11-2014

Majority of Cells Lining the Walls of the 3rd Ventricle in the Adult Rat Brain are not Neural Progenitor Cells

C. Hasken
DePauw University

S. Vermilyea

M. Hendrickson

R. Kalil

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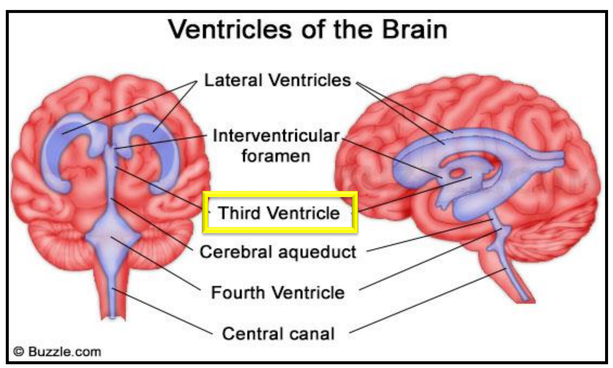
Hasken, C.; Vermilyea, S.; Hendrickson, M.; Kalil, R.

Department of Ophthalmology and Visual Sciences, University of Wisconsin – Madison

Introduction

- The subventricular zone (SVZ) along the lateral wall of the lateral ventricles of the adult brain, as well as the subgranular zone (SGZ) of the dentate gyrus of the hippocampus, are sources of neurogenesis[1].
- However, it remains unclear whether ependymal cells and tanycytes, the cells lining the walls of the third ventricle, function as neural progenitor cells (NPCs)[2,3,4].
- Tanycytes and some ependymal cells express nestin, a class VI intermediate filament widely accepted as a marker for NPCs[4].
- However, Hendrickson et al. found that few to none of the proliferating cells (BrdU+) in the third ventricle walls were cells that expressed nestin[4].
- Therefore, we used a different cell proliferation marker, Ki67, to quantitatively measure the number of nestin-positive cells that proliferate in the third ventricle walls. We compared with BrdU results using fluorescence immunohistochemistry.

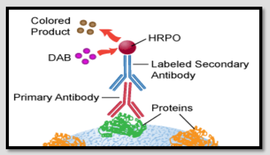
Question: *Do cells lining the third ventricle walls of the adult rat brain act as neural progenitor cells?*



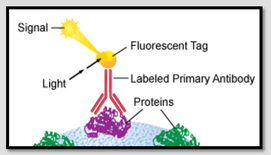
Methods

One adult male Sprague-Dawley rats brain was preserved in 4% paraformaldehyde and stored in 0.05% sodium azide until sectioning with a vibrating blade microtome (Leica VT1000 S). Chromogenic immunohistochemistry (IHC) was performed on 40 µm sections against the nuclear protein antigen Ki67. 10 mM sodium citrate buffer with heat was used for antigen retrieval. We used the primary antibody rabbit monoclonal anti-Ki67 (Thermo Scientific; clone SP6) at 1:500 dilution. The secondary antibody used was biotinylated donkey anti-rabbit IgG (Chemicon) at 1:250 dilution. Light cressyl staining was performed. Images were taken using a Nikon basic digital camera and widefield bright microscope.

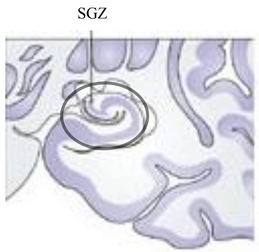
Fluorescence IHC was performed on 40 µm sections to double stain for Ki67 and nestin. 10 mM sodium citrate buffer with heat was used for antigen retrieval. We used the primary antibody rabbit monoclonal anti-Ki67 (Thermo Scientific; clone SP6) at 1:500 dilution . The secondary antibody used was Alexa 488-conjugated goat anti-rabit (Molecular Probes) at 1:500 dilution. For nestin, we used primary antibody mouse monoclonal anti-nestin at 1:1000 (Rat-401, Millipore). The secondary antibody we used was biotinylated donkey anti-mouse (Jackson ImmunoResearch) at 1:250, followed by tyramide treatment using the TSA Kit (PerkinElmer). Cell nuclei were stained with DAPI (Sigma). Images were produced using a Nikon A1R confocal fluorescence microscope coupled with a Bio-Rad MRC-1024 laser confocal scanning system. Every third section was stained through the entire ventricle, and Ki67+/nestin- and Ki67+/nestin+ cells were counted.



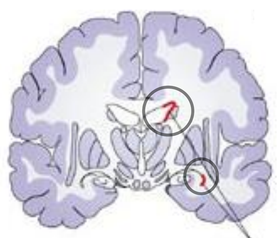
Chromogenic Immunohistochemistry



Fluorescence Immunohistochemistry

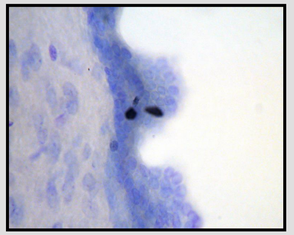


subgranular zone (SGZ) of the hippocampus of the human brain[1]

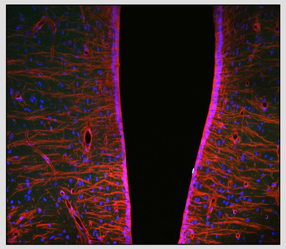


subventricular zone (SVZ) of the human brain[1]

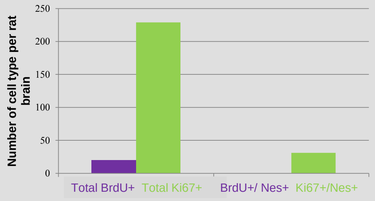
Results



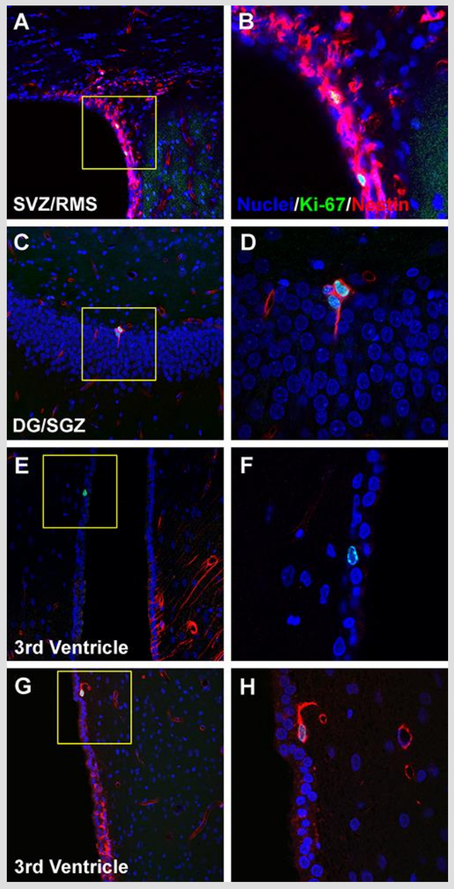
Chromogenic staining in the wall of the third ventricle. The cressyl violet stain in the background marks rough ER in cells, and the dark black stain depicts Ki67-positive cell nuclei.



Fluorescence IHC staining in the walls of the third ventricle. Nestin+ (red), Ki67+ (green), and DAPI+ (blue) regions are indicated in the third ventricle. The long red cellular processes of tanycytes are present in the ventral portion of the ventricular walls, as shown. Ciliated ependymal cells line the dorsal portion (not shown).



Results using BrdU vs Ki67. Both BrdU and Ki67 are cell proliferation markers, but we saw drastic differences in the number of marked cells in the 3rd ventricle. Purple= BrdU as marker; Green= Ki67 as marker.



Cell proliferation in three brain regions. The images were produced with a Nikon A1 confocal microscope coupled with a Bio-Rad MRC-1024 laser confocal scanning system. Proliferation in three brain regions is indicated by fluorescence IHC staining for Ki67 (green), nestin (red), and nuclei (blue using DAPI) within the (A and B) subventricular zone (SVZ) and initial portion of the rostral migratory stream (RMS) adjacent to the lateral ventricle, (C and D) dentate gyrus (DG) and subgranular zone (SGZ) of the hippocampus, and (E-H) wall of the third ventricle. Boxed regions in A, C, E, and G are shown at three-fold higher magnification in B, D, F, and H..

Conclusions

- The majority of nestin-positive cells in the walls of the 3rd ventricle do not proliferate under normal conditions[5]
- Of the cells that did proliferate, only a small percentage may be producing neurons[5]
- Tanycytes may play a significant structural and/or secretory role in brain function[6]
- Different results using the different proliferation markers (BrdU and Ki67)[7]
- Next Step
  - To determine the exact function of nestin-positive cells lining the walls of the 3rd ventricle

Acknowledgements and References

This research project was funded by the United States Department of Defense.

[1]Vescovi, A. et al. 2006. Brain tumor stem cells. *Nature Reviews Cancer* 6, 425-436.
[2]Chouaf-Lakhdar, L. et al. 2003. Proliferative activity and nestin expression in periventricular cells of the adult rat brain. *Neuroreport* 14, 633-636.
[3]Xu, Y. et. al. 2005. Neurogenesis in the ependymal layer of the adult rat 3rd ventricle. *Experimental Neurology* 192, 251-264.
[4]Hendrickson, M. et al. 2011. Expression of nestin by neural cells in the adult rat and human brain. *PLoS ONE* 6(4), e18535.
[5]Robins SC, Stewart I, McNay DE, Taylor V, et al. (2013) α-Tanycytes of the adult hypothalamic third ventricle include distinct populations of FGF-responsive neural progenitors. *Nature Communications* 4: 2049
[6]Rodriguez, E. et al. 2005. Hypothalamic Tanycytes: A key component of brain-endocrine interaction. *International Review of Cytology* 247, 89-164.
[7]Olariu, A. et al. 2007. Decreased neurogenesis in aged rats results from loss of granule cell precursors without lengthening of the cell cycle. *Journal of Comparative Neurology* 501, 659-667.
Bora, Chandramita. 2013. Ventricles of the brain. *Buzzle.com*.
Leinco Technologies, Inc. Immunohistochemistry Protocol.