Neural Stem Cells

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SUMMARY

Neural stem cells (NSCs) are the self-renewing, multipotent cells that generate neurons, astrocytes, and oligodendrocytes in the nervous system. In the fetus, NSCs participate to the development of the nervous system. Stem cells are present in many tissues of adult mammals where they contribute to cellular homeostasis and regeneration after injury. The central nervous system (CNS), unlike other adult tissues, elicits limited capacity to recover from injury. It was believed contrary to other adult tissues that the CNS lacks stem cells, and thus the capacity to generate new nerve cells. In the 1960s, preliminary studies by Altman and Das gave the first evidence that new neuronal cells were being generated in the adult brain. In the following decades, with the emergence of new technologies for identifying and characterizing neural progenitor and stem cells in vivo, and in vitro, new studies have contributed to confirm that neurogenesis occurs in the adult brain, and that NSCs reside in the adult CNS. Thus overturning the long-held dogma that we are born with a certain number of nerve cells and that the brain cannot generate new neurons and renew itself. In this chapter, we will review the evidences that neurogenesis occurs throughout adulthood in discrete regions of the adult brain and that NSCs reside in the CNS of mammals, including human beings. We will review and discuss the different theories regarding the origin of NSCs in the adult in the brain and spinal cord.

Key Words: Mammals; multipotential; self-renewal; progenitor cell; spinal cord.

1. ADULT NEUROGENESIS AND NEURAL STEM CELLS OF THE MAMMALIAN CENTRAL NERVOUS SYSTEM

The first evidence that neurogenesis occurs in the adult mammalian brain came from studies conducted by Altman and Das (1). Altman and Das reported, using [3H]-thymidine autoradiographic labeling, evidences that new neuronal cells are generated in the adult rat dentate gyrus (DG) of the hippocampus. In a second study, Altman reported evidence of cell proliferation in the ventricular zone, migration, and persisting neurogenesis in the olfactory bulb (OB) (2). Similarly, in a previous study, Adrian and Walker (3) failed to observe neurogenesis, but reported cell genesis of glial cells and inflammatory cells in the mouse spinal cord. Until the early 1990s, these studies were marginal, though a few reports supported the seminal work of Altman and Das that neurogenesis occurs in the adult mammalian brain (4). Two major advances in the early 1990s contributed to the emergence of adult neurogenesis and neural stem cells (NSCs) as a major field for biological research and cellular therapy: the validation and wide use of bromodeoxyuridine (BrdU), a marker for dividing cells, as a tool for studying adult neurogenesis and the isolation and characterization of neural progenitor and stem cells in vitro from adult mouse brain by Reynolds and Weiss (5).
1.1. Labeling of Dividing Cells in the Central Nervous System

The currently used protocol for characterizing neurogenesis in vivo consists of administering a marker of cellular division: BrdU, to perform histological labeling with antibodies against BrdU and other markers of nerve cells, and to perform analysis by confocal microscopy. BrdU is a thymidine analog that incorporates into DNA during S phase of the cell cycle that can be used to visualize cell proliferation (6). BrdU is administered intraperitoneally in the animals and inserted into the DNA of dividing cells, including in the central nervous system (CNS) (7). Histological studies allow the characterization of the newly generated neuronal cells and their fates, by multiple labeling with antibodies against BrdU and markers of interest, such as nestin (8–12), sox-2 (13–17), and oct-3/4 (18), markers of neural progenitor and stem cells, β-tubulin type III (19–21), neuronal nuclear antigen (22), microtubule-associated protein-2 (23) and calbindin (24), markers of neuronal cells, and O4 (25), NG2 (26–29), and glial fibrillary acidic protein (30), markers of glial cells. BrdU has some limitations as a marker for dividing cells. Indeed, BrdU can also label DNA undergoing repair and cells that are initiating cell death by apoptosis (31). Thus, when using BrdU for characterizing cell division additional controls must be performed to confirm the specificity of the labeling.

To this aim, other markers of the cell cycle, such as Ki-67, and proliferating cell nuclear antigen (PCNA), are being used to further confirm that cells are dividing, rather than in the process of DNA repair. Ki-67 is a nuclear protein expressed in all phases of the cell cycle except the resting phase (32,33). PCNA is a cell cycle-dependent nuclear protein that serves as a cofactor of DNA polymerase and has a role in ensuring the fidelity of DNA replication (34). PCNA is expressed in the S phase of cell division (35). Ki-67 is consistently absent in quiescent cells and is not detectable during DNA repair processes. Thus, Ki-67 offers a reliable marker for cell division. Other markers of the cell cycle, such as PCNA, are also detected in cells undergoing DNA repair (36) and have the same limitation as BrdU regarding its specificity as a marker for studying cell division. The technique known as “terminal deoxynucleotidyltransferasemediated dUTP-biotin nick-end labeling” allows determining cells undergoing apoptosis (37). Cell death can also be characterized by the identification of proteases, such as caspases (38–40). All this labeling can be performed simultaneously with BrdU labeling and allow to confirm cell-division analysis. Lastly, one of the most convincing techniques for identifying newly generated cells involves administering retrovirus-carrying genes such as the gene of the green fluorescent protein. Retrovirus infect only dividing cells (41,42), and thus not only offer a strategy for identifying newly generated cells’ origin and fate, but also for tracking cell migration and physiological studies. Such protocols have been applied to characterize neurogenesis in the CNS and have confirmed that BrdU is a valid marker for studying neurogenesis in situ (43–48).

1.2. Neurogenesis in the Adult CNS

Neurogenesis occurs mainly in two areas of the adult brain: the subgranular zone (SGZ) of the DG of the hippocampus (49), and the anterior part of the subventricular zone (SVZ) (44), along the ventricle, of several species including human (50–52). Newly generated neuronal cells in the SGZ migrate to the granular layer of the DG, where they extend axonal projections to the CA3 area. Newly generated neuronal cells in the SVZ migrate to the OB, through the rostromigratory stream, where they differentiate into interneurons of the OB (53–55). Particularly, the BrdU labeling paradigm has been used to label newly generated neuronal cells in the adult human CNS. Eriksson et al. (50) reported neurogenesis in the human DG from postmortem patient who had been treated with BrdU during the course of cancer treatment. More recently, Sanai et al. (51) reported neurogenesis in the human SVZ from organotypic slices of human adult SVZ. Newly generated neuronal cells in the DG and OB establish synaptic contacts and functional connections with neighboring cells (56–61). Thus, it is a functional neurogenesis. It is estimated that as many as 9000 new cells are generated per day in a young adult rat DG (62), though a significant proportion of these newly generated neuronal cells are lost within 2 wk in
the DG (62). Hippocampal neurogenesis contributes about 3.3% per month or about 0.1% per day of the granule cell population (63). As in the DG, a significant fraction of newly generated neuronal cells in the adult SVZ are believed to undergo programmed cell death rather than achieving maturity (64). It is estimated that 65.3–76.9% of the bulbar neurons are replaced during a 6-wk period in the adult rodent (65). It has been reported that in the adult DG, newborn granule cells survive for at least 8 mo in rodents (1), 12 wk in the macaque (66), and 2 yr in humans (50). Thus, the granule cells born during adulthood that become integrated into circuits and survive to maturity are very stable and may permanently replace granule cells born during development (67).

More recent studies have reported that neurogenesis occurs in other areas of the adult mammalian brain, albeit at lower levels. Rietze et al. (68) reported low level of neurogenesis in the Ammon’s horn CA1 of the adult mouse hippocampus. Gould et al. (66,69) reported that neurogenesis occurs in the neocortex of adult primates. Bedard et al. (70) and Bernier et al. (71) reported that neurogenesis occurs in the adult monkey striatum and amygdala, respectively. More recently, Zhao et al. (72) reported that neurogenesis occurs also in the adult mice substantia nigra. Some of these results have been contradicted by other studies. Kornack and Rakic (73) reported cell proliferation without neurogenesis in adult primate neocortex, whereas Lie et al. (74) and Frielingsdorf et al. (75) did not report evidence for new dopaminergic neurons in the adult mammalian substantia nigra. Thus, the confirmation of neurogenesis in these two later areas of the adult mammalian brain remains questionable.

In the adult spinal cord, cell genesis occurs without neurogenesis (3). Horner et al. (76) reinvestigated neurogenesis in the adult spinal cord by BrdU labeling and confocal microscopy. Cell division occurs throughout the adult spinal cord and is not restricted to the lining of the central canal, with the majority of dividing cells residing in the outer circumference of the spinal cord. Horner et al. confirmed that newly generated cells in the spinal cord express markers of both immature and mature glial cells, astrocytes and oligodendrocytes, but not of neurons. It is estimated that 0.75% of all astrocytes and 0.82% of all oligodendrocytes are derived from a dividing population over a 4-wk period. These data confirmed that gliogenesis, but not neurogenesis, occurs in the adult spinal cord.

Thus, neurogenesis occurs in the adult mammalian brain, and it is hypothesized that neurogenesis arises from residual stem cells in the brain (77,78). In contrast to the adult brain, newly generated cells in the adult spinal cord give rise to new cells restricted to the glial phenotype. Two hypotheses can be formulated to explain such discrepancies. First, the adult spinal cord, as opposed to the adult brain, does not contain NSCs, but restricted glial progenitor cells. Alternatively, the adult spinal cord would contain NSCs, but the environment would prevent these cells to differentiate into neuronal lineage. Thus, the presence of NSCs in the adult CNS remains to be resolved and the mechanisms of NSCs’ fate determination remains to be characterized.

1.3. Neural Stem and Progenitor Cells of the CNS

The demonstration that NSCs exist in the adult CNS lie on two main criteria: self-renewal and multipotentiality. The demonstration that putative NSCs are multipotent relies on showing that the three main phenotypes of the CNS, neurons, astrocytes, and oligodendrocytes can be generated from single cells. The demonstration that putative NSCs can self-renew relies on showing that cells maintain their multipotentiality over time. These two criteria have not been established yet in vivo; however, cells with self-renewing and multipotential properties have been isolated from the adult brain and characterized in vitro. In 1992, Reynolds and Weiss (5) were the first to isolate and characterize in vitro, a population of undifferentiated cells, from adult mouse striatal tissue including the SVZ, capable of generating the three main phenotypes of the CNS. This population of cell was termed neural progenitor cells (NPCs) because their stem cell properties had yet to be demonstrated. The NPCs were found to be immunoreactive for the intermediate filament protein nestin, a marker of neural progenitor and stem cells (4,8–12). In 1995, Gage et al. (79) isolated a population
of cells with similar properties from the adult rat hippocampus, the second neurogenic area of the adult CNS. In both models, NPCs were isolated and cultured in vitro, in defined medium in the presence of trophic factors. The two models of NPCs differ by the trophic factors used to isolate and expand them and by the growth characteristics of the cells. Whereas NPCs isolated by Reynolds and Weiss (5) grow as neurospheres in the presence of epidermal growth factor, NPCs isolated by Gage et al. (79) grow as monolayer in the presence of fibroblast growth factor (FGF-2). In 1996, Gritti et al. (80) isolated and characterized in vitro self-renewing, multipotent NSCs from adult mouse striatal tissue including the SVZ, and in 1997, Palmer et al. (81) reported the isolation and characterization of self-renewing, multipotent NSCs from adult rat hippocampus. Because NPCs and self-renewing multipotent NSCs have been isolated and characterized in vitro from different areas of the adult CNS, including the spinal cord, and from different species, including human (4,74,82–90).

One of the limitations in characterizing self-renewal, multipotential properties of putative NSCs in vitro is the difficulty of culturing isolated single cells. Epidermal growth factor and FGF-2, regulate the proliferation of NPCs in vitro (5,79). Other studies have shown that unknown factors, particularly derived from conditioned medium, are required to stimulate NSC proliferation in vitro from single cells (81,86,91–95). Taupin et al. (96) purified and characterized a factor derived from the conditioned medium of adult hippocampal-derived NPCs, and required with FGF-2 for the proliferation of NSCs in vitro, from a single cell, and to stimulate adult neurogenesis in vivo. The isolated factor is the glycosylated form of the protease inhibitor cystatin C (97), whose N-glycosylation is required for its activity as a cofactor of FGF-2 (CCg) (96). Thus, FGF-2 requires an autocrine/paracrine cofactor, CCg, for its mitogenic activity on NSCs. It has been a result of the isolation and characterization of CCg that we have been able to isolate adult human progenitor cells from autopsy and biopsy brain (88).

In vivo data show that gliogenesis, but not neurogenesis, occurs in the adult spinal cord. It is hypothesized that the adult spinal cord, as opposed to the adult brain, does not contain NSCs, but restricted glial progenitor cells (76). Alternatively, the adult spinal cord would contain NSCs, but the environment would prevent these cells to differentiate into neuronal lineage. The isolation and characterization of self-renewing, multipotent NSCs from the adult spinal cord suggest that the adult spinal cord contains putative NSCs, and that the environment would prevent these cells to differentiate into neuronal lineage (84,87). In support of this contention, Shihabuddin et al. (87) reported that with transplantation in the adult spinal cord, adult spinal-cord-derived neural progenitor and stem cells elicited only glial phenotypes, whereas when transplanted into the DG, neuronal phenotypes were also observed. Thus, the clonally expanded spinal-cord-derived neural progenitor and stem cells, when transplanted in the adult spinal cord, behave like endogenous proliferating spinal-cord cells, by differentiating into glia only (76). The ability of the cells to differentiate into neuronal phenotype in heterotypic transplantation studies suggest that adult spinal-cord-derived neural progenitor and stem cells are induced to express mature neuronal phenotype by environmental signals.

Thus, putative NSCs reside in the adult brain not exclusively in the neurogenic areas in the adult brain, but also in nonneurogenic areas where they will remain quiescent. However, the criteria used to characterize self-renewing, multipotent NSCs, although are well accepted to show that a single cell is a NSC in vitro, are not absolute. The main criticism resides in the number of subcloning steps that one must show to qualify a cell as self-renewing in vitro (84). Recent reports have challenged the isolation and characterization of self-renewing, multipotent NSCs from the adult DG, claiming the DG contains restricted progenitors, highlighting the limitation of in vitro studies to identify putative NSCs, but also differences in isolation procedures (98,99).

2. ORIGIN OF NSCs IN THE ADULT CNS

The fact that a cell can be labeled in vivo by administration of [3H]-thymidine, BrdU, or retroviral labeling does not mean that it is a stem cell. Self-renewing, multipotent NSCs can be isolated from the adult brain and expanded in vitro, hence NSC research has aimed at identifying the origin of the newly generated neuronal cells in the adult mammalian brain. It is currently
hypothesized that neurogenesis arises from residual stem cells in the adult brain. There are several hypotheses and theories regarding the identity and origin of NSCs in the adult brain. One theory contends that the NSCs of the adult SVZ are differentiated ependymal cells that express the intermediate filament protein nestin (96). The other theory identifies them as astrocyte-like cells expressing glial fibrillary acidic protein and nestin in the SVZ (100–106) and that would originate from a pool of slowly dividing cells (107). An astroglial origin for NSCs in the hippocampus has also received much of support (108).

In the adult spinal cord, it has been hypothesized that the central canal is the presumed location of the putative NSCs, because cells in the corresponding region of the brain, that is, the SVZ, can proliferate and differentiate to neurons and glia postnatally (95,100–106,109). Regarding the location of the progenitor cells in the spinal cord, the data from Horner et al. (76) predicts otherwise. Horner et al. reported that cell division occurs throughout the adult spinal cord, and is not restricted to the lining of the central canal, with the majority of dividing cells residing in the outer circumference of the spinal cord. Thus, glial progenitor cells exist also in the outer circumference of the spinal cord. Horner et al. (76) proposed two models regarding the origin of glial progenitor cell in the adult spinal cord. One model contends that a stem cell exists at the ependymal layer, and divides asymmetrically. A daughter cell then migrates to the outer circumference where it exists as a bipotent or glial progenitor cell and begins to divide more rapidly. The other model predicts that a glial progenitor and stem cell population may exist in the outer circumference where cell division is more common. This model functionally separates ependymal cell division from the proliferative zone of the outer annuli. Yamamoto et al. (110) isolated and characterized neural progenitor and stem cells from the periventricular area, but also from other regions of the parenchyma, supporting previous evidence by Horner et al. (76) that NSCs in the adult spinal cord are not restricted to the periventricular area, although putative NSCs in the adult spinal cord remain to be identified.

3. CONCLUSIONS

Neurogenesis occurs in the adult brain and NSCs reside in the adult CNS. The identification of the putative NSCs in the adult CNS remains the source of intense debate. The identification of molecular markers will ultimately define such cells. Several teams have attempted to identify specific markers of NSCs by gene profiling (111–114). However, the reported results are questionable owing to the heterogeneity of such culture; they contain NSCs, and more mature, yet undifferentiated, cells termed NPCs (4,115–121). Homogeneous populations of NSC/NPCs have been isolated using cell surface markers from human fetal spinal cord and brain tissues (122), by promoter-targeted selection from adult rat (123) and human SVZ (124), and from the lateral ventricle wall of adult mice by negative selection (125). These studies, by providing homogeneous populations of NSCs, will allow us to further study the origin and molecular identity of the NSCs.

ACKNOWLEDGMENT

PT was supported by grants from the NMRC, BMRC, and the Juvenile Diabetes Research Foundation.

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The Cell Cycle in the Central Nervous System
Janigro, D. (Ed.)
2006, 584 p., Hardcover
ISBN: 978-1-58829-529-3
A product of Humana Press