



TDG is a novel tumor suppressor of liver malignancies

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ABSTRACT

In a recent publication, we demonstrated that conditional deletion of the gene encoding thymine DNA glycosylase (TDG) leads to a late onset of hepatocellular carcinoma (HCC). TDG loss causes disruption in active DNA demethylation in the liver and dysregulation of the farnesoid X receptor and small heterodimer partner (FXR-SHP) regulatory cascade. This leads to a loss of bile acid and glucose homeostasis, which predisposes mice to HCC.

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The methylation of cytosine at 5th carbon (5-mC) is an epigenetic mark that occurs predominantly in a CpG context. In mammalian cells, 70–80% of all CpG dinucleotides are methylated and are found within gene bodies, repetitive elements and regulatory elements such as enhancers and promoters.¹ Numerous studies have shown that 5-mC, in combination with other epigenetic modifications such as histone tail modifications, regulate gene expression patterns that are essential for a multitude of cellular processes. By regulating these processes, DNA methylation dictates parameters essential in diverse biological programs from development to normal physiological homeostasis.

The 5-mC mark is deposited by DNA methyltransferases (DNMTs), which transfer the methyl group from S-adenosylmethionine to cytosine. Although tissue-specific DNA methylation patterns are established early during embryogenesis, at many regulatory sites the 5-mC mark varies and can be removed via passive or active processes.¹ In passive DNA demethylation, 5-mC is diluted over successive replication cycles by limiting the activity of DNMTs or their associated co-factors. In contrast, active DNA demethylation is replication independent and involves the coordinated action of ten eleven translocation (TET) enzymes and members of the base excision repair (BER) machinery. In active DNA demethylation, 5-mC is oxidized by TETs to 5-hydroxymethylcytosine (5-hmC). The 5-hmC is further oxidized to 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC), which are specifically recognized and excised by thymine DNA glycosylase (TDG). This generates an apurinic site, which is repaired by the BER machinery to restore unmethylated cytosine. Loss of *Tdg* is embryonic lethal at embryonic age 11.5 and causes DNA hypermethylation and dysregulation of numerous developmentally regulated genes.² Importantly, *Tdg* null embryonic stem cells fail to differentiate

normally in response to retinoic acid (RA) and 5-fC/5-caC levels increase five-fold genome wide.^{2,3} These studies demonstrate that *Tdg* is essential for survival in part by maintaining epigenetic stability in cells.

It is well established that the DNA methylation landscape is dramatically altered in most cancers. 5-mC derivatives are largely depleted in tumors compared to normal tissues and TET mutations are common in several types of cancers. We have previously shown that the Hypermethylated in cancer 1 (*Hic1*) tumor suppressor gene undergoes active demethylation in response to RA and that loss of TDG precedes hypermethylation and silencing of *Hic1* *in vivo*.⁴ Recent studies in mice bearing a truncated adenomatous polyposis coil protein (*Apc^{min}*) and a colon-specific deletion of *Tdg* demonstrate a 2-fold increase in the number of small intestinal adenomas compared to *Wildtype* mice.⁵ Collectively, these studies suggest that *Tdg* has tumor suppressive functions. However, a direct role for *Tdg* as a tumor suppressor has not been formally demonstrated. In our recent *Cell Reports* publication,⁶ we addressed this question using a conditional knockout mouse model of *Tdg* (*Tdg_{CKO}; Tdg^{fl/fl}; UBC-creERT2⁺*). We observed efficient excision of *Tdg* in response to tamoxifen. Importantly, the loss of TDG persisted during the entire course of our aging study. Surprisingly, *Tdg_{CKO}* mice developed a late-onset (17–24 months post tamoxifen) of hepatocellular carcinoma (HCC) and hepatoblastoma (HB) with a male sex bias. The *Tdg_{CKO}* male mice displayed increased body weight, glucose intolerance and increased hepatic and serum bile acids (BA) with age. This was an important finding because the administration of exogenous BA or loss of BA homeostasis have been linked to several types of cancers including HCC.⁷ High-throughput transcriptomic analysis of the male livers followed by gene-set enrichment analysis identified metabolism as the most dysregulated pathway. In

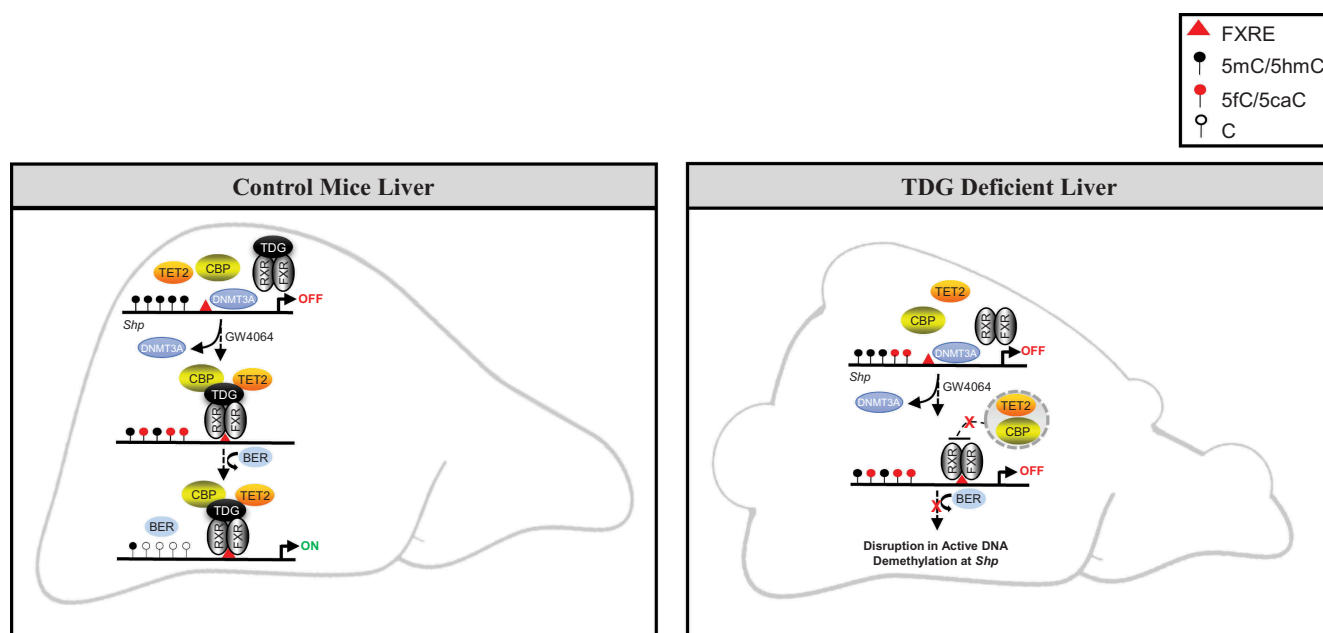


Figure 1. Mechanism of active DNA demethylation by FXR/TDG signaling. In the absence of ligand, the small heterodimer partner (*Shp*) promoter is hypermethylated and is repressed due in part to the binding of DNA methyltransferase 3A (DNMT3A). Binding of GW4064 (A bile acid receptor agonist) ligand to farnesoid X receptor (FXR) activates the FXR and retinoid X receptor (RXR) heterodimer which causes the assembly of thymine DNA glycosylase (TDG), ten eleven translocase 2 (TET2) and CREB-binding protein (CBP) to the FXR response element (FXRE) at the *Shp* locus. TET/TDG activities are coupled and act in concert to oxidize 5-methylcytosine (5-mC) to 5-formylcytosine (5-fC) or 5-carboxylcytosine (5-caC) followed by excision. The excised intermediates trigger recruitment of the base excision repair (BER) machinery to restore unmethylated DNA. The conditional deletion of *Tdg* in mice leads to a higher basal level of 5-fC/5-caC at *Shp*. In addition, co-activator complex assembly and active DNA demethylation at *Shp* in response to GW4064 are blocked, thereby, leading to a loss of *Shp* expression.

contrast, the *Tdg*_{CKO} females showed no statistically significant differences in weight and displayed higher insulin sensitivity relative to their age/sex matched controls. These findings demonstrate that deletion of *Tdg* leads to a late-onset HCC, and that sexual dimorphism may contribute to the phenotype in *Tdg*_{CKO} mice.

Using pathway analysis, we also identified dysregulation in nuclear hormone receptor signaling including the retinoic acid receptors (RAR) and farnesoid X receptor (FXR) signaling. FXR is a nuclear receptor that regulates BA homeostasis in the liver through upregulation of the small heterodimer partner (SHP). SHP is an orphan nuclear receptor that functions to transrepress cytochrome P450 family 7 subfamily A member 1 (CYP7A1), which is the rate-limiting enzyme in the primary BA synthesis pathway. Interestingly, both *Shp*^{-/-} and *Fxr*^{-/-} mice display many of the phenotypes observed in *Tdg*_{CKO} mice, such as glucose intolerance, increased BAs and a late-onset HCC.^{7,8} Mechanistically, we provide evidence that active DNA demethylation and co-activator complex assembly at *Shp*, and other FXR target genes, are TDG dependent events *in vivo* (Figure 1). Immunohistochemistry and dot-blot analysis of *Tdg*_{CKO} livers showed statistically significant accumulation of 5-caC in a subpopulation of cells in the liver. This provides evidence that the deletion of *Tdg* blocks active DNA demethylation, leading to an accumulation of 5-caC in the liver. Finally, we show that the phenotypes observed in *Tdg*_{CKO} mice were recapitulated using a liver-specific knockout mouse model (*Tdg*^{f/f}; *Alb-cre*[±]). Collectively, these findings demonstrate that a loss of *Tdg* leads to a dysregulation in the FXR-SHP axis in the liver and that *Tdg*_{CKO} mice exhibit an increased prevalence of HCC in a background of elevated serum and intrahepatic BAs.

The onset of liver cancer in *Tdg*_{CKO} mice is in stark contrast to *Tet* knockouts, which result in predominantly hematopoietic malignancies.⁹ These mice may get other cancers, but the resulting leukemia appears first and compromises survival. Unlike the hematopoietic system, the liver typically exhibits limited ongoing turnover, but following injury or in disease various cell types can undergo substantial expansion. Under normal conditions, hepatocytes are mitotically dormant and mostly found in the quiescent state (G₀) with only 0.025% of the cells undergoing DNA replication at a given time and a cell turnover of approximately 400 days.¹⁰ These unique growth properties of hepatocytes may provide a more favorable environment for the abnormal accumulation of 5-fC/5-caC in *Tdg*_{CKO} livers during active demethylation, which is a replication independent process. Additionally, loss of the protein scaffolding functions of TDG and associated transcriptional consequences may also contribute to the phenotypes observed in *Tdg*_{CKO} mice.

Overall, our study provides strong evidence that TDG-dependent active DNA demethylation is involved in regulating genes important for glucose and BA homeostasis in the liver, and that disruption of this process can lead to metabolic dysregulation and HCC. Future studies exploiting the role of active demethylation in disease pathology may elucidate novel diagnostic and therapeutic strategies for the treatment of HCC and other cancers.

Disclosure of potential conflict of interest

The authors declare no conflict of interest.

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