Review

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Connections between TET proteins and aberrant DNA modification in cancer

Yun Huang[1,2*] and Anjana Rao[1,2]

[1] La Jolla Institute, La Jolla, CA 92037, USA
[2] Sanford Consortium for Regenerative Medicine, La Jolla, CA 92037, USA

DNA methylation has been linked to aberrant silencing of tumor suppressor genes in cancer, and an imbalance in DNA methylation–demethylation cycles is intimately implicated in the onset and progression of tumors. Ten-eleven translocation (TET) proteins are Fe(II)- and 2-oxoglutarate (2OG)-dependent dioxygenases that successively oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), thereby mediating active DNA demethylation. In this review, we focus on the pathophysiological role of TET proteins and 5hmC in cancer. We present an overview of loss-of-function mutations and abnormal expression and regulation of TET proteins in hematological malignancies and solid tumors, and discuss the potential prognostic value of assessing TET mutations and 5hmC levels in cancer patients. We also address the crosstalk between TET and two critical enzymes involved in cell metabolism: *O*-linked β-*N*-acetylglucosamine transferase (OGT) and isocitrate dehydrogenase (IDH). Lastly, we discuss the therapeutic potential of targeting TET proteins and aberrant DNA methylation in cancer.

TET proteins oxidize 5-methylcytosine in DNA

DNA methylation controls diverse biological processes, including X chromosome inactivation, gene expression, and genomic imprinting [1]. Dysregulated DNA methylation is frequently observed in cancer, and comprises aberrant silencing of tumor suppressor genes due to increased DNA methylation at their promoters as well as global DNA hypomethylation leading to decreased genome stability. Both processes contribute to oncogenesis and tumor progression [2,3]. In normal cells, DNA methylation is mediated through the coordinated actions of several DNA methyltransferases (DNMTs) that transfer a methyl group from S-adenosyl methionine (SAM) to the carbon-5 position of cytosine [4] (Figure 1). DNA methylation occurs primarily in the CpG context; replication of symmetrically methylated CpG dinucleotides leads to the production of daughter strands bearing unmethylated CpGs. The resulting hemimethylated DNA strands are normally restored to their symmetrical methylation status by the maintenance DNA methyltransferase complex (DNMT1/UHRF1), which recognizes hemimethylated CpGs [5,6]. If this maintenance methylation does not occur, DNA becomes progressively demethylated through a 'passive' replication-dependent mechanism.

Recently, proteins of the ten-eleven translocation (TET) family were identified as dioxygenases that utilize two key co-factors: Fe(II) and 2-oxoglutarate (2OG), to oxidize successively the methyl group of 5-methylcytosine (5mC) to hydroxymethyl, formyl, or carboxyl groups, thus forming the oxidized methylcytosines 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) (together termed oxi-mC) [7–11] (Figure 1). It is now clear that these oxi-mC intermediates facilitate DNA demethylation in at least two ways. First, they potentiate passive DNA demethylation by interfering with maintenance DNA methylation by the DNMT1/UHRF1 complex, but they also effect 'active', replication-independent DNA demethylation as discussed below, reviewed in [10,11]. Second, two of the oxi-mC intermediates, 5fC and 5caC, can be excised by the DNA repair enzyme thymine-DNA glycosylase (TDG), followed by replacement with umodified cytosine through a base excision repair mechanism [8,12–15]. TDG was originally identified as an enzyme that excised thymine from T:G mismatches, but it is also able to bind and excise 5fC and 5caC with comparable affinity and efficiency, even though these modified bases are fully base-paired with G [8,13,14]. Other putative active DNA demethylation mechanisms are discussed elsewhere [10,11,15].

In this review, we focus on the role of TET proteins in cancer. TET proteins were named because of the rare ten-eleven translocation associated with myeloid and lymphoid malignancies that fuses the N-terminal region of the mixed lineage leukemia (MLL) gene (encoded on chromosome 10) to the C-terminal catalytic domain of TET1 (encoded on chromosome 11) [16,17]. More recently, the gene encoding TET2 was found to be frequently mutated or deleted in a variety of hematological malignancies [18,19]. Here, we provide an overview of TET loss-of-function mutations and aberrant TET expression or regulation in hematopoietic and solid cancers, describe the crosstalk between TET proteins and aberrant cancer metabolic pathways, and discuss the exciting possibility of targeting TET proteins with novel anticancer therapeutics.

Corresponding authors: Huang, Y. (yun.huang@ibt.tamhsc.edu); Rao, A. (arao@liai.org).

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*Present address: Institute of Biosciences & Technology, Texas A&M University Health Science Center, Houston, TX 77030, USA

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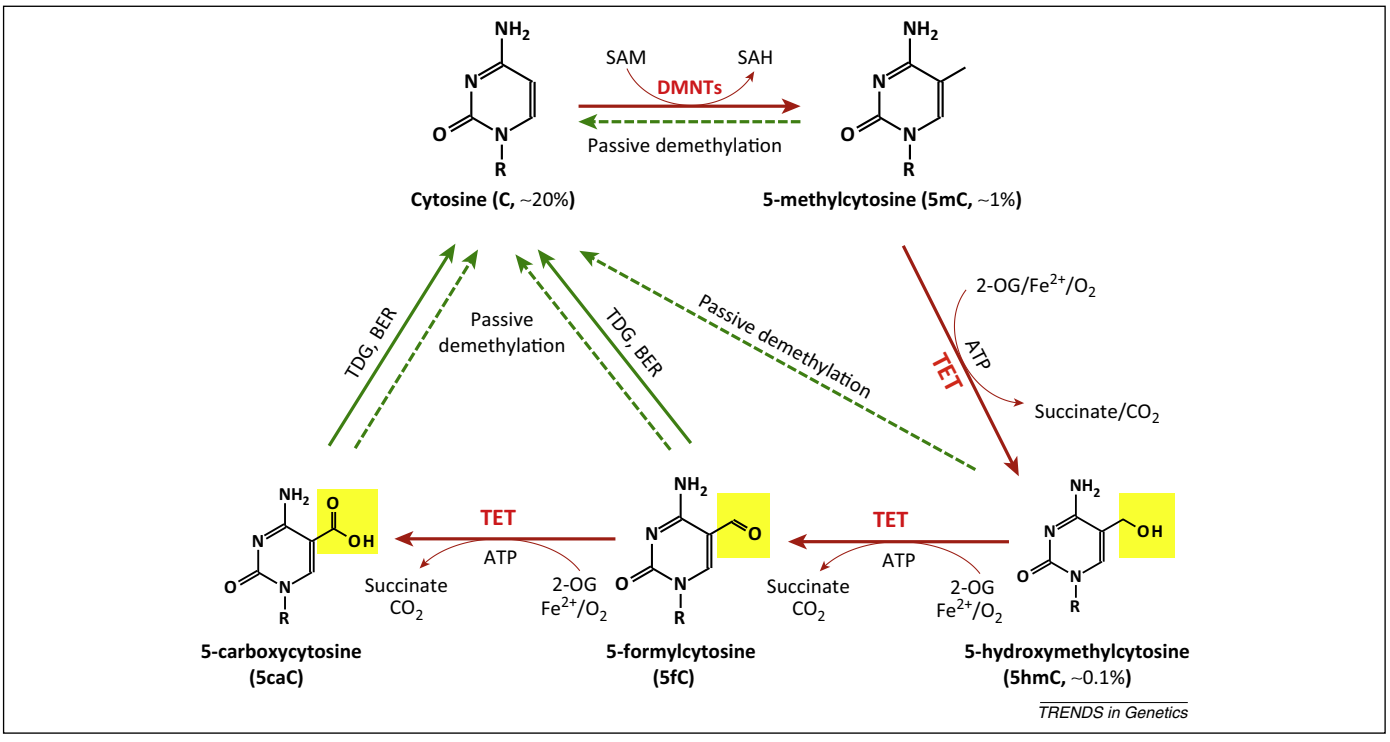

Figure 1. Schematic of major DNA methylation and demethylation pathways in mammals. DNA methylation occurs almost exclusively as symmetrical methylation at the carbon-5 position of cytosine in the context of the dinucleotide CpG. DNA methyltransferases (DNMTs) methylate cytosine (C; ~20% of all bases) to yield 5-methylcytosine (5mC; ~1% of all bases and ~60% of all CpGs) by transferring the methyl group from S-adenosylmethionine (SAM) to cytosine. Ten-eleven translocation (TET) enzymes oxidize 5mC to 5-hydroxymethylcytosine (5hmC; ~0.1% of all bases), 5-formylcytosine (5fC), 5-carboxylcytosine (5caC) (together: oxi-mC). Through oxi-mC production, TET proteins mediate multiple pathways of DNA demethylation, including thymine DNA glycosylase (TDG)-mediated base excision repair (BER) of 5fC:G and 5caC:G base pairs and replication-dependent passive demethylation. A recent study showed that TET proteins can oxidize thymine to 5-hydroxymethyluracil (5hmU) [128], and other studies have suggested that activation-induced deaminase (AID)/APOBEC can mediate the deamination of 5hmC to 5hmU followed by TDG-mediated BER, reviewed in [11]. Although 5hmU:G mismatches can also be excised by TDG [129], these pathways are less-well-characterized and so are not depicted here.

Mutations in TET proteins in hematological malignancies

TET1 and *TET3* are rarely mutated in hematological malignancies [20] (Box 1). By contrast, the 4q24 region of human chromosome 4 that harbors the *TET2* gene recurrently undergoes microdeletions and copy-number-neutral loss-of-heterozygosity (also termed uniparental disomy) in myelodysplastic syndromes (MDS) and myeloid malignancies [18,19]. *TET2* was originally identified as the relevant tumor suppressor gene in this region through the discovery of a patient with a myeloproliferative disorder who exhibited a 325 kb somatic microdeletion in 4q24 that encompassed only the *TET2* gene [18]. Since then, large-scale whole-exome sequencing studies by many groups have confirmed that *TET2* is one of the most frequently mutated genes in chronic myelomonocytic leukemia (CMML; ~50%) [21–23], acute myeloid leukemia (AML; ~20%) [24–27], and myelodysplastic syndromes (MDS; ~20%) [18,19,22,28,29]. In many cases, deletion of *TET2* in the 4q24 region is associated with a *TET2* mutation on the other allele [19]. Deletion of *Tet2* in mouse models is also associated with dysregulated hematopoiesis (Box 2).

So far, >700 *TET2* mutations have been identified in more than 2000 leukemia patients [26,30,31]. The majority of missense mutations impair the enzymatic activity of TET2, with a resultant decrease in 5hmC levels and aberrant DNA methylation [27,32]. The missense mutations tend to be clustered in two highly conserved regions of the human TET2 protein (amino acids 1104–1478 and 1845–2002) that correspond almost exactly to the well-structured regions observed in a recently determined crystal structure of the TET2 catalytic domain [33] (Figure 2). Based on the crystal structure, many of the residues affected by the missense mutations are located on the surface of the TET2 catalytic domain [33] (Figure 2); these residues might be important for protein–protein interactions and/or may be subject to post-translational modifications (e.g., phosphorylation, ubiquitylation, SUMOylation, glycosylation).

Whether or not *TET2* mutations have prognostic value for cancer patients is not yet entirely clear. A meta-analysis of a large cohort of AML patients described in eight published studies revealed a robust correlation between *TET2* mutations and poor prognosis, as judged by overall survival as well as event-free survival [30]. In a smaller cohort of MDS patients, *TET2* mutations significantly decreased the time of transformation of MDS to secondary AML as well as the probability of survival [34]. However, the prognostic potential of *TET2* mutations in MDS and CMML is still controversial. Based on studies of 96 and 88 patients with MDS and CMML, respectively, *TET2* mutations were reported to be predictive of a favorable prognosis in MDS [35] but were negatively correlated with overall survival in CMML [36]. By contrast, results from two other groups suggested no significant correlation between *TET2* mutations and overall prognosis in MDS or CMML [21,28,37]. Some of these discrepancies may be due to small sample sizes and could potentially be resolved by meta-analyses in