Brain stem cell division and maintenance studied using multi-isotope imaging mass spectrometry (MIMS)

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ABSTRACT:
New neurons are continuously produced from neural stem cells in specific regions of the adult brain of animals and humans; one of these regions is the hippocampus, a region crucial for cognitive function. Adult hippocampal neurogenesis responds to a multitude of extrinsic stimuli and emerging evidence indicates that it may be important for behavior, pathophysiology, brain repair, and response to drugs. G. Enikolopov lab developed an approach to identify and quantify the cellular targets of pro- and anti-neurogenic stimuli, based on reporter transgenic mouse lines in which neural stem and progenitor cells or their progeny are marked by fluorescent proteins. It enabled them to dissect neurogenesis in the adult hippocampal dentate gyrus (DG) into distinct steps, and to find that age-related decline in hippocampal neurogenesis is driven by the continuous depletion of the neural stem cell pool [1]. Here, we demonstrate the feasibility of using MIMS for studying adult neurogenesis. Mice were pulsed with \(^{15}\)N-thymidine for 8 weeks and chased for 4 weeks. Tissue was fixed by perfusion, brains removed, and sectioned with a vibratome at 40 µm thickness directly affixed to the surface of silicon chips, without embedding. Multi-isotope imaging mass spectrometry (MIMS) analysis uncovered \(^{15}\)N-labeled DNA of actively proliferating cells. The majority of labeled cells lie within the granular cell layer of the dentate gyrus. MIMS is exceptionally well suited for measuring the changes in the amount of label as a function of divisions because the method is: (a) highly sensitive, with the level of detection orders of magnitude lower than for the conventional autoradiography or immunocytochemistry; (b) highly quantitative over several orders of magnitude scale of values; (c) allows multiple labels to be resolved simultaneously; (d) compatible with direct or immunocytochemical detection of the fluorescent signal; (e) allows 3D reconstruction of the imaged cell, thus enabling analysis of organelles or their subregions.

CONCLUSION:
For the first time we are directly locating and measuring stem cell generation in the hippocampus. We will recognize stem and progenitor cells by combining MIMS with the reporter lines (Nestin-GFP and Nestin-mCherry).

REFERENCES: