Fig. 1C). The rapidly dividing immature precursors are closely associated with the chains of newly generated neurons that migrate through the glial tubes formed by the processes of SVZ astrocytes. They are scattered in focal clusters along the network of chains. The multiciliated ependymal cells line the ventricular cavity.

Surprisingly, slowly dividing SVZ astrocytes act as stem cells in this region. After specific labeling in vivo with a fluorescent marker, they generate fluorescent neurospheres that can be passaged and give rise to both neurons and glia. Furthermore, when they are specifically infected in vivo with a retroviral tracer, they generate new neurons that migrate to the olfactory bulb via the rapidly dividing type C cells. SVZ astrocytes are also capable of tissue regeneration, a feature often exhibited by stem cells. We have shown that when the SVZ is destroyed by administration of the antimitotic drug Ara-C that kills dividing cells, only SVZ astrocytes and ependymal cells remain. The SVZ network is rapidly regenerated within 10 days. This regeneration occurs simultaneously over the entire wall of the ventricle in small focal spots, suggesting that the stem cells are widely distributed. By tracing which cells divide and following their offspring with time-lapse photography, we showed that SVZ astrocytes, but not ependymal cells, begin to divide rapidly after the antimitotic treatment is stopped and fully regenerate the SVZ network of chains. When pulsed with a tracer, labeled SVZ astrocytes give rise to the rapidly dividing type C cells that in turn divide to generate the neurons that form the network of chains. This pattern of genesis follows that of other stem cell systems, namely, a relatively quiescent cell type divides to give rise to a rapidly dividing amplifying cell population that in turn gives rise to more differentiated cells. The multiciliated ependymal cells have also been postulated to be stem cells in the adult brain; however, this finding has not been confirmed by other groups.

The finding that SVZ astrocytes are stem cells in the adult brain was most unexpected, given that astrocytes and neurons are believed to arise from different lineages during development. However, it raises the hopeful possibility that astrocytes throughout the brain may have a latent capacity to give rise to neurons. Multipotential precursors can be cultured even from non-neurogenic regions of the brain, including the spinal cord, septum, and striatum, as well as from the neurogenic hippocampal formation. It is tempting to speculate that these as yet unidentified cells are similar to SVZ astrocytes.

Recent work hints that neural stem cells might not be restricted to the generation of brain cells, but may be able to cross lineage boundaries after culturing in vitro. Experiments suggest that neural stem cells may be able to generate blood and muscle cells when transplanted into the adult and to contribute to various lineages when grafted into a developing embryo. However, the precise identity of the transplanted cells needs to be elucidated to substantiate these potentially exciting results.

For stem cells to persist in the adult brain, they must reside in a molecular niche that permits their maintenance but also prevents unrestricted proliferation. As such, strict control must be exerted over their division and differentiation. When such control goes awry, unchecked proliferation could result in the formation of brain tumors, including gliomas. The glial nature of the stem cells in the adult brain is consistent with such a possibility. As we begin to understand how neurogenesis is regulated in the adult brain, it may be possible to manipulate SVZ cells to arrest unchecked proliferation or to stimulate these cells to be used for brain repair. Purification of adult stem cells and identification of factors that induce their differentiation along distinct lineages are paths currently being examined in the hope of using these cells for therapeutic purposes. Endogenous SVZ precursors can be expanded in vivo by administering known growth factors and can also remyelinate axons.

We are left with the concept of a dynamic brain, one in which memories are perhaps formed by the addition of new cells, and possibility of a brain with a latent potential for self-repair. The adult brain is no longer the static entity it once was thought to be.

WEB SITES OF INTEREST

http://www.nih.gov/news/stemcell/scireport.htm
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Accepted November 12, 2001.

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