

RESEARCH HIGHLIGHT

# Balance of DNA methylation and demethylation in cancer development

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## Abstract

Genome-wide 5-hydroxymethylome analysis of a rodent hepatocarcinogen model reveals that 5-hydroxymethylcytosine-dependent active DNA demethylation may be functionally important in the early stages of carcinogenesis.

Epigenetic information is crucial for eukaryotic organisms as it impacts a broad range of biological processes from gene regulation to disease pathogenesis. This information is mainly embodied in DNA methylation, carried by 5-methylcytosine (5mC, the fifth base), and various histone modifications. It is well-established that epigenetics can play critical roles in cancer development; a highly distorted epigenome (including aberrant DNA methylation and histone modification patterns) is now accepted to be a general feature of many cancers [1,2]. Understanding the molecular mechanisms of epigenetic alterations at the early stages of tumorigenesis may therefore be important in developing new cancer treatments.

A cell's DNA methylation pattern is a dynamic status balanced by methylation and demethylation, and aberrant DNA methylation has been attributed to either excessive methylation or deficient demethylation. A study by Meehan, Moggs and colleagues, published in this issue of *Genome Biology* [3], now links active demethylation with the early stages of carcinogenesis by investigating the non-genotoxic carcinogen phenobarbital (PB)-induced rodent hepatocarcinogen model.

## Active DNA demethylation and 5-hydroxymethylcytosine

DNA methylation is established during early development and maintained through generations by DNA methyltransferases (DNMTs). DNA methylation can be erased during replication if DNMTs fail to methylate the daughter strand, a process named passive demethylation. However, in multiple instances, DNA demethylation in mammalian cells has been observed in the absence of DNA replication. The mechanisms for the active DNA demethylation pathways that must be at work in these non-replicating cells had been elusive for decades, until 5-hydroxymethylcytosine (5hmC) was identified as the so-called sixth base in 2009 [4,5]. 5hmC is oxidized from 5mC by the TET (ten-eleven translocation) family of iron(II)/ $\alpha$ -ketoglutarate-dependent dioxygenases, and is proposed to be a new epigenetic mark that constitutes the first step in an active pathway for DNA demethylation. Indeed, subsequent studies revealed that 5hmC can be further oxidized by TET enzymes to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). 5fC and 5caC can be excised by thymine DNA glycosylase (TDG), and subsequently converted to cytosine through base excision repair, thus concluding the first biochemically validated active demethylation pathway in mammalian cells (for review, see [6]).

The aberrant DNA methylation landscape in cancer cells has long been imputed to the dysfunction of the methylation machinery, in the form of the DNMT enzymes. The discovery of 5hmC, 5fC and 5caC, however, prompts a re-evaluation of the relationship between DNA demethylation and cancer development, as it raises the possibility that impaired function of the demethylation machinery could equally lead to an imbalance and reprogramming of the DNA methylation status. Indeed, in human cancer cells, 5hmC is largely depleted compared with normal tissues, and the expression of *TET* genes is substantially reduced. Notably, *TET2* is frequently mutated or inactivated in leukemia, but is required for normal hematopoiesis (for

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review, see [7]). Together, these recent observations suggested that functionally active demethylation is crucial in maintaining the dynamic balance of DNA methylation status and, as a consequence, in suppressing tumor development.

### Active DNA demethylation and early carcinogenesis

While these recent studies linked the dysfunction of the active demethylation machinery to cancer, the detailed molecular mechanisms leading to carcinogenesis remained unclear. The new study by Meehan, Moggs and colleagues [3] now sheds light on the 5hmC-dependent active demethylation pathway during the early stages of hepatocarcinogenesis, by using a rodent model of non-genotoxic carcinogenesis with PB. PB-mediated tumor promotion is a well-characterized rodent model of non-genotoxic liver carcinogenesis, in which epigenetic alterations can be profiled at different stages. Using this model, the authors previously investigated DNA methylation changes in the mouse liver during a short term (28 days) exposure to PB, and discovered that 5mC levels only became reduced in the promoter regions of a small subset of PB-induced genes [8]. *Cyp2b10*, a direct and early target of the PB-induced signaling pathway, exhibited both the strongest transcriptional upregulation and the most significant promoter demethylation, associated with a repressive-to-active switch of histone marks [8].

To further elucidate whether 5hmC is involved in PB-induced carcinogenesis, in the new study the authors use a genome-wide hMeDIP (hydroxymethylated DNA immunoprecipitation)-coupled microarray to profile the 5-hydroxymethylome in mouse liver, both before and after a 28-day PB exposure [3]. These data represent the first report of the genome-wide distribution of 5hmC in mouse liver, which is found to be generally similar to those of mouse embryonic stem cells and cerebellum tissue, although some distinct features were also observed [3]. The study goes on to investigate the relationship between the 5-hydroxymethylome and 5mC, histone marks and gene expression levels.

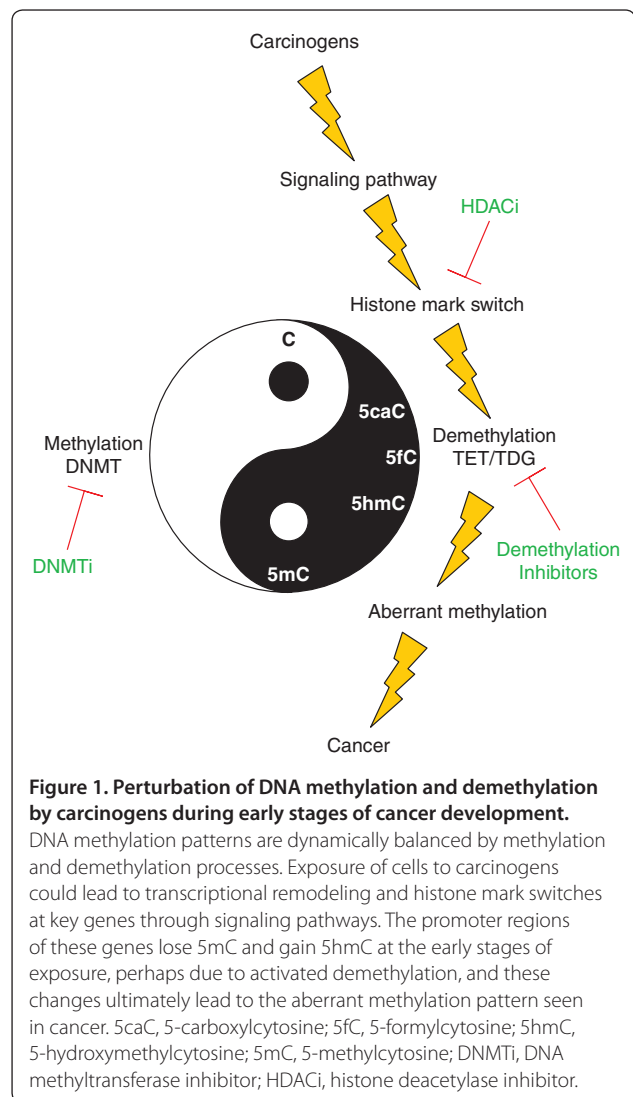
Following the 28-day PB treatment, the 5hmC signal in promoter proximal regions is elevated specifically in PB-induced genes, and this upregulation is reciprocal to decreases in the level of 5mC.

Returning to the previous study's focus on the *Cyp2b10* promoter [8] the authors are able to confirm that its strong demethylation is associated with a significant increase in the level of 5hmC [3]. This is an interesting example of apparent active demethylation through 5hmC at a specific locus. To further support the hypothesis that 5hmC acts as an intermediate of active demethylation in tumorigenesis, the authors track 5mC/5hmC changes at

the *Cyp2b10* promoter during a longer, 91-day PB exposure. This prolonged PB treatment leads to complete demethylation (loss of both 5mC and 5hmC) in the center of the promoter region, which resembles the general features of aberrant methylation and depleted 5hmC in cancer. Collectively, the data in this work suggest that a 5hmC-dependent active demethylation pathway is involved in the early stages of PB-induced carcinogenesis.

### Perspectives and insights

The results in [3] depict an interesting model for early cancer development (Figure 1). According to this model, exposure to carcinogens, and subsequent mutations in and perturbations of signaling pathways, could alter the short-term histone mark distribution in affected cells, and this redistribution in turn disequilibrates the dynamic DNA methylation balance through



**Figure 1. Perturbation of DNA methylation and demethylation by carcinogens during early stages of cancer development.**

DNA methylation patterns are dynamically balanced by methylation and demethylation processes. Exposure of cells to carcinogens could lead to transcriptional remodeling and histone mark switches at key genes through signaling pathways. The promoter regions of these genes lose 5mC and gain 5hmC at the early stages of exposure, perhaps due to activated demethylation, and these changes ultimately lead to the aberrant methylation pattern seen in cancer. 5caC, 5-carboxylcytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; DNMTi, DNA methyltransferase inhibitor; HDACi, histone deacetylase inhibitor.

5hmC-dependent active demethylation. Imbalanced active demethylation could yield complete demethylation or aberrant methylation, which in turn would promote cancer. Although the model is overly preliminary and simplified, it offers a starting point for future research. For example, if the base excision repair pathway mediated by TDG is indeed the active demethylation pathway downstream of 5hmC, the functional interplay between TDG and PB-induced gene activation would warrant investigation. 5hmC is not necessarily a committed intermediate towards active demethylation, as the further oxidized bases 5fC and 5caC provide alternative candidates as the committed intermediate toward demethylation. Profiling of 5fC and 5caC could therefore further reveal active demethylation at specific promoters. Another question raised by the model is which TET enzyme (or enzymes) is responsible for 5hmC generation in the PB-induced changes.

An additional angle to pursue is whether 5hmC has an epigenetic function that directly regulates gene expression, rather than simply acting as a demethylation intermediate. In this regard, two very recent studies provide evidence for a hypothesis in which 5hmC itself can activate target genes. The first study showed that a 5mC regulatory enzyme (Parp1) and a 5hmC-generating TET enzyme (Tet2) functioned separately during somatic cell reprogramming, whereas redundancy would be expected were 5hmC simply to be an intermediate [9]. In the second study, 5hmC regeneration was found to be a potent suppressor of melanoma progression [10].

Finally, the study by Meehan, Moggs and colleagues [3] may provide early biomarkers for cancer diagnostics and prognostics, although it must first be determined whether 5hmC changes during the early stages of carcinogenesis are recurring events in other non-genotoxic carcinogenesis exposure models. 5hmC holds promise not only in diagnostics, but also in therapeutics. Current epigenetic therapy efforts have mainly focused on targeting the DNA methylation and histone modification machineries, by using DNA methylation inhibitors and histone deacetylase inhibitors, respectively (Figure 1) [1]. From the results described in [3], however, it is tempting to speculate that, in certain cases, using

TET or TDG inhibitors to target the DNA demethylation machinery may also prevent cancer development (Figure 1).

#### Abbreviations

5caC, 5-carboxylcytosine; DNMTs, DNA methyltransferases; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; PB, phenobarbital; TET, ten-eleven translocation; TDG, thymine DNA glycosylase.

#### Competing interests

The authors declare they have no competing interests.

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