Quantifying cell division with D₂O and multi-isotope imaging mass spectrometry (MIMS)

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ABSTRACT: Quantifying cell division has traditionally relied upon incorporation of label in newly synthesized DNA by nucleotide salvage, including labeled thymidine or thymidine analogs[1]. An alternate approach is to use precursors of the de novo nucleotide synthesis pathway, such as glucose or water[2,3]. Because such precursors are not specific for DNA synthesis, studies utilizing this approach have analyzed isolated genomic DNA to exclude background incorporation in other molecules. Due to the rapid rate of DNA synthesis during S-phase of mitosis and to the inherent stability of DNA, we hypothesized that pulse-chase analysis of stable isotope labeled water would result in sufficient nuclear labeling to enable discrimination of recently divided cells by NanoSIMS ion microscopy. We administered deuterated (D)-water and (15N)-thymidine to mice concurrently, guided by the rationale that (15N)-thymidine incorporation would serve as a "gold standard" to identify dividing cells[1]. Mice were labeled for 3 days, followed by a 3-day label-free chase. We analyzed the small intestine, due to its recognized propensity for cell turnover. After fixation, small intestinal samples were embedded in LR white, sectioned to 0.5µm, and analyzed with a NanoSIMS 50L (Cameca). The instrument was carefully tuned to study the D/H ratio in two ways: by sequentially recording D- and H- followed by (15)N/14N and (15)N/14N or by simultaneously capturing C(13)D and C-H along with (15)N/14N and (15)N/14N [4]. We show both qualitative and quantitative that dividing cells in the small intestine (15N-labeled) reveal a clearly discernible (15N signal in the nucleus that was not observed in undivided cells (15N-unlabeled). Correlation with 15N- and 14N/15C/14C images demonstrate preferential localization of (15N labeling in regions of the nucleus with high DNA content as expected of labeling being incorporated during DNA synthesis and cell division. These data support the concept that stable isotope tagged precursors of the de novo nucleotide synthesis pathway can be used in concert with NanoSIMS to study cell division in vivo. This approach is particularly relevant to study tissues characterized by low labeling frequency, where the ongoing turnover is eminently translatable to humans.

REFERENCES:

SUMMARY:
- MIMS distinguishes dividing cells from quiescent cells after in vivo D-water labeling.
- Labeled nuclei become more evident during a label-free chase period as background labeling of non-genomic molecules fades due to ongoing turnover.
- The use of labeled precursors to the de novo nucleotide synthesis pathway represents a practical approach to study slowly dividing cell populations, due to the ease of long-term label administration.
- Because stable isotopes are non-toxic, the use of D-water to study cell turnover is eminently translatable to humans.