

## REVIEW ARTICLE

## TET2: A cornerstone in normal and malignant hematopoiesis

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**Abstract**

Regulation of genome-wide DNA methylation is fundamental for a variety of biological processes such as mammalian development, stem cell function, cellular proliferation/differentiation, and oncogenesis. Among the regulators of DNA methylation, ten-eleven translocation 2 (*TET2*) is one of the most frequently mutated genes in clonal hematopoiesis of indeterminate potential and in various hematological malignancies, underscoring a pivotal role for *TET2* in blood homeostasis and hematopoietic transformation. *TET2* oxidizes methylated cytosines to further modify cytosines, which behave as intermediates in active/passive DNA demethylation processes. *TET2* itself associates with histone modifiers, thereby regulating histone modifications and expression of target genes. A number of studies have reported pleiotropic effects of *TET2* on hematopoietic stem cell self-renewal, hematopoietic differentiation, genome instability and inflammatory response. Recent single-cell genomics studies have identified gene promoters as well as transcription factor binding sites as *TET2*-targeted genetic loci in which disruption of DNA methylation can fundamentally modify hematopoietic differentiation and promote leukemogenesis. *TET2* mutations show convergent cooperativity with other disease alleles in signaling molecules, epigenetic modifiers, and spliceosome factors in hematopoietic transformation. Future studies focusing on the molecular basis of stem cell and immune regulation by *TET2* loss will further deepen our understanding of the entire landscape of pathophysiology and molecular vulnerabilities of *TET2*-mutated hematological malignancies.

**KEYWORDS**

cytosine demethylation, hematological malignancy, hematopoietic stem cell, *TET2*

**1 | INTRODUCTION**

Epigenetic modifiers that regulate DNA cytosine methylation and histone methylation/acetylation are recurrent targets of genetic aberrations in hematological malignancies.<sup>1</sup> Ten-eleven translocation 2 (*TET2*) is one of the TET family proteins that either catalyzes serial oxidation of 5-methylcytosine (5mC) or mediates

histone modifications.<sup>2-6</sup> Genomic studies have uncovered recurrent *TET2* loss-of-function mutations in various hematological malignancies as well as in clonal hematopoiesis of indeterminate potential (CHIP),<sup>7-17</sup> suggesting a critical role for *TET2* in both normal and malignant hematopoiesis. In this review, we will summarize the most recent updates regarding *TET2* biology, focusing on its key roles in hematopoietic stem cell (HSC) self-renewal/differentiation, inflammatory response, and leukemogenesis.

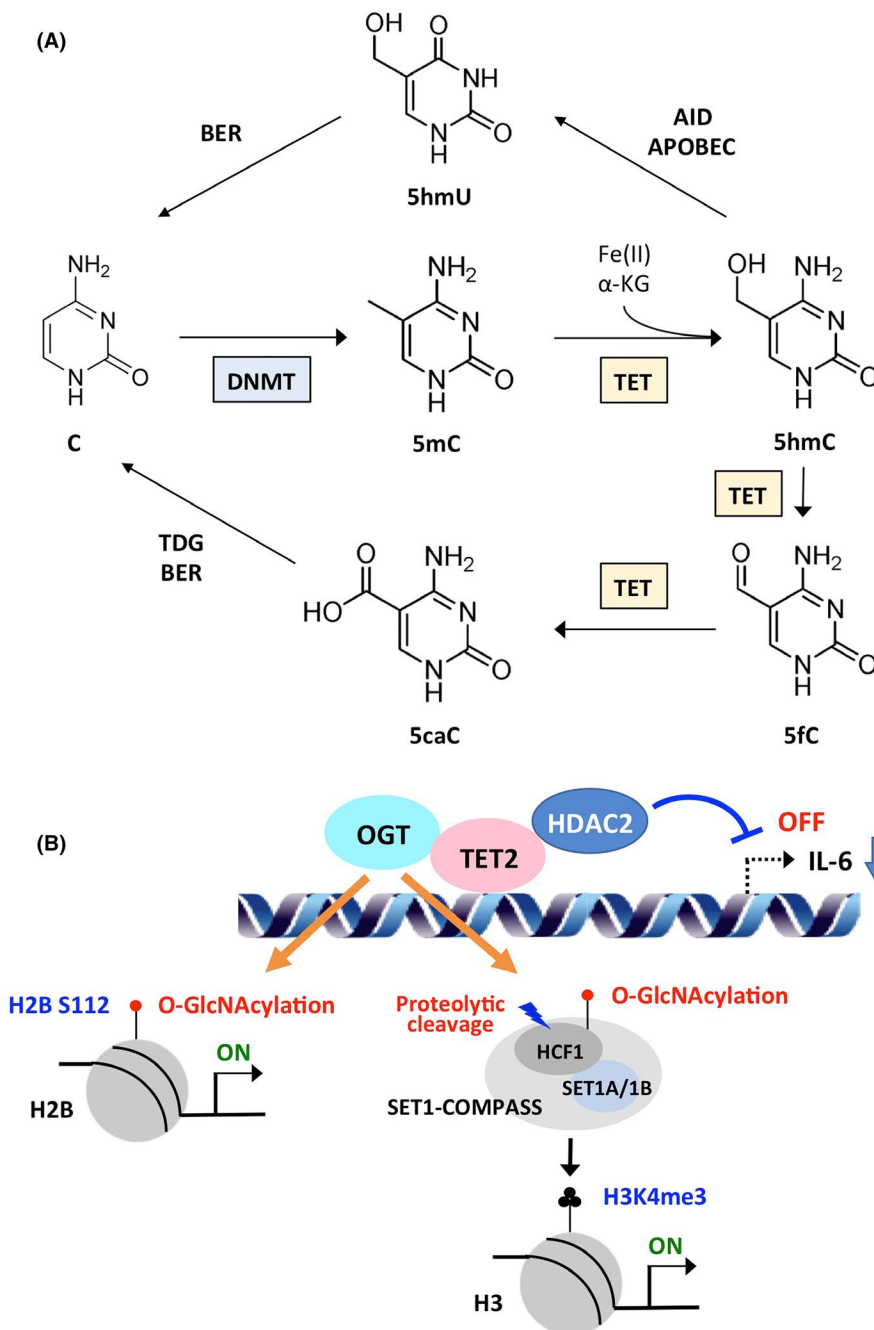
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## 2 | BIOCHEMICAL FUNCTIONS OF TET2

The TET family of proteins was first reported with the cloning of *TET1* as a fusion partner of *MLL1* in patients with t(10;11)(q22;q23) acute myeloid leukemia (AML).<sup>18</sup> TET proteins (TET1-3) are Fe(II)- and  $\alpha$ -ketoglutarate ( $\alpha$ -KG)-dependent mammalian DNA oxidases that catalyze the conversion of 5mC to 5-hydroxymethylcytosine (5hmC) (Figure 1A).<sup>2</sup> The discovery of this new modification on DNA methylcytosine has provided a novel insight into DNA demethylation pathways. The TET enzymes can further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), which can be directly recognized and repaired by thymine DNA glycosylase

(TDG)-mediated base-excision repair (BER) to generate unmethylated cytosines, leading to active DNA demethylation (Figure 1A).<sup>3,19</sup> Alternative active DNA demethylation through the activation-induced cytidine deaminase (AID)-APOBEC DNA repair pathway has also been reported. The first step of this pathway is the conversion of 5hmC to 5-hydroxymethyluracil (5hmU) by AID/APOBEC, followed by TDG- or single-strand-selective monofunctional uracil DNA glycosylase (SMUG1)-mediated BER to generate unmethylated cytosines (Figure 1A).<sup>20,21</sup> Conversely, 5hmC might also lead to passive DNA demethylation, as DNMT1, a maintenance methyltransferase that methylates unmethylated cytosines in the daughter strand upon DNA replication, cannot recognize 5hmC.<sup>22</sup>



**FIGURE 1** DNA demethylation and histone modification by ten-eleven translocation 2 (TET2). A, The biochemical process of DNA methylation/demethylation and the enzymes involved in each step are shown. Cytosine can be methylated into 5-methylcytosine (5mC) by DNA methyltransferases (DNMTs) and 5mC can be iteratively oxidized into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) by TETs. 5caC can be converted to unmethylated cytosine by the thymine DNA glycosylase (TDG)-mediated base-excision repair (BER) pathway. Alternatively, 5hmC can be deaminated into 5-hydroxymethyluracil (5hmU) by activation-induced cytidine deaminase (AID)/APOBEC, followed by TDG- or SMUG1-mediated BER to generate unmethylated cytosine.  $\alpha$ -KG,  $\alpha$ -ketoglutarate. B, TET2 interacts with O-linked  $\beta$ -N-acetylglucosamine (O-GlcNAc) transferase (OGT), tethering OGT to the target gene promoters and regulating gene transcription through histone H2BO-GlcNAcylation or H3K4 trimethylation mediated by proteolytic activation/O-GlcNAcylation of host cell factor 1 (HCF1), an integral component of H3K4 methyltransferase complex SET1/COMPASS. TET2 also recruits histone deacetylase 2 (HDAC2) to inactivate interleukin-6 (IL-6) transcription through histone deacetylation

Recent studies have also uncovered a novel role of TET proteins in histone modifications. Several groups have reported that TET2 interacts with O-linked  $\beta$ -N-acetylglucosamine (O-GlcNAc) transferase (OGT), tethering OGT to the target gene promoters and regulating gene transcription through histone H2B O-GlcNAcylation or O-GlcNAcylation/proteolytic activation of HCF1, an integral component of H3K4 methyltransferase complex SET1/COMPASS (Figure 1B).<sup>4-6</sup> Notably, TET2 was shown to enhance catalytic activity of OGT. However, Tet2 has been shown to recruit histone deacetylase 2 (Hdac2) to the interleukin-6 (*Il-6*) promoter and repress transcription of *Il-6* through histone deacetylation (Figure 1B).<sup>23</sup> These data indicate that TET2 modifies both chromatin landscape and DNA methylation, thereby regulating gene transcription.

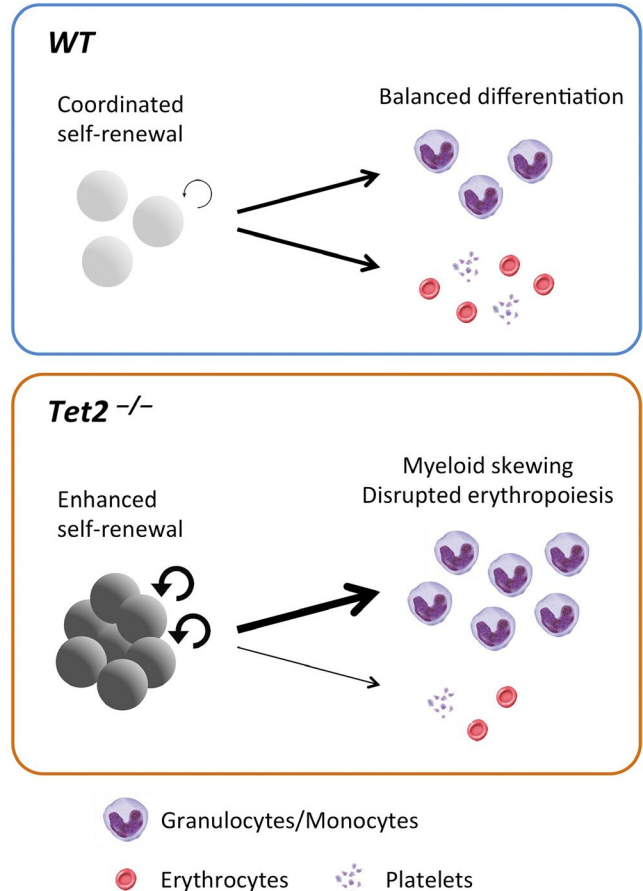
### 3 | TET2 MUTATIONS IN CHIP AND HEMATOLOGICAL MALIGNANCIES

TET2 is one of the major epigenetic modifiers recurrently mutated in individuals with CHIP.<sup>15-17</sup> Previous studies using high throughput genome-wide sequencing have also identified somatic deletions and loss-of-function mutations in the TET2 gene in 10%-20% of patients with myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPN),<sup>7,8</sup> in 10%-20% of patients with AML, and in 40%-50% of patients with chronic myelomonocytic leukemia.<sup>9,10</sup> Additionally, TET2 mutations are also reported in lymphoid malignancies, especially at high frequency in angioimmunoblastic T-cell lymphoma.<sup>11-14</sup> These data clearly suggest a common key role for TET2 as a tumor suppressor in hematological malignancies.

### 4 | PLEIOTROPIC EFFECTS OF TET2 LOSS ON HSC HOMEOSTASIS

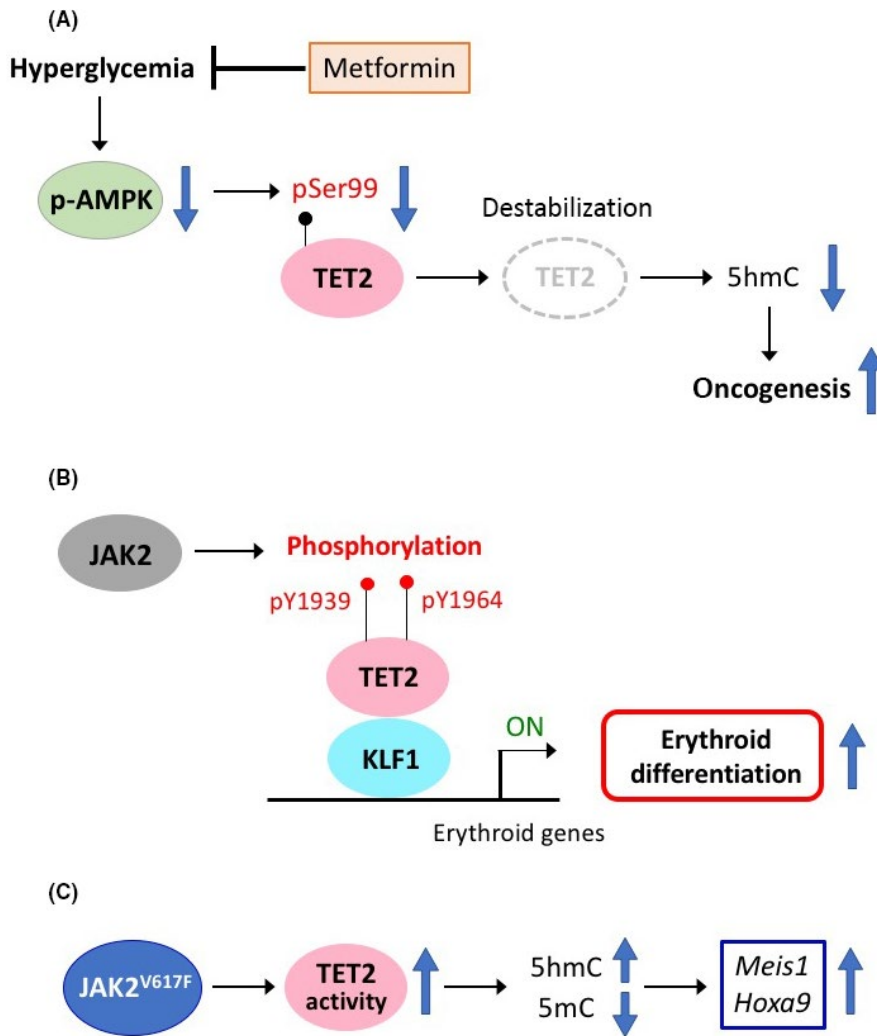
A number of studies have explored the functional role of TET2 in hematopoiesis and leukemogenesis. Initial study on TET2 mutations in myeloid malignancies has shown enhanced repopulating capacity of HSCs derived from TET2-mutated MPN patients in xenograft assay.<sup>7</sup> Tet2-silenced murine bone marrow (BM) hematopoietic stem/progenitor cells (HSPCs) showed preferential differentiation toward myeloid lineage in vitro.<sup>24</sup> Consistent with murine data, TET2 silencing in human cord blood CD34<sup>+</sup> cells led to skewed differentiation toward CD14<sup>+</sup> monocytic lineage in ex vivo culture.<sup>25</sup> In addition, *Aid*-deficient mice as well as *AID*-silenced human BM CD34<sup>+</sup> cells showed myeloid skewing.<sup>26</sup> These data suggest that active DNA demethylation mediated by the TET2/AID pathway functions as a safeguard mechanism against aberrant HSC self-renewal and their perturbation skews differentiation toward myeloid lineage (Figure 2).

In line with the above observations, several groups have generated mouse models of germline or conditional *Tet2* loss and reported common features in these mice, including disrupted hematopoietic differentiation, expansion of HSPC (LSK; Lin<sup>-</sup> Sca-1<sup>+</sup> c-Kit<sup>+</sup>) compartment and enhanced HSC self-renewal.<sup>11,27-31</sup> Furthermore,



**FIGURE 2** Ten-eleven translocation 2 (*TET2*) loss leads to clonal expansion and altered hematopoietic differentiation. Upper panel, normal hematopoietic stem cells (HSCs) maintain coordinated self-renewal and balanced hematopoietic differentiation, contributing to blood homeostasis. Lower panel, *Tet2*-deficient HSCs show enhanced self-renewal and altered hematopoietic differentiation, including myeloid skewing and disrupted erythropoiesis

some *Tet2*-null mice eventually developed myeloid malignancies in vivo.<sup>11,27,28</sup> Of note, *Tet2*-disrupted fetal liver common myeloid progenitors showed enhanced replating capacity in vitro, implying that *Tet2* loss could potentially transform more differentiated myeloid progenitors.<sup>32</sup> Additionally, microRNA-22 (miR-22), which targets TET2, was reported to be upregulated in MDS patient samples.<sup>33</sup> Mice conditionally expressing miR-22 displayed increased HSC self-renewal with defective hematopoietic differentiation and developed MDS similar to *Tet2*-deficient mice, confirming the functional relevance of the miR-22/TET2 regulatory network in myeloid transformation.<sup>33</sup> Interestingly, a recent study using transcriptomic/epigenomic profiling and mathematical modeling has shown that TET2 loss in human AML cell lines increases stem-like signatures and that TET2 loss of function alters the cellular switching dynamics between stem-like and differentiated cell states, thereby enhancing population fitness during drug treatment.<sup>34</sup> TET2 deficiency is also associated with disordered erythropoiesis in both human and mouse models.<sup>25,28,35,36</sup> Together, these data clearly show that *Tet2* regulates both myeloid/



**FIGURE 3** Role of ten-eleven translocation 2 (TET2) modification in leukemogenesis and erythropoiesis. A, Hyperglycemia impedes AMP-activated kinase (AMPK)-mediated phosphorylation of TET2 at serine 99, leading to destabilization of TET2. This results in dysregulation of both 5-hydroxymethylcytosine (5hmC) and the tumor-suppressive function of TET2, leading to oncogenesis. B, Cytokine receptor-associated JAK2 phosphorylates TET2 at tyrosines 1939 and 1964 following erythropoietin stimulation. Phosphorylated TET2 interacts with the erythroid transcription factor KLF1, thereby promoting transcription of erythroid genes and differentiation of erythroid progenitors. C, JAK2<sup>V617F</sup> mutation is associated with increased TET2 activity and cytosine hydroxymethylation as well as genome-wide loss of cytosine methylation, leading to increased expression of several oncogenic transcripts such as *Meis1* and *Hoxa9*. 5mC, 5-methylcytosine

erythroid differentiation and clonal hematopoietic expansion, and thereby functions as a tumor suppressor (Figure 2).

Recent studies have also uncovered novel aspects of Tet2 biology in blood homeostasis and hematopoietic transformation. Ito et al<sup>37</sup> analyzed *Tet2* catalytic-mutant and KO mice and reported that Tet2 enzymatic activity is critical for myelopoiesis, whereas noncatalytic activity is mainly associated with augmented HSC self-renewal and aberrant lymphopoiesis. Intriguingly, loss of Tet2 in BM mesenchymal stromal cells increased their proliferation, self-renewal, and osteoblast differentiation potential, which in turn accelerated *Tet2*-deficiency-mediated myeloid malignancy progression in vivo.<sup>38</sup> Notably, Tet2 loss leads to hypermutagenicity in HSPCs, as TET2-mutated myeloid tumors had significantly more mutational events than the tumors with WT TET2, particularly at the genomic loci enriched in 5hmC.<sup>39</sup> Consistent with this notion, a recent study has shown that TET2 mutant HSPCs are highly sensitive to topoisomerase 1 (TOP1)-targeted drugs and poly(ADP-ribose) polymerase 1 (PARP1) inhibitors due to low levels of tyrosyl-DNA phosphodiesterase and inability to remove TOP1 cleavage complexes, leading to DNA double-strand breaks and cell death.<sup>40</sup> These data suggest a novel functional relevance of noncatalytic activity and cell-extrinsic

effect and a role for genome stability of TET2 in HSC homeostasis and myeloid transformation.

## 5 | TET2 MEDIATES ANTILEUKEMIC ACTIVITY OF ASCORBATE

Ascorbate is a cofactor for several  $\alpha$ -KG-dependent dioxygenases, including TET oxidases.<sup>41</sup> Importantly, ascorbate is enriched in HSCs to promote *Tet2* activity in vivo, limiting HSC frequency and suppressing leukemogenesis.<sup>42</sup> Of note, vitamin C treatment mimics *Tet2* restoration to block leukemia progression and enhances sensitivity of leukemia cells to PARP inhibition.<sup>43</sup> These data suggest that TET2 mediates antileukemic activity of ascorbate in HSCs.

## 6 | TET2 MODIFICATION LINKS TO ONCOGENESIS AND ERYTHROPOIESIS

Two recent studies shed light on the functional link between TET2 modification and oncogenesis. Epidemiologically, diabetes is

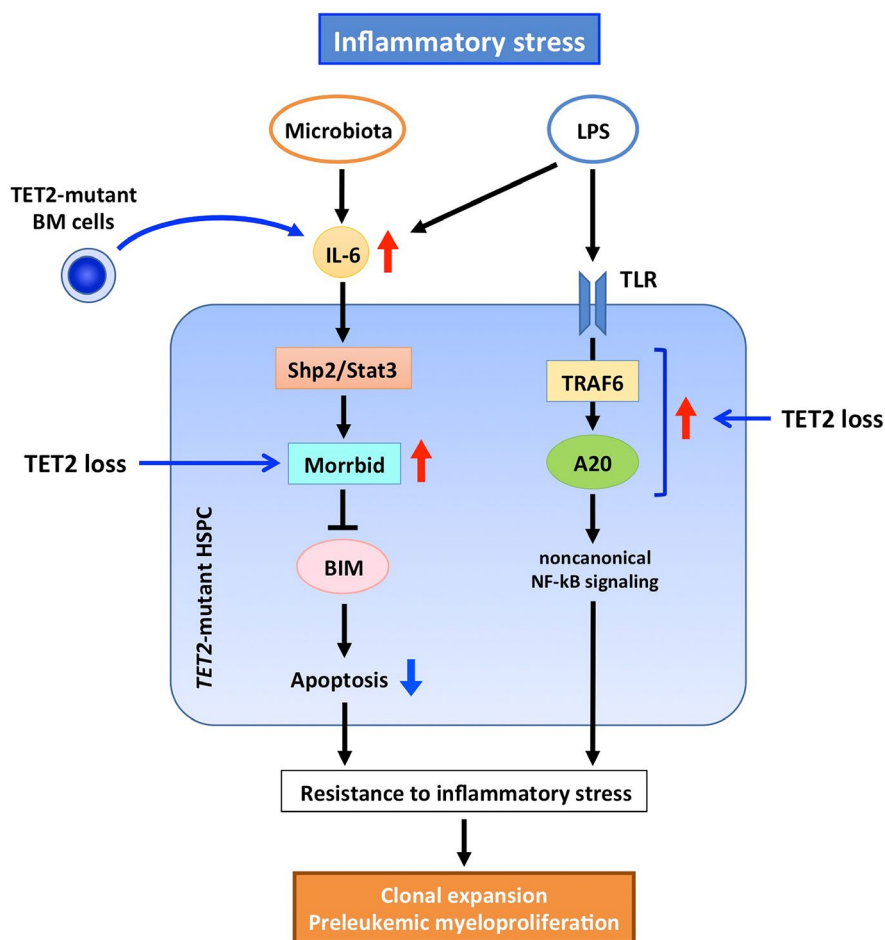
associated with increased risk of cancer.<sup>44</sup> Wu et al have shown that hyperglycemic conditions impede AMP-activated kinase (AMPK)-mediated phosphorylation of TET2 at serine 99, leading to its destabilization. This leads to dysregulation of both 5hmC and the tumor-suppressive function of TET2, mechanistically linking diabetes with cancer (Figure 3A).<sup>45</sup> Treatment with the antidiabetic drug metformin protects AMPK-mediated phosphorylation of TET2 at serine 99, thereby increasing TET2 stability and 5hmC levels (Figure 3A).<sup>45</sup>

Modification of TET2 is also involved in JAK2-mediated erythropoietin signaling as well as oncogenic JAK2<sup>V617F</sup> signaling. Jeong et al<sup>46</sup> have reported that cytokine receptor-associated JAK2 phosphorylates TET2 at tyrosines 1939 and 1964 upon erythropoietin stimulation. Phosphorylated TET2 interacts with the erythroid transcription factor (TF), KLF1, thereby promoting transcription of erythroid genes and differentiation of erythroid progenitors (Figure 3B).<sup>46</sup> Interestingly, CD34<sup>+</sup> cells derived from MPN patients with activating JAK2<sup>V617F</sup> mutation and murine HSPCs expressing *Jak2*<sup>V617F</sup> showed increased TET2 activity and cytosine hydroxymethylation as well as genome-wide loss of cytosine methylation, leading to increased expression of several oncogenic transcripts, such as *Meis1* and *Hoxa9* (Figure 3C).<sup>46</sup>

These results underscore a critical role for TET2 modification in oncogenesis and erythropoiesis.

## 7 | ROLES OF INFLAMMATORY SIGNALS IN CLONAL EXPANSION OF TET2-DEFICIENT HSPCS

Recent studies have addressed how inflammatory signals affect myeloproliferation and clonal expansion of *Tet2*-deficient HSPCs. It has been shown that microbial-dependent inflammation in small intestine and the resultant increase of IL-6 production are critical for the development of preleukemic myeloproliferation in *Tet2*-deficient mice.<sup>47</sup> Another study showed that the frequency and numbers of *Tet2*-deficient mature myeloid cells and HSPCs rapidly increase in response to acute inflammatory stress by lipopolysaccharide. Lipopolysaccharide induced enhanced production of IL-6 in *Tet2*<sup>-/-</sup> mice and *Tet2*-null HSPCs showed hyperactivation of the Shp2/Stat3/Morrbid pathway in response to IL-6. In addition, TET2 loss itself might also enhance *Morrbid* expression. As *Morrbid* is a prosurvival, long noncoding RNA that selectively suppresses *BIM* transcription, TET2 loss-induced enhanced expression of *Morrbid* led to reduced apoptosis and promoted survival of *Tet2*<sup>-/-</sup> HSPCs under basal conditions as well as inflammation (Figure 4).<sup>48</sup> Of note, *MORRBID* is overexpressed in murine *Tet2*<sup>-/-</sup> *Flt3*<sup>TD</sup> AML and a subset of human AML with *TET2* mutation.<sup>49</sup> These results together



**FIGURE 4** Inflammatory signals drive ten-eleven translocation 2 (*TET2*) loss-driven clonal expansion and preleukemic myeloproliferation. *Tet2*-null hematopoietic stem/progenitor cells (HSPCs) show hyperactivation of interleukin-6 (IL-6)/Shp2/Stat3/Morrbid pathway, which reduces apoptosis and promotes cell survival, clonal expansion, and preleukemic myeloproliferation of *Tet2*-deficient cells under basal conditions as well as inflammation. In addition, noncanonical nuclear factor-κB signaling mediated by Toll-like receptor (TLR)-TRAF6-A20 axis protects *Tet2*-deficient HSPCs from chronic inflammation and drives competitive advantage of these cells in an inflammatory milieu. BM, bone marrow; LPS, lipopolysaccharide



suggest that *Tet2* loss confers HSPCs with survival advantage during inflammation in an IL-6-dependent manner.

Similar to IL-6, cell-intrinsic noncanonical nuclear factor- $\kappa$ B signaling protects MDS HSPCs, including *Tet2*-deficient HSPCs, from chronic inflammatory stress driven by LPS (Figure 4).<sup>50</sup> In addition, both *Tet2*-deficient murine and *TET2*-mutant human HSPCs showed clonal advantage in an in vitro environment containing the proinflammatory cytokine, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ).<sup>51</sup>

These observations clearly depict emerging roles of inflammatory signals in *TET2* loss-mediated myeloproliferation and clonal expansion.

## 8 | TARGETS OF TET2-MEDIATED CYTOSINE MODIFICATION AND ITS FUNCTIONAL RELEVANCE

Exploring the exact genetic loci of TET2-mediated cytosine modification has been a focus of intense study for more than a decade. Recent study using a mammalian two-hybrid screen has revealed that SMAD nuclear interacting protein 1 (SNIP1) recruits TET2 to the promoters of *c-MYC* target genes, including those involved in DNA damage response and cell viability, thereby connecting epigenetic control to maintenance of genome stability.<sup>52</sup>

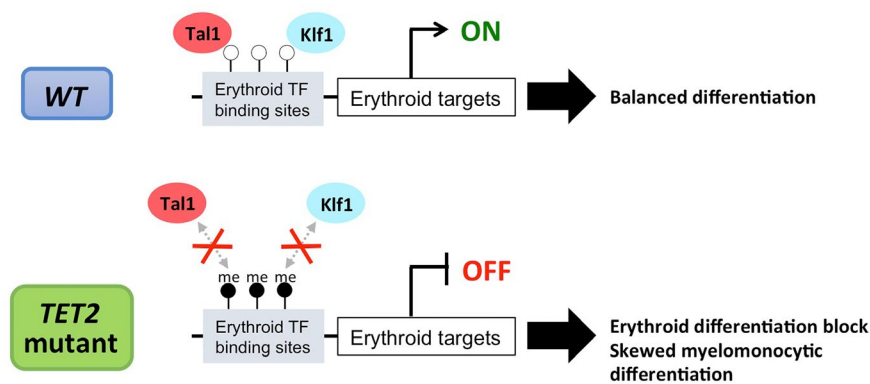
Ostrand et al.<sup>53</sup> compared functional difference between *Dnmt3a*-null and *Tet2*-null HSPCs and found that *Tet2* loss induced profound myeloid lineage skewing, whereas *Dnmt3a* loss conferred limitless self-renewal to HSCs. RNA sequencing (RNA-seq) and assay for transposase-accessible chromatin-sequencing (ATAC-seq) deciphered divergent transcriptomes and chromatin accessibility underlying these functional differences.<sup>53</sup> Genome-wide mapping of TET2 binding sites through ChIP and sequencing, together with ATAC-seq and RNA-seq, uncovered that TET2 localizes to regions of open chromatin and cell type-specific enhancers.<sup>54</sup> Following loss of TET2, chromatin binding of the members of basic helix-loop-helix (bHLH) TF family was attenuated in multipotent progenitors as well as in fully

transformed AML cells.<sup>54</sup> More recently, single-cell analyses for RNA-seq, DNA methylation, and ATAC-seq have shown that *Tet2* loss drives methylation of accessible TF binding sites, such as those of erythroid TFs. This leads to attenuated binding of erythroid TFs, including Klf1 and Tal1, to CpG-rich erythroid motifs, which then induces block of erythroid differentiation and skewed differentiation to myelomonocytic lineage in *Tet2*-null HSCs (Figure 5).<sup>55</sup> Together, these data suggest that *Tet2* loss-mediated disruption of DNA methylation in gene promoters as well as in TF binding sites alters the landscape of hematopoietic differentiation and promotes hematopoietic transformation.

## 9 | COOPERATIVITY AND EXCLUSIVITY OF MUTATED TET2 WITH OTHER DISEASE ALLELES IN LEUKEMOGENESIS

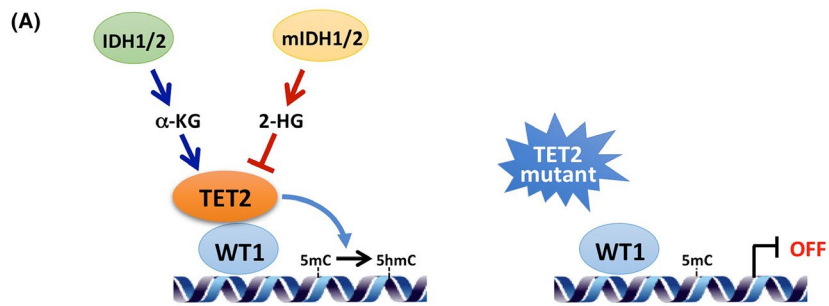
Recent genomic studies have uncovered mutual exclusivity and cooperativity with other disease alleles in *TET2*-mutant hematological malignancies.

Isocitrate dehydrogenase 1 and 2 (*IDH1/2*) and Wilms tumor 1 (*WT1*) mutations, recurrently seen in AML patients, are known to be mutually exclusive with *TET2* mutations in AML.<sup>1</sup> Interestingly, all these mutations are associated with global reductions in 5hmC levels in primary AML samples, suggesting a functional link between *IDH1/2*, *WT1*, and *TET2* in DNA hydroxymethylation.<sup>56</sup> Consistent with this notion, oncometabolite 2-hydroxyglutarate, produced by *IDH1/2* mutations, inhibits TET2 function as well as other  $\alpha$ -KG-dependent oxygenases.<sup>57,58</sup> In addition, WT1 physically interacts with and recruits TET2 to regulate its target gene expression. Furthermore, AML-derived *TET2* mutations disrupt its binding to WT1, and in turn, *WT1* mutations lead to impaired TET2 function and decreased 5hmC levels.<sup>56,59</sup> These studies revealed that *IDH1/2* mutations and *WT1* mutations share, at least partially, common epigenetic pathogenesis in AML with *TET2* mutations through altered DNA hydroxymethylation (Figure 6A).



**FIGURE 5** Ten-eleven translocation 2 (*TET2*)-mediated regulation of erythroid differentiation. Upper panel, in WT cells, TET2 demethylates erythroid transcription factor (TF) binding sites and allows erythroid TFs (Tal1 or Klf1) to bind these sites. This induces transcription of erythroid target genes and enables balanced differentiation between erythroid and myelomonocytic lineages. Lower panel, in *TET2*-mutant cells, erythroid TF binding sites are hypermethylated, which prevents erythroid TFs from binding these sites. This suppresses transcription of erythroid target genes and leads to erythroid differentiation block as well as skewed differentiation to myelomonocytic lineage

**FIGURE 6** Mutual exclusivity and cooperativity of ten-eleven translocation 2 (*TET2*) mutation with other disease alleles. A, left panel, Wilms tumor 1 (*WT1*) physically interacts with and recruits *TET2* to regulate its target gene expression through facilitating conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). Oncometabolite 2-hydroxyglutarate (2-HG) produced by isocitrate dehydrogenase 1 and 2 (*IDH1/2*) mutations inhibits *TET2* function by competing  $\alpha$ -ketoglutarate ( $\alpha$ -KG). A, right panel, acute myeloid leukemia (AML)-derived *TET2* mutations disrupt its binding to *WT1*, and in turn, *WT1* mutations lead to impaired *TET2* function, both of which lead to decreased 5hmC and increased 5mC levels, causing silencing of target gene expression. B, Cooccurring mutations with mutated *TET2* and their mouse models. CMML, chronic myelomonocytic leukemia; HSC, hematopoietic stem cell; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm



(B)

Gene	Function	Human disease	Mouse model	Phenotype of the mouse model	Reference#
FLT3-ITD	Signaling	AML	<i>Tet2</i> <sup>-/-</sup> x <i>FLT3-ITD</i>	AML through synergistic remodeling of DNA methylation	60
NRAS	Signaling	AML, MDS, MPN	<i>Tet2</i> <sup>-/-</sup> x <i>Nras</i> <sup>G12D</sup>	lethal CMML-like disease with decrease in <i>Spry2</i> and activation of MAPK signaling	61
KIT	Signaling	AML, MDS, MPN	<i>Tet2</i> <sup>-/-</sup> x <i>Kit</i> <sup>D814V</sup>	mastocytosis-like MPN partially due to PI3K activation	62
JAK2	Signaling	MPN	<i>Tet2</i> <sup>-/-</sup> x <i>Jak2V617F</i>	aggressive MPN by clonal HSC dominance and expansion of downstream precursors	63, 64
EZH2	Histone modification	MDS, MDS/MPN	<i>Tet2</i> <sup>-/-</sup> x <i>Ezh2</i> <sup>-/-</sup>	MDS or MDS/MPN through derepression of oncogenic polycomb targets	65
BCOR	Histone modification	AML, MDS, MPN	<i>Tet2</i> <sup>-/-</sup> x <i>Bcor</i> <sup>ΔE9-104</sup>	MDS or MDS/MPN	66
TET1	DNA methylation	B cell lymphoma	<i>Tet2</i> <sup>-/-</sup> x <i>Tet1</i> <sup>-/-</sup>	B-cell malignancies	67
DNMT3A	DNA methylation	AML	<i>Tet2</i> <sup>-/-</sup> x <i>Dnmt3a</i> <sup>-/-</sup>	T-cell lymphoma/leukemia	69, 70
TET3	DNA methylation	-	<i>Tet2</i> <sup>-/-</sup> x <i>Tet3</i> <sup>-/-</sup> <i>Tet2</i> <sup>+/-</sup> x <i>Tet3</i> <sup>-/-</sup>	lethal AML with spontaneous inactivation of residual non-targeted <i>Tet2</i> or <i>Tet3</i> allele	73
SF3B1	RNA splicing	MDS	<i>Tet2</i> <sup>-/-</sup> x <i>Sf3b1</i> <sup>K700E</sup>	early onset MDS, rescue of the competitive disadvantage of <i>Sf3b1</i> <sup>K700E</sup> stem cells	74
AML1-ETO	Oncogenic fusion gene	AML	<i>Tet2</i> <sup>-/-</sup> x <i>AML1-ETO</i>	fully penetrant AML, partially due to silencing of tumor suppressors	75, 76

*Tet2* mutations frequently cooccur with various other mutations, and functional cooperativity between these alleles in inducing hematological malignancies has been rigorously tested in mouse models.

Signaling molecules are frequently comutated with *TET2* in myeloid neoplasms, and studies have indicated epigenetic remodeling by *TET2* mutation synergizes with enhanced signaling to drive myeloid malignancies. A recent study has reported the cooperative function of *Tet2* loss and *Flt3*<sup>ITD</sup> mutation to induce AML in vivo through synergistic remodeling of DNA methylation by both mutations, resulting in reduced expression of *Gata2* by promoter methylation.<sup>60</sup> Concurrent *Tet2* loss and *Nras*<sup>G12D</sup> expression in hematopoietic cells induced lethal chronic myelomonocytic leukemia-like disease in vivo through decrease in negative regulators of MAPK signaling, including *Spry2*, thereby causing synergistic activation of MAPK signaling.<sup>61</sup> Concurrent *Tet2* loss and *Kit*<sup>D814V</sup> expression in hematopoietic cells induced mastocytosis-like MPN in vivo, partially due to PI3K activation.<sup>62</sup> Furthermore, studies from two independent groups have shown that combination of *Tet2* loss and *Jak2V617F* resulted in aggressive MPN phenotype through both clonal HSC dominance and expansion of downstream precursor populations.<sup>63,64</sup>

Mutations in epigenetic modifiers, such as the members of polycomb group complex, are another well-known partner for *TET2*

mutation. In agreement with cooccurrence of *TET2* and *EZH2* mutations in MDS and MDS/MPN patients, concurrent depletion of *Tet2* and *Ezh2* in mice developed MDS or MDS/MPN through derepression of oncogenic polycomb targets.<sup>65</sup> Tara et al<sup>66</sup> generated mice with concurrent *Tet2* loss and *Bcor* insufficiency, which developed MDS or MDS/MPN, further confirming functional cooperativity of these two alleles in myeloid transformation.

*TET2* mutations also cooccur with changes in other *TET* members or *DNMT3A* mutations in human acute B-lymphocytic leukemia and T-cell lymphoma.<sup>67,68</sup> Concordant with this, *Tet1/2* double-KO mice developed lethal B cell malignancies and *Tet2* loss combined with *Dnmt3a* mutation caused T-cell lymphoma/leukemia in vivo, possibly due to dysregulated *Bcl6*/*Myc* and *Notch* pathways, respectively.<sup>67,69,70</sup> Although *TET3* mutations are infrequent in hematological malignancies, its expression declines with age in mouse HSCs as well as in human peripheral blood T cells.<sup>71,72</sup> Consistent with this observation, both *Tet2*<sup>+/-</sup> *Tet3*<sup>-/-</sup> and *Tet2*<sup>-/-</sup> *Tet3*<sup>+/-</sup> mice developed lethal AML in vivo with spontaneous inactivation of residual nontargeted *Tet2* or *Tet3* allele, respectively, suggesting that this phenomenon is a recurrent genetic event during myeloid transformation with *Tet* insufficiency.<sup>73</sup>

*TET2* loss was also shown to cooperate with spliceosome factor mutations in MDS pathogenesis. The combination of the *Sf3b1*<sup>K700E</sup> mutation and *Tet2* deletion causes an early onset of MDS characteristics and rescues the competitive disadvantage of *Sf3b1*<sup>K700E</sup> stem cells in vivo.<sup>74</sup> Oncogenic fusion gene associated with chromosomal translocation also cooperates with *TET2* mutation. Combined *Tet2* loss with *AML1-ETO* expression leads to fully penetrant AML in vivo, partially due to hypermethylation of enhancer regions, thereby silencing tumor suppressors.<sup>75,76</sup>

Collectively, these data suggest that functional convergent cooperativity of *TET2* mutations and cooccurring disease alleles drives hematopoietic transformation (Figure 6B).

## 10 | CONCLUSION

Biochemical analyses have revealed fundamental roles of *TET2* in active/passive DNA demethylation processes and in histone modification, thereby regulating expression of target genes. Previous genomics studies have identified somatic deletions and loss-of-function mutations in the *TET2* gene in various hematological malignancies. Since the first report of *TET2* mutations in myeloid malignancies in 2009, a number of groups have explored the functional relevance of *TET2* loss in normal and malignant hematopoiesis. These studies uncovered pleiotropic effects of *TET2* on HSC self-renewal, myeloid/erythroid differentiation, genome instability, and inflammation. Recent seminal studies have identified gene promoters as well as TF binding sites as key genetic loci in which disruption of DNA methylation can significantly alter the hematopoietic differentiation landscape and promote hematopoietic transformation. Finally, mutations in signaling factors, epigenetic modifiers, and spliceosome factors have been shown to cooperate with *TET2* loss in the pathogenesis of hematological malignancies. Deeper insights into the molecular basis of stem cell and immune regulation by *TET2* loss will allow us to decipher the exact pathophysiology and molecular vulnerabilities of *TET2*-mutant hematological malignancies.

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## CONFLICT OF INTEREST

The authors have no conflict of interest.

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