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The Human Brain Subventricular Zone: Stem Cells in This Niche and Its Organization

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The subventricular zone (SVZ) of the adult mammalian brain harbors neural stem cells that may be a source of progenitor cells for future neuroregenerative therapy [1–3] as well as the potential source of brain tumors (see the review by Sanai and colleagues [4]). It is now understood that the organization of the SVZ in the adult human brain differs significantly from that of any other studied vertebrate [5–11]. Specifically, this region in the adult human brain contains a unique ribbon of astrocytes that proliferate in vivo and can function as neural stem cells in vitro [10].

It is known that explants of intraoperative and postmortem adult human SVZ are capable of generating neurons in vitro [10,12–16]. The extent of this astrocyte ribbon and its relations to other cellular elements of the human SVZ remained unknown until recently, however [10,17]. To define these qualities, the authors have examined the cellular composition of the walls of the human lateral ventricles using immunohistochemistry and electron microscopy and have reported the existence of stem cells in the human SVZ from intraoperative tissue [10,17]. In this article, they review the evidence supporting the existence of stem cells in the human SVZ as well as the current understanding of the cytoarchitecture and organization of the human SVZ.

Stem cells in the human subventricular zone

The authors collected normal adult human SVZ specimens from neurosurgical resections, dissociated them, and grew them as neurospheres (Figs. 1 and 2A). Human SVZ neurospheres differentiated into glial fibrillary acidic protein (GFAP)-positive astrocytes, O4+ oligodendrocytes, and TuJ1+ neurons and were passaged at least three times with no change in multipotency or self-renewing capacity [10].

When these astrocytes were purified to greater than 98% [18], as determined by GFAP staining, the authors found that multipotent self-renewing neurospheres had been produced. Furthermore, they found that single human SVZ astrocytes could produce multipotent self-renewing neurospheres

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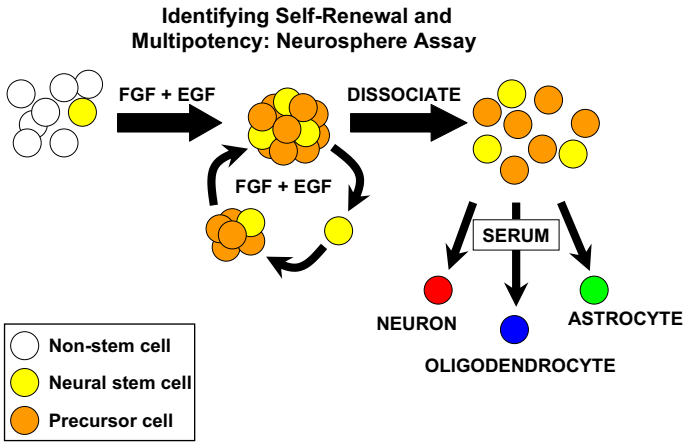

Fig. 1. Neurosphere assay. Start with a heterogeneous population of cells, some of which are neural stem cells (*red*). Add growth factors to this population, and the neural stem cells proliferate into floating spheres. These neurospheres can be broken down, and more growth factors can be added to obtain more neurospheres. This assay allows one to measure the self-renewing capacity of neural stem cells. The neurospheres can be then broken down, growth factors removed, and serum added; the cells within the neurospheres then differentiate into the three cell types of the central nervous system. EGF, epidermal growth factor; FGF, fibroblast growth factor.

as well. Dissociation of these neurospheres derived from single astrocytes resulted in the production of multiple secondary neurospheres. On differentiating primary and secondary neurospheres, the authors identified astrocytes, oligodendrocytes, and neurons in each colony (Fig. 2B). The clonal production of neurospheres from adult human GFAP-labeled SVZ astrocytes but not from astrocytes of other regions demonstrated that neural stem cells correspond to SVZ astrocytes.

Under direct fluorescent visualization and without epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF), single green astrocytes (Fig. 2C) were placed into individual wells of confluent human cortical astrocyte monolayers along with serum-free neurobasal/B27 media, and it was found that these astrocytes indeed generated colonies with bipolar or multipolar neurons expressing 4′-6-Diamidino-2-phenylindole (DAPI) and TuJ1 (Fig. 2D). The results of this experiment indicated that individual human SVZ astrocytes give rise to neurons without exogenous EGF or bFGF.

Cytoarchitecture and organization of the human subventricular zone

Intraoperative tissues as well as postmortem pathology specimens were collected to study the organization and cytoarchitecture of the human SVZ. These brain tissue specimens had no clinical or postmortem evidence of brain pathologic changes. The cerebral hemispheres of postmortem specimens were fixed by bilateral perfusion with 4% paraformaldehyde (PFA) through the internal carotid arteries. Intraoperative and postmortem tissue was further immersed in 4% PFA for 3 to 14 days depending on whether it was a small piece or a hemisphere. Some postmortem and intraoperative tissue was fixed in 2% glutaraldehyde and 2% PFA for electron microscopy. Intraoperative tissues and postmortem tissue specimens were then cryoprotected in 30% sucrose for cryostat sectioning.

All fixed specimens were cut on a cryostat (10–50-μm sections). Tissue was frozen in OCT compound (Tissue-Tek, Hatfield, Pennsylvania). Tissue sections were thoroughly rinsed in phosphate-buffered saline (PBS) and incubated for 1 hour at room temperature in blocking solution (PBS, 0.1% Triton, and 10% normal goat serum). Sections were then incubated for 24 hours at 4°C in primary antibody GFAP (1:500 ratio; Chemicon, Billerica, Massachusetts) diluted in blocking solution, rinsed in PBS, and then incubated using the fluorescent secondary antibody (Molecular Probes, Carlsbad, California and Jackson Laboratories, West Grove, Pennsylvania) at a dilution of 1:500 for 24 hours at 4°C. Sections were counterstained with DAPI (40 ng/mL) for 5 minutes at room temperature and mounted in Aqua

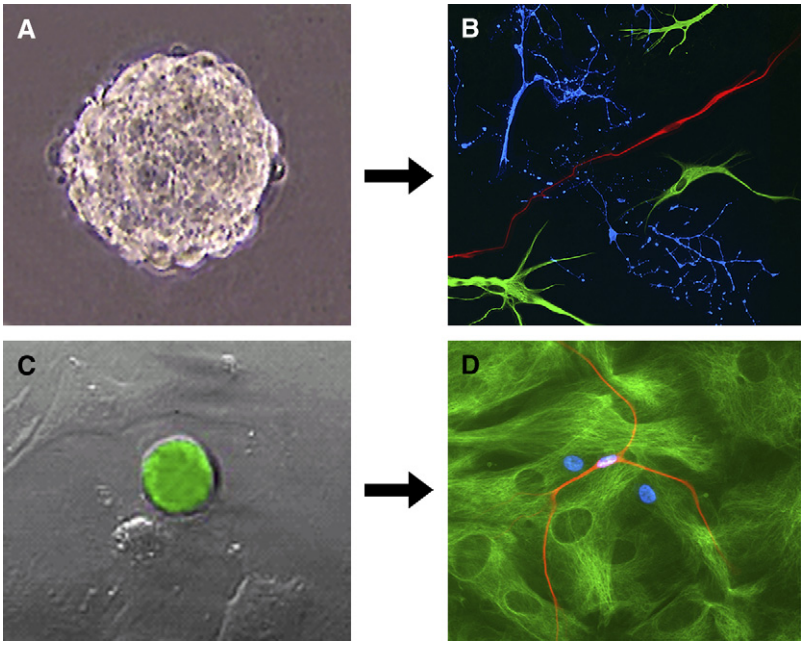

Fig. 2. Single human neurosphere derived from temporal horn SVZ astrocytes that has been grown in growth factors (*A*) and then differentiated to form astrocytes (*green*), oligodendrocytes (*blue*), and neurons (*red*) in each colony (*B*). Single SVZ astrocytes transfected with a glial fibrillary acidic protein (GFAP)–positive–glial fibrillary protein (GFP) adenovirus (differential interference contrast and green fluorescent images superimposed) were traced with DAPI (*blue*) (*C*) and then placed on a cortical astrocyte monolayer (*D*), where a resultant DAPI-positive colony grows on GFAP-positive cortical astrocytes (*green*) and yields a DAPI-positive cell colocalizing with the young neuronal marker TuJ1+ (*red*). (*Adapted from* Sanai N, Tramontin AD, Quinones-Hinojosa A, et al. Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature 2004;427:743; with permission.)

Polymount (Polysciences, Warrington, Pennsylvania). In all cases, omission of the primary antibody resulted in no labeling. Additionally, Western blot analysis and immunoabsorption immunohistochemistry control experiments were performed. Bright-field images were taken with a digital camera (SPOT camera; Diagnostic Instruments, Sterling Heights, Michigan) using a light microscope (AX70; Olympus, Center Valley, Pennsylvania) and then exported to Photoshop (Adobe Systems, San Jose, California).

As detected by GFAP and DAPI (nuclear marker), the lateral ventricular wall consisted of four identifiable layers throughout the length of the ventricle from the frontal horn to the temporal horn, as has been reported previously (Table 1) [10,17]. Layer I was a monocellular layer of ependymal cells. Layer II was a hypocellular layer consisting of minimal amounts of myelin and sporadic cells; this layer had a dense network of GFAP-positive processes. In comparison to layer II, layer III contained many more cell bodies, but the organization of this layer varied with location. In this layer, the authors found mainly cells with astrocytic characteristics. Further away from the ventricular surface, the astrocytic cellularity diminished and the appearance resembled that of the underlying brain parenchyma, including the striatum, corpus callosum, and hippocampus as well as neuronal bodies. The authors found marked differences in the thickness of the hypocellular layer and the astrocytic ribbon [10] along the rostrocaudal extent of the lateral wall.

Table 1
Layers of the wall of the lateral ventricle corresponding to the outermost 100 μm

Layers	Description
I	Ependyma
II	Hypocellular layer
III	Ribbon of cells
IV	Transitional zone to the parenchyma

With respect to SVZ wall organization, the authors identified mainly four types in the adult human brain: types A through C line the striatum from dorsal (type A), to middle (type B), to ventral (type C) regions along the lateral wall of the lateral ventricle. Type D wall lines the floor of the temporal horn over the hippocampus (Figs. 3 and 4).

This architecture remained consistent across specimens from different ages and genders. There was variability in the thickness of the hypocellular layer (II), the thickness of the GFAP band within this hypocellular layer, and the thickness of the ribbon of cells that comprised layer III (Fig. 5). Around blood vessels, astrocytes and their processes were more prominent (Fig. 6). In their model of human SVZ organization, the authors also include a previously published observation, wherein small clusters of ependymal cells were noted within the SVZ [17]. These cells were clearly ependymal cells, because they retained their tuffs of cilia with 9 plus 2 microtubule organization. The authors found these displaced ependymal cells in all studied brain specimens irrespective of age or gender, and, interestingly, they were always in a similar ventral location.

A detailed analysis of the cytoarchitecture and cellular organization of the adult human SVZ

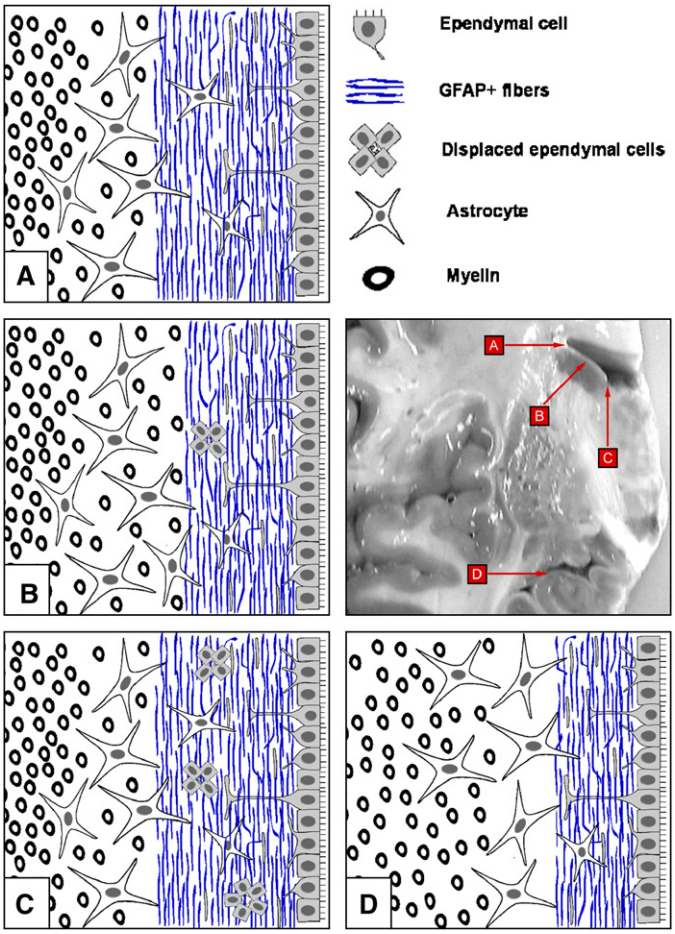

Fig. 3. Four types of SVZ walls in the human brain (*A–D*). The overall organization of the human SVZ can be better demonstrated in this summary figure of four different walls. The thickness of the GFAP dorsally to ventrally should be noted. It should then be compared with the hippocampus. The displaced ependymal cells and their distribution quantification in the different types of walls should also be noted. Within the photo *A–D* (in the red boxes) corresponds with the four parts of the brain: *A*, dorsal, near the corpus collosum; *B*, middle, over the caudate; *C*, ventral; and *D*, hippocampus.

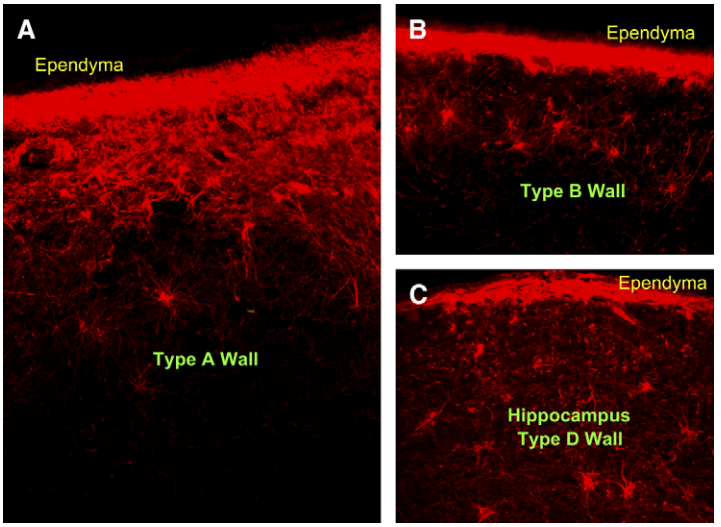

Fig. 4. Immunohistochemistry (GFAP, *red*) of the human SVZ illustrates the different types of walls encountered throughout the different regions of the SVZ, as illustrated schematically in Fig. 3. (*A*) Area around the ventral region of the ventricle (type A wall) is shown. (*B*) Region right at the middle of the caudate (type B wall) is shown. (*C*) Region over the hippocampus (type D wall) is shown.

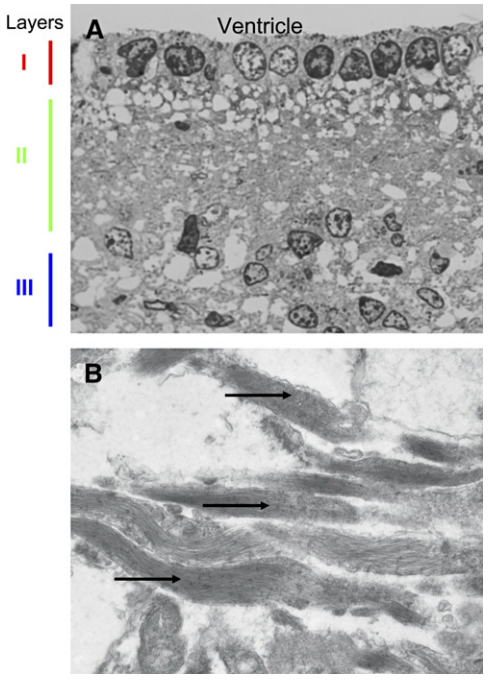

Fig. 5. (*A*) Semithin section of the ependyma (layer I) and hypocellular gap (layer II) and part of the ribbon of cells (layer III). (*B*) Immunogold stain for GFAP (*arrows*) in layer II.

reveals considerable variability along the length of the lateral ventricle. Although select structural and functional features of the human SVZ have been identified [6,10,19], this germinal region remains poorly understood. The authors have identified four different types of walls lining the lateral ventricles of the adult human brain. Three of these four wall types are found along the SVZ overlying the striatum, with the fourth wall type mainly found along the floor of the temporal horn in the region of the hippocampus. Although this organization seems to be unique to the adult human brain, some features are comparable to those reported in other vertebrates. For example, in a recent study of bovine SVZ [20], a hypocellular layer similar to that described in human beings has been reported over the striatum. One notable difference in this comparison is that the authors found the human SVZ hypocellular layer to be omnipresent, whereas it is sporadic in the bovine SVZ. Although this distinction remains constant despite specimen age or gender, its functional implications remain unknown.

Summary

Taken together, these observations offer direct evidence that this germinal region is not only organized differently but contains cells with

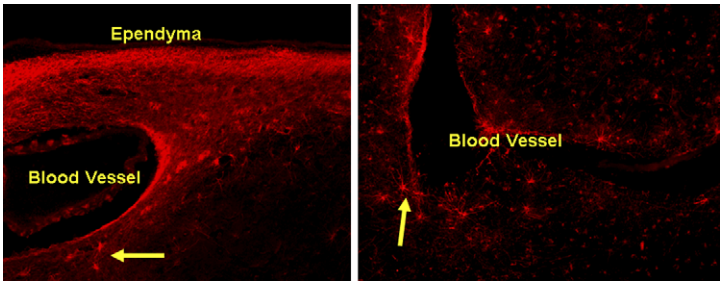

Fig. 6. Astrocytes (*arrow*) and their processes were more prominent around blood vessels. (*Left*) Around the SVZ, the GFAP-positive processes surrounded the blood vessels and appeared to thicken. (*Right*) Deeper into the SVZ, some astrocytes (*arrow*) appeared to extend their processes to the blood vessel.

astrocytic characteristics that function as neural stem cells. Considering the size of the human lateral ventricular system, this work suggests that a substantial number of neural stem cells exist in the adult brain throughout life. Understanding the organization of the adult human SVZ represents a necessary first step in understanding the cellular proliferation, precursor migration, and neurogenic niche of the largest known germinal region in the adult human brain—a foundation on which to develop novel neuroregenerative therapies.

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