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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

LETTERS TO THE EDITOR

Detecting Adult Enteric Neurogenesis in the Context of Adult ENS Homeostasis

Dear Editor:

In a recent study, Virtanen et al[1] failed to detect incorporation of thymidine analogues in the adult Enteric Nervous System (ENS) of mice that were dosed with these chemicals, and concluded that murine small intestinal myenteric neurons do not replicate at health. By contrast, we previously identified enteric neuronal precursor cells, and observed the incorporation of thymidine analogues in large numbers of adult small intestinal myenteric neurons, thus describing one possible homeostatic mechanism that maintains the structural integrity of the healthy ENS.[2] The nature and the ability of adult enteric neuronal precursor cells to cycle at steady state conditions was independently validated.[3]

Detecting the incorporation of thymidine analogues often requires exposure to extremely acidic pH to access and denature nuclear DNA. These protocols, optimized for tissue type, often vary in the time the tissue was exposed to acid (15–90 minutes), molarity (1–4 N HCl), and temperature of the acid (20°C–70°C).[4,5] Our protocol was within the range of conditions of prior protocols. We credit Virtanen et al[1] for examining one part of our work, which is important for testing and refining the framework our study established. However, we observed gaps between our methodologies and inferences. Virtanen et al[1] fixed their tissue overnight, which is far longer than performed in prior studies.[6,7] We used overnight fixation only for optically clarified full-thickness tissue, which required extended times for tissue permeabilization and antibody incubation. Indeed, Hayat[8] warns against overfixation, which causes "weak or absent immunostaining" because "prolonged fixation introduces excessive protein crosslinking, which hampers antigen accessibility to the antibodies."

Although the Virtanen et al[1] study was able to detect thymidine analogues in the epithelium of these overfixed tissues, the location of epithelium and ENS with relationship to the extracellular matrix, which is made of diverse structural proteins, differs significantly.[9,10] Dora et al[11] in this journal showed the presence of a protein-rich basement membrane–rich barrier that cocoons the ENS, but not the mucosa. These significant differences in the extracellular matrix composition between the intestinal mucosa and the gut wall explain why aberrations in tissue processing asymmetrically affect one gut layer and not the other. Indeed, although the overfixation of tissues did not alter the ability of Virtanen et al[1] to detect thymidine analogues in epithelial cells, their failure to detect thymidine analogues in any cell within the myenteric ganglia, and especially in myenteric glial cells that also cycle at steady state,[12,13] suggests that their methods may not have been optimized. These results further necessitate the need to differentially optimize tissue fixation, antigen retrieval, and other staining protocols specific to the layer of the gut studied. Without adequate optimization, it would be incorrect to use cells from one layer as a positive or negative control for studying the biology of cells from a different gut layer.

The framework of adult ENS homeostasis our study proposed studies ENS neurogenesis in the context of a continual neuronal loss at steady state. Our observations on the high rate of myenteric neuronal apoptosis (~11%) at steady state have been independently validated.[14] Neurons are terminally differentiated cells that do not "replicate" (as Virtanen et al[1] imply) to maintain their populations but are generated from other cells. Maintenance of enteric neuronal numbers therefore necessitates neurogenesis, even though the exact mechanisms may seem controversial at first glance. The current study does not seem to dispute the high rate of ongoing neuronal loss but suggests cell-cycle independent neurogenesis. Cells that transdifferentiate into neurons, but do not cycle to maintain their own population will also be lost in a short time-frame, necessitating the need for cells that cycle and differentiate to generate neurons, which is a definition of a neuronal precursor cell.

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Conflicts of interest
The authors disclose no conflicts.

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