5-Hydroxymethylcytosine Is a Stable DNA Modification

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HIGHLIGHTS

5-Hydroxymethylcytosine (hmC) is an oxidation product of 5-methylcytosine (mC) present in DNA of most mammalian cells. Reduction of hmC levels is a hallmark of cancers. Elucidating the dynamics of this oxidation reaction and the lifetime of hmC in DNA is fundamental to understanding hmC function.

We have developed:

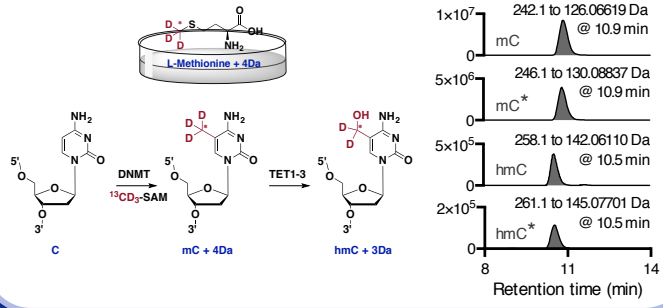
- **a sensitive LCMS method for quantification of DNA modifications**
- **a labelling strategy to track the fate of modified cytosines**

We have found that:

- **mC oxidation does not occur on nascent DNA during replication**
- **hmC is stable in DNA, and not an intermediate of demethylation**
- **the rate of proliferation governs the global levels of hmC**

ISOTOPIC LABELLING OF DNA AND LCMS

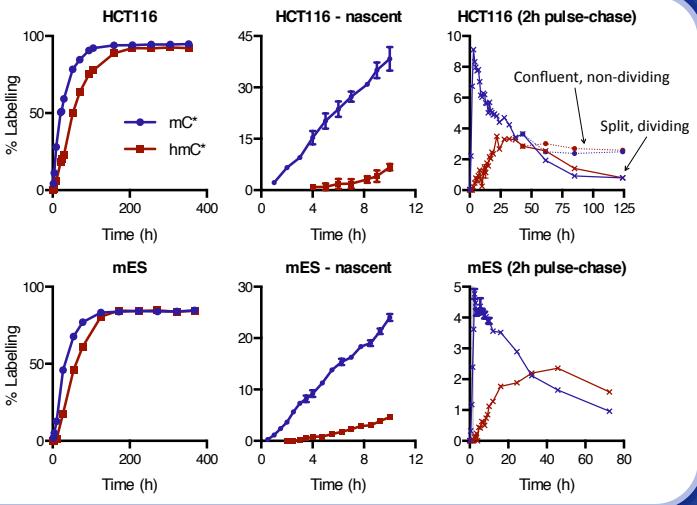
Cells can be grown in custom media containing **L-methionine($^{13}C,D_3$)**, a source of methyl group for mC in DNA. Accurate LCMS analysis then provides ratios of labelled to total (labelled + unlabelled) modification (% labelling), and therefore **information about DNA methylation and mC oxidation activity.**



TIMING OF mC OXIDATION ON NASCENT DNA

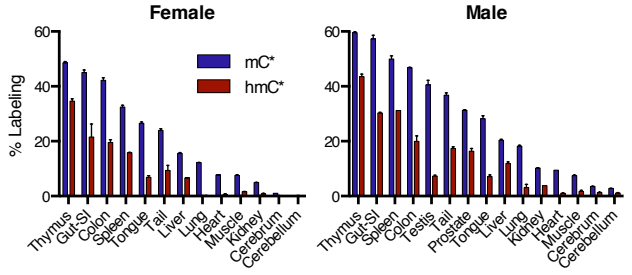
Plots on the right show mC and hmC labelling in 2 different cell lines:

- mC labelling is always higher or equal to hmC, consistent with **hmC** being a **product of mC** (left plots)
- A gap between labelling curves reflects a **difference in timing between mC formation and its oxidation to hmC** on a newly synthesized DNA strand (left and middle plots)
- Focus on early time points confirms that the **first mC oxidation activity happens several hours after replication** (middle plots)
- Pulse-chase experiments with a 2h labelling pulse allow tagging a small proportion of mC and hmC and follow their lifetime in DNA
- Labelled hmC is present >125h after labeling pulse, **hmC is** therefore **a stable modification** in cultured cells



hmC IS STABLE IN DNA *In vivo*

Custom mouse diet containing **L-methionine($^{13}C,D_3$)** is a useful tool to study the **dynamics of DNA modifications *in vivo***.

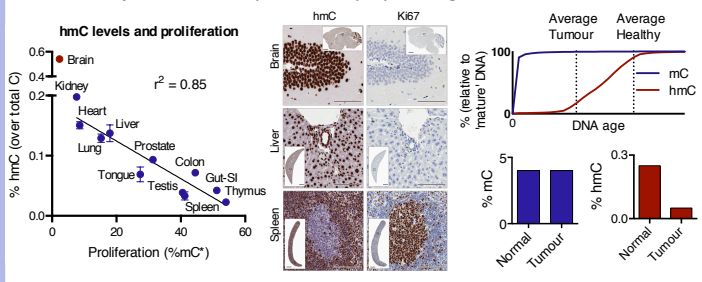


mC and hmC labelling in adult mice fed with custom diet for 4 months:

- Gap between mC and hmC labelling >> **delayed hmC 'maintenance'**
- Lack of labelling in slow-/non-dividing tissues >> **hmC must be stable**

GLOBAL hmC LEVELS AND PROLIFERATION

Plotting global levels of hmC against proliferation rate shows a **linear relationship**, confirmed qualitatively by **IHC against hmC and Ki67**.



hmC stability and mC oxidation timing are responsible for:

- Tissue-specific levels of hmC
- Reduced levels of hmC reported in all studied cancers

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