



Commentary

A catalytic dependent role for DNMT3B in tumor suppression

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While cancer is a genetic disease, epigenetic programs are intimately involved in the neoplastic state. This is especially true in the case of the DNA methylation. Epigenomic patterns of CpG methylation are markedly altered in transformed cells compared to healthy ones [1], and DNA methylation modifiers are commonly mutated in human cancers [2]. Understanding the mechanisms of DNA methylation in cancer is of paramount importance, as multiple inhibitors of DNA methylation are currently in use in the clinic [3].

In mammals, most (~70%) of cytosines in the CpG dinucleotide context are methylated on the 5' carbon. As DNA methylation is transcriptionally repressive, this helps to suppress spurious transcription and expression of endogenous retroviruses (ERVs). At promoter regions, however, densely packed "CpGs islands" are typically unmethylated, to avoid impeding transcription. These programs are markedly dysregulated in cancer [1]. Globally, the cancer epigenome is hypomethylated compared to normal counterparts, disrupting genome stability. In contrast, promoter CpG islands are hypermethylated in cancer, providing a critical mechanism for silencing tumor suppressor genes (TSGs). DNA methylation inhibitors such as Azacitidine and Decitabine can de-repress TSGs, but global DNA demethylation can be highly toxic [3]. Accordingly, there is significant need for a greater understanding of the multifaceted role of DNA methylation in cancer.

DNA methylation is catalyzed by the *de novo* methyltransferases DNMT3A and DNMT3B, as well as the maintenance methylase DNMT1, which propagates existing CpG methylation across semi-conservative DNA replication. In addition to their catalytic activity, the DNMTs also play structural roles in mediating transcriptional silencing via interactions with repressor complexes [4]. All three enzymes have been implicated in human cancers [2]; however, the role of DNMT3B is less well understood than that of DNMT3A or DNMT1. While experimental studies suggest a tumor suppressive role for DNMT3B in acute myeloid leukemia (AML) [5] and MYC-induced T-cell lymphomagenesis (MTCL) [6], these results were

based on *Dnmt3b* full knockout models, which do not discriminate between effects from total loss of DNMT3B protein vs. loss of DNMT3B catalysis.

In this issue of EBioMedicine, Lopusna et al. [7] investigate the role of DNMT3B in cancer, with a focus on disentangling the role of its catalytic vs. structural functionality in oncogenesis. The authors employ an elegant mouse model featuring homozygous expression of catalytically inactive DNMT3B (*Dnmt3b^{Cl/Cl}*) with a two amino acid substitution in the active site. This study builds on their previous work showing that, while full knock out of *Dnmt3b* is embryonic lethal, *Dnmt3b^{Cl/Cl}* mice survive, highlighting the importance of the non-catalytic "accessory function" of DNMT3B [8].

In the current study, the authors first demonstrate that DNMT3B catalysis is dispensable for postnatal development, and then examine the role of DNMT3B catalysis in oncogenesis. They find that *Dnmt3b^{Cl/Cl}* MEFs are more susceptible to oncogenic transformation *in vitro* and tumor formation *in vivo*. Turning to the hematological context, the authors then employ the commonly used MLL-AF9 model of AML, and the Myc overexpression model of Myc-induced T-cell lymphomagenesis (MTCL). In both cases, they find that *Dnmt3b^{Cl/Cl}* mice have decreased survival times. Together, these models support the catalytic-dependent tumor suppressive role of DNMT3B in oncogenesis.

The authors next investigate the role of DNMT3B in regulating the DNA "methylome" of MCTL lymphoma cells. *Dnmt3b* full knockout mice (*Dnmt3b^{-/-}*) have extensive losses of methylation, and one might expect similar losses in *Dnmt3b^{Cl/Cl}* mice. Surprisingly, despite lacking catalytic activity, expression of DNMT3B^{Cl/Cl} restores roughly 5/6th of the methylation losses. However, the remaining 1/6th – the putative direct catalytic targets of DNMT3B – are markedly enriched for the C-Met oncogenic pathway, and experimental activation of this pathway accelerates disease progression in *Dnmt3b^{+/-}* wild type mice. Thus, Lopusna et al. provide compelling evidence that DNMT3B suppresses oncogenesis via direct repression of the c-Met pathway, and their approach underscores the importance of using models that can distinguish the enzymatic activity of chromatin regulator from its non-catalytic structural role.

Questions remain regarding the tumor suppressive role of DNMT3B. First, how is DNMT3B mediating the methylation of many loci independently of its catalytic activity? Is it possible that DNMT3B interacts with another DNMT – possibly DNMT3A, as has been shown *in vitro* [9] – and it is this other DNMT that methylates these loci? Secondly, it will be important to determine the oncogenic contexts in which DNMT3B is most active as a tumor suppressor. The authors focus on AML and lymphoma, but their approach can be extended to

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other cancers as well. Finally, what are the mechanisms by which DNMT3B directly targets the c-Met pathway for methylation-based inactivation? DNMTs lack DNA sequence specificity and instead interact with histone modifications and other chromatin regulators to access their target loci [10]. How do these interactions recruit DNMT3B specifically to the regulatory regions of c-Met pathway genes? Such open questions carry clinical significance. DNA methylation inhibitors are in clinical use [3], but the tumor suppressive activity of DNMT3B suggests they should be used with caution.

Author contributions

H.L. - Literature review, assistance with manuscript writing
M.A.B. – Conceptualization, manuscript writing

Declaration of Competing Interest

The authors have nothing to declare.

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