ABSTRACT

For decades, it was believed that the adult brain was a quiescent organ unable to produce new neurons. At the beginning of the 1960’s, this dogma was challenged by a small group of neuroscientists. To date, it is well-known that new neurons are generated in the adult brain throughout life. Adult neurogenesis is primary confined to the subventricular zone (SVZ) of the forebrain and the subgranular zone of the dentate gyrus within the hippocampus. In both the human and the rodent brain, the primary progenitor of adult SVZ is a subpopulation of astrocytes that have stem-cell-like features. The human SVZ possesses a peculiar cell composition and displays important organizational differences when compared to the SVZ of other mammals. Some evidence suggests that the human SVZ may be not only an endogenous source of neural precursor cells for brain repair, but also a source of brain tumors. In this review, we described the cytoarchitecture and cellular composition of the SVZ in the adult human brain. We also discussed some clinical implications of SVZ, such as: stem-cell-based therapies against neurodegenerative diseases and its potential as a source of malignant cells. Understanding the biology of human SVZ and its neural progenitors is one of the crucial steps to develop novel therapies against neurological diseases in humans.

Introduction

At the beginning of the twentieth century, Santiago Ramon y Cajal was one of the stronger supporters of the dogma that the brain, as a quiescent organ, was not able to generate postnatal neurons. This belief was challenged by Joseph Altman and coworkers, when they described the presence of thymidine-labeled neurons under the ependymal layer located at the ventricular wall of the brain [1-3]. These initial findings were later confirmed by electron microscopy screenings [4]. Soon later, several groups described the presence of mitotic neurons in several species such as birds [5], lizards [6] and most of the mammalian species [7-12]. Finally, a decade ago, it was described the presence of stem-cell-like progenitors in the human subventricular zone (SVZ) [13]. These outstanding findings ignited the interest of a number of groups to study human neural stem cells in the adult brain.

To date, it is well accepted that adult neurogenesis is a lifetime process, which is primary confined into the SVZ (at the forebrain) and the subgranular zone (SGZ) in the hippocampus [14-17]. The SVZ is the largest source of new neurons in the adult brain, which has a subpopulation of glial cells with stem-cell-like features both in vivo and in vitro [18-22].

Keywords: Subventricular zone; subgranular zone; neural stem cells; human; brain tumor; neurodegenerative diseases
Herein, we describe some of the astrocytic characteristics of the primary neural progenitors and the cytoarchitecture and cellular composition of the human SVZ. We also discuss some potential implications of adult neural stem cells on the clinical treatment of neurodegenerative diseases and brain tumor formation.

Defining neural stem cells
To date, one of the main experiments in vitro for determining the presence of neural stem cells is the ‘neurosphere’ assay [23], which consists in plating a suspension of cells in serum-free conditions. These floating cells are primarily supplemented with cytokines (epidermal growth factor -EGF, and fibroblast growth factor -FGF). With these non-adherent tissue culture conditions, stem-like cells divide and give rise to multipotent clones known as primary neurospheres [24]. When the first generation of neurospheres is dissociated to get single-cell clones, the progeny of these single clones can generate new spheres, also known as secondary neurospheres [23]. After the removal of mitogens and transfer to adherent plates, secondary neurospheres give rise to neurons and glia (oligodendrocytes and astrocytes) [24, 25]. Despite neurosphere assay is extensively used as a method to indicate the presence of stem cells in neural tissue. Increasing evidence indicates that this analysis may not accurately reflect the ‘stemness’ potential of SVZ cells in vivo. In consequence, in vivo evidence is required to completely demonstrate the presence of stem cells in the adult brain. The capacity of SVZ progenitor cells to behave as putative stem cells in vivo has been demonstrated in rodents [20, 22, 26]. In the human brain, the existence of neural stem cells has not been demonstrated in vivo, but indirect evidence supports the presence of stem cells in the adult brain.

Neurogenesis and stem cells in humans
Using neurosphere approach, neural stem cells have been isolated from human brain fetuses [27]. When maintained under growth-factor-enriched conditions, multipotent fetal cells are able to self-renewal and divide indefinitely [28]. The presence of neural stem cells in the adult brain was suggested at the end of the 90’s, when cells isolated from the SVZ and dentate gyrus were able to generate neurons and glia in vitro [29]. Additional in vitro evidence indicated that tissue explants isolated from the ventricular wall of the temporal lobe of epileptic patients were able to generate neurospheres [30, 31]. Later, a striking study showed that astrocytes, isolated from different segments of the lateral wall of cerebral ventricles, were capable of forming neurospheres and generating neurons, oligodendrocytes and astroglia [13]. This study also suggests that subpopulations of SVZ astrocytes are the putative neural stem cells in the human brain [13]. At the end of the 20th century, the presence of adult neurogenesis in vivo was suggested when the presence of neurons (NeuN-expressing cells) co-labeled with bromodeoxyuridine (BrdU, a proliferation marker) was found in the adult human hippocampus [32]. This postmortem evidence was obtained from neural tissue derived from patients with lung carcinomas, who received a dose of bromodeoxyuridine for diagnostic purposes. Neurogenesis in the adult cortex is under debate in rodents and nonhuman primates but, thus far, studies in humans have not revealed any evidence for the occurrence of cortical neurogenesis (Eriksson et al., 1998) and by determining the level of the isotope14C into the DNA of individual cortical neurons [33]. In addition, cell proliferation in the hippocampus, but not in the cortex has also been described by in vivo imaging studies such as magnetic resonance spectroscopy [34, 35]. Yet, the existence of bona fide neural stem cells (namely with self-renewal and multipotency capacity) [36] has not been fully demonstrated in the human brain and further studies are needed to elucidate this item.

The rodent subventricular zone
To understand the cellular composition and cytoarchitecture of the human SVZ is necessary to know the organization of the rodent SVZ, which is better studied and described. The SVZ is adjoined to the ependymal layer at the lateral wall of the cerebral ventricles (Figure 1). The SVZ contains four basic cell types: the type-E cells or ependymal cells; the Type B cells, the primary progenitors or bona fide neural stem cells; the type-C cells are highly proliferative progenitors, also known as transit amplifying progenitors; and migrating neuroblasts defined as type-A cells (Figure 2). Each cell type in the SVZ is composed by cell subpopulations, which are still being identified by morphology, molecule expressions and genetic backgrounds.

The ependymal cells (type-E cells) are post-mitotic and multiciliated, which mobilize the cerebrospinal fluid and modulate the SVZ proliferation [37-40]. By morphology, it has been described two types of ependymal cells: multiciliated (Type-E1 cells) and bi-ciliated (Type-E2 cells), which surround the niches of type-B cells and form a ‘pinwheel’ cellular organization (Figure 1). Type-E1 and type-E2 cells express S100β and CD24 [40, 41].

In the adult brain, the slowly dividing type-B cells have been identified as bona fide neural stem cells [21]. Type-B progenitors are a subpopulation of astrocytes that by morphology can be subdivided in type-B1 and type-B2 cells [42]. Type-B1 cells are in closed contact with the ependymal layer via both gap and adherens junctions, and have a non-motile primary cilium that reaches the ventricular lumen (Figure 1) [40]. Type-B1 astrocytes also contact blood vessels bordering the SVZ [40, 43]. In contrast, type B2 astrocytes are only located at the brain parenchyma surrounding type-A cells (Figure 1) [40].

As mentioned above, type-B cells have morphology, ultrastructure and marker expression that have been usually associated with astroglia. Some of the proteins that type-B cells express are: the glial fibrillary acidic protein (GFAP), vimentin, the astrocyte-specific glutamate transporter (GLAST), nestin, connexin 30, and the brain-lipid-binding protein (BLBP) [21, 44, 45]. Type-B1 astrocytes also express the stem-cell markers: carbohydrate Lewis X (LeX) [46] and prominin-1 (CD133) [47-49]. Despite these advances there is not a good marker available to categorically identify multipotent type-B1 cells, yet.

Type-B1 cells give rise to type C cells, also known as transit amplifying progenitors (Figure 2). These cells also display characteristic of neural stem cells in vitro and it seems they are a heterogeneous population of rapidly dividing progenitors [50]. Some markers used to identify

**Figure 1.** Three-dimensional model of the rodent subventricular zone (SVZ). Type-B cells (shown in blue) have a long basal process that contacts blood vessels and an apical ending at the ventricle surface. Note the pinwheel-like organization made by multiciliated (Type-E1 shown in brown) and bi-ciliated (Type-E2 shown in pink) ependymal cells encircling Type-B apical surfaces. Type-C cells are in green and type-A cells in red.
type-C cells are the Ascl1 transcription factor (Mash1), the epidermal growth factor receptor (EGFR), the Dlx2 transcription factor [25, 51] and the orphan nuclear receptor Tlx [52]. Subsequent division of type-C cells originate type-A cells (Figure 2)[45].

Figure 2. SVZ cell progeny. Neural stem cells or type-B cells (depicted in blue) have astrocytic characteristics. These cells give rise to Type-C cells called transit-amplifying progenitors (shown in green) that, in turn, generate neuroblasts (Type-A cells; shown in red), which migrate to the olfactory bulb and differentiate into neurons.

Type-A cells are young neurons that migrate rostrally toward the olfactory bulb, where they differentiate in mature interneurons [42, 53, 54]. Type-A cells can be easily identified by their strongly expression of doublecortin and the polysialylated neural cell adhesion molecule [16, 55, 56]. Finally, oligodendrocyte progenitors have also been found in the adult SVZ, but specific markers to identify them are not well-known [50, 57-59].

The human subventricular zone
The human SVZ is positioned within the lateral wall of the lateral ventricles but, in contrast to rodent SVZ, it has four well-defined layers (Figure 3) [60]. The first layer (Layer I) adjacent to the lateral ventricle is the multiciliated ependymal cells with radial or tangential processes. The Layer II, also known as hypocellular layer, has a few astrocytic and neuronal cell bodies, but a number of cytoplasmic expansions of ependymal cells interdigitated with astrocytic ramifications (Figure 3) [60]. This hypocellular gap appears to be a remnant of the neuronal formation and migration that have been observed at embryonic stages [61]. These interconnections between the astrocytic and ependymal processes might help preserve metabolic homeostasis and neuronal functioning in the SVZ [62, 63]. The Layer III is a ribbon of proliferative astrocyte somata (GFAP-Ki67-expressing cells) (Figure 3) [13, 60]. Remarkably, multipotent neurospheres appear to be generated by a subpopulation of astrocytes resident within this ribbon [13, 64]. Interestingly, some oligodendrocyte-like precursors and clusters of displaced ependymal cells with abundant microvilli, cilia and junctional complexes are also found within this ribbon (Figure 3). Finally, the most internal layer (the Layer IV), a transition zone between the astrocyte ribbon and the brain parenchyma, is primary comprised of many myelin tracts and neuronal bodies [64].

Figure 3. The human SVZ niche has unique characteristics as compared to the rodent or primate SVZ. Note: 1) The layer II devoid of cell bodies, 2) type B cells (GFAP-expressing cells shown in blue) are organized as a ‘ribbon’ in the layer III and, 3) No chains of migrating neuroblasts are found along the ventricular wall.
In summary, the human SVZ display fundamental differences respect to the rodent SVZ. Briefly, the presence of a layer with very few cells (Layer II), only reported in bovines [65], which contrast with the SVZ of the most of the mammals that show a close contact between ependymal and type-B cells [42]. Second, the human SVZ devoid of chains of migrating neuroblasts [13, 60, 66, 67]. However, some reports suggest that other brain regions may have migrating cells in the adult brain [68, 69]. Third, the number of proliferating cells in the human SVZ is very low as compared to the rodent SVZ [13, 60, 67]. Fourth, the rodent SVZ has a high number of neurons as compared to the human SVZ [13, 42, 60, 67]. Therefore, these obvious differences in the SVZ cell composition between humans and other mammals may be reflecting functional differences that are mandatory to be investigated.

Neural stem cells and brain disorders
Cumulative data indicates that adult neurogenesis can be influenced by a number of pathological conditions, including epilepsy, stroke, infections, inflammation, drug addiction, neurodegenerative diseases, demyelinating disorders, tumor development, and other [70]. However, the biological meaning of SVZ cellular responses is not well-understood. Some of these pathological changes and its relationship with neural stem cells are discussed below.

Epilepsy
Experimental epilepsy induces proliferation of neural progenitors and neuroblasts that eventually declines to basal levels [71, 72]. The morphological features of these new neurons are aberrant. Seizure-induced neurons show basal dendrite formation, mossy fiber sprouting and ectopic hilar migration [71, 73]. Remarkably, despite their abnormal connectivity, these new neurons are able to form functional circuits into the hippocampus [72]. Interestingly, the antiepileptic treatment counteracts seizure-induced neurogenesis and improves hippocampal-dependent memory. Nevertheless, the precise role of new-formed neurons induced by epileptic seizures is still unclear and further research is needed to elucidate this item.

Cerebral ischemia
There exist some evidence indicating that focal and global ischemia promotes neurogenesis in the SVZ and the SGZ [74]. After brain ischemia, neuroblast-like cells migrate through blood vessels from the SVZ to the injury site [75]. Therein, SVZ progenitors appear to differentiate into mature neurons [75, 76]. However, it is still not clear whether these migrating cells survive or integrate into the existing circuitry in situ. Moreover, the recruitment of newborn neurons to the infarct site in human stroke patients needs further clarification.

Neurodegenerative Diseases
There are several attempts to elucidate the role of both SVZ and SGZ neural progenitors in neurodegenerative disorders, such as: Multiple sclerosis, Parkinson’s disease, Alzheimer’s disease and others [77-81]. Promising results have been obtained against demyelinating disorders. For instance, experimental models indicate that SVZ progenitors play a role in myelination [58, 82]. Epidermal growth factor administration significantly increases the production of oligodendrocytes in the SVZ and promotes re-myelination upon demyelinating injuries [50, 83]. Less promising results have been reported in mice genetically modified to overexpress a mutant form of human α-synuclein [84], as observed in multiple system atrophy (MSA), Parkinson’s disease and dementia with Lewy bodies (DLB) [85]. Therefore, this system has been used as a mouse model of Parkinson’s disease and, interestingly, these animals show a significant decrease in the
survival and proliferation of neuroblast in the SVZ [86, 87]. In humans, it was found a decrease in the number of SGZ and SVZ progenitors in postmortem brain tissue obtained from Parkinson’s patients, although no functional correlations between the behavioral dysfunction and the cellular changes in both neurogenic niches have been done, yet [88]. In contrast, increased cell proliferation in the SVZ of Huntington’s patients and in the SGZ of Alzheimer’s patients has been reported in postmortem tissue [88]. Experimental evidence in mouse models is very controversial; in fact, transgenic mice that have parenchymal accumulation of the amyloid precursor protein into the brain exhibit decreased neurogenesis [89, 90]. However, presenilin mutant mice may have increased or decreased neurogenesis [91]. Therefore, the role of SVZ or SGZ progenitors in the pathogenesis of neurodegenerative disorders or in the treatment of them is yet to be determined.

Neuroinflammation
From the beginning of the present century, growing evidence indicates that immune cells, the complement system, serum cytokines, and Toll-like receptor-mediated innate immunity modulate neurogenic regions and control cell survival, proliferation, migration and fate of neural stem cells [57]. Cytokines and chemokines are immune modulators that regulate cellular communication and are present predominantly in macrophages, microglia cells, endothelium and epithelial cells [92, 93]. Under normal conditions, only macrophages, T lymphocytes and dendritic cells can enter into the brain [93-95], but after damage, an inflammatory process is initiated by the activation of astrocytes and local microglia. This event is followed by parenchymal infiltration of macrophages and lymphocytes. These activated and recruited cells release a number of anti- and pro-inflammatory substances, neurotransmitters, chemokines and reactive oxygen species. Then, more inflammatory factors are released, creating a positive feedback loop that results in neural damage and causes both detrimental and positive consequences to neurogenesis [92, 95, 96]. These changes in neurogenesis resulting from inflammatory mediators are likely to be caused by selective death of certain neuronal phenotypes. Interestingly, stem cell proliferation can be restored by anti-inflammatory treatments [97, 98].

The alteration in the function of neural stem cells appears to occur through the Janus kinase-signal transducer and JAK/STAT pathway [92, 99]. Thus, adult neural stem cells might be differentially regulated by the immune system via different cytokines and signaling pathways.

Brain tumors
A recent hypothesis suggests that the SVZ may be not only a source of neural precursor for brain repair, but also a source of brain tumors [100-102]. These tumor stem cells should be a relatively quiescent population of cells that consequently are able to escape the antimitotic effects of cancer drugs [26, 103]. Although certain clinical and experimental models support these assumptions no categorical evidence has been obtained yet. Therefore, the proposed contribution of neurogenic niches to the pathology cancer is to be demonstrated.

Conclusions
Since the discovery of adult neurogenesis amazing progresses have been done in this field. To date, it is though that neurogenesis is present in all mammalian species. The term “neural stem cells” refers to the properties of multipotentiality and self-renewal in cell culture. To determine whether neural stem cells exist in vivo and how to identify them and to prospectively isolate them is the one of the main challenges for researchers. On this regard, markers for stem cells identify overlapping but not identical subpopulations of SVZ cells; therefore, researchers have to be cautious when assigning biological characteristics to any subset
of SVZ progenitors [104]. Neural stem cells are instructed \textit{in vivo} through extracellular signals or cell-to-cell contacts with ependymal cells, the extracellular matrix, neuronal inputs, immune cells, local vasculature and the cerebrospinal fluid [50, 57, 63]. Following this strong regulation the SVZ progenitor cells generate neurons and oligodendrocytes, which are critical for preserving existing neural circuits and myelination in the adult brain. Therefore, despite all the scientific advances, the exact nature of their contribution to normal brain functions remains to be addressed. Understanding the functional role of neural stem cells in both pathological and physiological conditions will also help us to develop new therapies against neurological diseases in humans, such as: neuroinflammation, Alzheimer's disease, multiple sclerosis, stroke, brain tumors, Parkinson's disease, multiple system atrophy and many others.

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REFERENCES


[61] Guerrero-Cazares H, Gonzalez-Perez O, Soriano-Navarro M, Zamora-Berridi G, Garcia-Verdugo JM, Quinones-Hinojosa A. Cytoarchitecture of the lateral ganglionic eminence and rostral extension of the lateral...


[67] Sanai N, Berger MS, Garcia-Verdugo JM, Alvarez-Buylla A. Comment on "Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension". Science. 2007 Oct 19;318(5849):393; author reply


