Review Article

TET2 as an epigenetic master regulator for normal and malignant hematopoiesis

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DNA methylation is one of the critical epigenetic modifications regulating various cellular processes such as differentiation or proliferation, and its dysregulation leads to disordered stem cell function or cellular transformation. The ten-eleven translocation (*TET*) gene family, initially found as a chromosomal translocation partner in leukemia, turned out to be a key enzyme for DNA demethylation. *TET* genes hydroxylate 5-methylcytosine to 5-hydroxymethylcytosine, which is then converted to unmodified cytosine through multiple mechanisms. Somatic mutations of the *TET2* gene were reported in a variety of human hematological malignancies such as leukemia, myelodysplastic syndrome, and malignant lymphoma, suggesting a critical role for *TET2* in hematopoiesis. The importance of the TET-mediated cytosine demethylation pathway is also underscored by a recurrent mutation of isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* in hematological malignancies, whose mutation inhibits TET function through a novel oncometabolite, 2-hydroxyglutarate. Studies using mouse models revealed that *TET2* is critical for the function of hematopoietic stem cells, and disruption of *TET2* results in the expansion of multipotent as well as myeloid progenitors, leading to the accumulation of premalignant clones. In addition to cytosine demethylation, TET proteins are involved in chromatin modifications and other cellular processes through the interaction with *O*-linked β-*N*-acetylglucosamine transferase. In summary, *TET2* is a critical regulator for hematopoietic stem cell homeostasis whose functional impairment leads to hematological malignancies. Future studies will uncover the whole picture of epigenetic and signaling networks wired with *TET2*, which will help to develop ways to intervene in cellular pathways dysregulated by *TET2* mutations.

E pigenetic modifications such as DNA methylation or histone methylation/acetylation play pivotal roles in cellular proliferation and differentiation, and their dysregulation leads to aberrant stem cell function and tumorigenesis.[1] Genome-wide whole exome sequencing has revealed recurrent mutations in epigenetic regulators (e.g. *TET2*, *DNMT3A*, or *ASXL1*) in hematological malignancies such as acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS).[2] Mutations of epigenetic regulators are so common in hematological malignancies that they constitute one of the largest groups of mutation, including those affecting splicing machinery, signaling pathways, or transcription factors.[2]
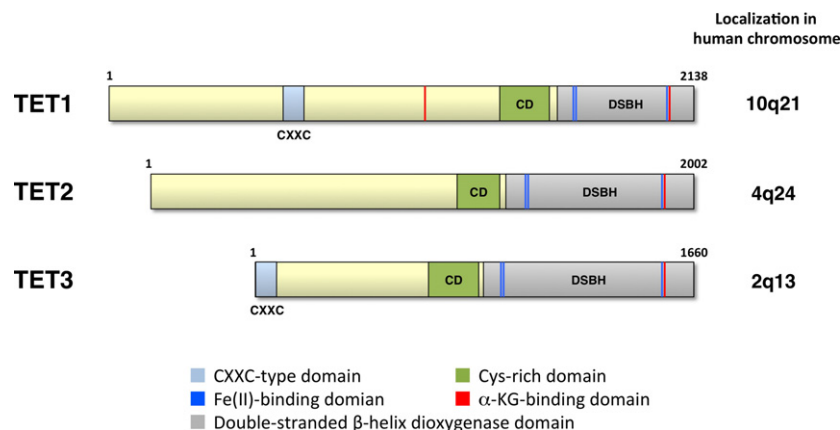
The ten-eleven translocation (TET) family of proteins are comprised of three family members, *TET1*, *TET2*, and *TET3*. The first, *TET1*, was cloned from the breakpoint of chromosomal translocation t(10;11) in infant AML, and subsequent homology searches have identified two additional members, *TET2* and *TET3*. TET proteins turned out to be key enzymes for DNA demethylation, and the discovery of recurrent mutations of *TET2* in hematological malignancies suggested that the regulation of cytosine methylation is critical for transformation of hematopoietic cells and normal hematopoiesis.

This review will summarize recent progress and current understanding of TET2, focusing on its roles in normal hematopoiesis and the pathophysiology of hematological malignancies.

TET Family Proteins – Structure and Function

The first TET family member, *TET1/LCX*, was originally cloned as a fusion partner of the *MLL* gene from the breakpoint of chromosomal translocation t(10;11)(q22;q23) found in infant AML.[3,4] Homology searches of *TET1* subsequently led to the discovery of two additional family members, *TET2* and *TET3*.

Structural analyses have revealed common features shared among TET family members (Fig. 1). The carboxyl (C)-terminal of TET proteins harbors the catalytic double-stranded β-helix dioxygenase domain, which demonstrates oxidating activity against 5-methylcytosine (5-mC). This domain contains binding sites for α-ketoglutarate (α-KG) and Fe(II), critically required for their catalytic function. In the middle of TET proteins, just before the double-stranded β-helix dioxygenase domain, resides a cysteine-rich domain with unknown function. The amino (N)-terminal region of TET1 and TET3 retains an evolutionarily conserved CXXC-type domain that is

Fig. 1. Structure and functional domains of ten-eleven translocation (TET) family proteins. α-KG, α-ketoglutarate; CD, cysteine-rich domain; Cys, cysteine; DSBH, double-stranded β-helix domain.

critical for binding to unmethylated cytosine residues in DNA.[5] Interestingly, TET2 does not have a CXXC-type domain. It is considered that the CXXC-type domain of *TET2* separated during evolution and became an independent, CXXC-containing gene called *IDAX* that is located at the 5′- end of the *TET2* gene in the opposite orientation.[6] *IDAX* was reported to negatively regulate *TET2* activity by facilitating its degradation through direct binding.[6]

Deregulation of DNA Methylation in Hematological Malignancies

Methylation of cytosine residue is one of the most critical epigenetic regulations for gene expression.[1,7,8] Cytosine methylation, which is mediated by DNA methyltransferase (*DNMT*), occurs frequently on CpG sites, alternate stretches of cytosine and guanine residues in DNA. Methylation of CpG clusters (called CpG islands) located at gene promoters suppresses transcription, and aberrant promoter methylation is commonly observed in tumor suppressor genes, such as *p16*, *PTEN*, *SFRP*, and *APC*, in various tumors including hematological malignancies.[9–11] It is therefore acknowledged that promoter methylation of tumor suppressor genes is one of the crucial mechanisms for tumor development.[11]

TET Proteins are Critical Regulators for DNA Demethylation

In 2009, it was reported that TET family proteins play critical roles in DNA demethylation by converting 5-mC to 5-hydroxymethylcytosine (5-hmC) in α-KG and in an Fe(II)-dependent manner.[12,13] A key active intermediate, 5-hmC is further processed by several pathways back to unmodified cytosine (Fig. 2). DNA replication-dependent, passive dilution of 5-hmC is considered one of the major mechanisms for demethylation after hydroxylation of 5-mC. Another pathway is deamination of 5-hmC by the activation-induced cytosine deaminase (AID)/apolipoprotein B mRNA-editing enzyme complex (APOBEC), which generates 5-hydroxymethyluracil. Subsequently, 5-hydroxymethyluracil may be repaired and converted to cytosine by base excision repair processes with thymine DNA glycosylase (TDG).[7,14–17] However, the physiological relevance of this pathway remains controversial, as deamination of 5-hmC by AID/APOBEC cannot be detected *in vitro* or in cells.[18,19] The other pathway involves TET-mediated successive conversion of 5-hmC to 5-formylcytosine and 5-carboxylcytosine, both of which are recognized and excised by TDG and are repaired back to unmodified cytosine by the base excision repair mechanism (Fig. 2).[20–22] DNA demethylation is therefore a dynamic, genome-wide process critically involving TET proteins.[23]

Mutation of *TET2* in Hematological Malignancies

Recurrent mutation of *TET2* in hematological malignancies was first reported in MDS patients with chromosome 4q24 abnormalities. *TET2* was identified from uniparental disomy or a commonly deleted region in chromosome 4q24 of MDS patients, and it was shown that approximately 26% of MDS patients harbored mutations (deletions or missense/nonsense
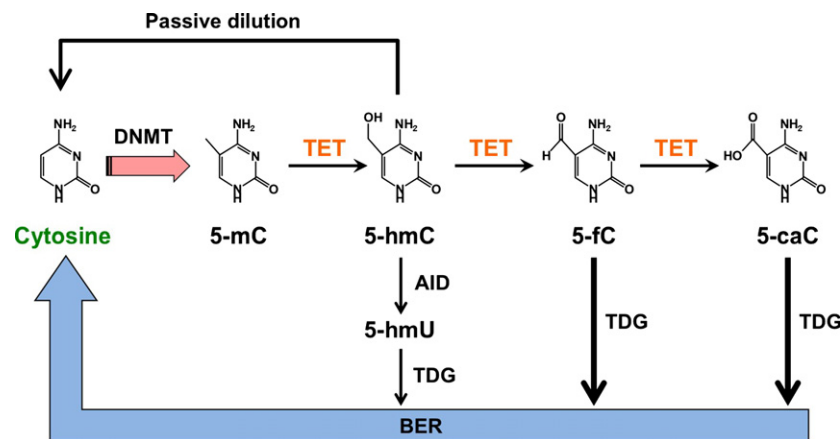
Fig. 2. Pathways of cytosine demethylation mediated by ten-eleven translocation (TET) proteins. AID, adenosine deaminase; BER, base excision repair; 5-caC, 5-carboxylcytosine; DNMT, DNA methyltransferase; 5-fC, 5-formylcytosine; 5-hmC, 5-hydroxymethylcytosine; 5-hmU, 5-hydroxymethyluracil; 5-mC, 5-methylcytosine; TDG, thymine DNA glycosylase.
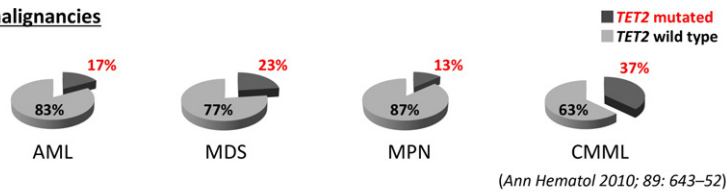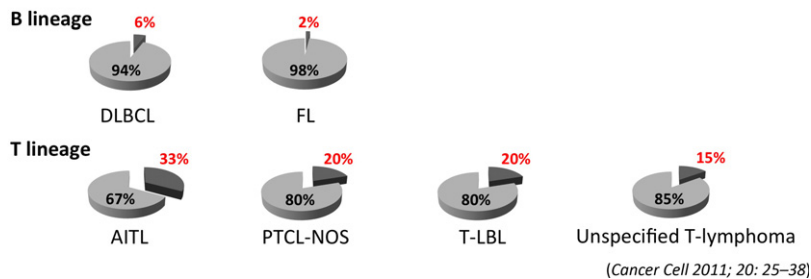
Fig. 3. Frequency of *TET2* mutation in various hematological malignancies. AITL, angioimmunoblastic T-cell lymphoma; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; T-LBL, T-lymphoblastic lymphoma.

mutations) in the coding region of *TET2*.[24] Another research group has also reported mutations or deletions of the *TET2* gene in patients with AML, MDS, or myeloproliferative neoplasm, suggesting their causative roles for disease initiation.[25] Subsequent studies have shown that *TET2* was mutated in a wide range of hematological malignancies including chronic myelomonocytic leukemia (CMML) and malignant lymphoma (Fig. 3).[26–28] The frequency of mutation is particularly higher in CMML (30–50%) and T-cell lymphoma, such as angioimmunoblastic T-cell lymphoma (50–80%) or peripheral T-cell lymphoma, not otherwise specified (40–50%).[27,29–31] It is considered that mutations disrupt the catalytic activity of *TET2*, as they are either missense mutations in the C-terminal catalytic domain or nonsense/frame-shift mutations in the N-terminal region leading to premature truncation before the catalytic core.[32] These findings suggest that disruption of *TET2*-mediated DNA demethylation processes constitutes a critical background for development of hematological malignancies.[32] Most of the *TET2* mutations are heterozygous, however, some mutations were found to be double heterozygous[30,33] or homozygous in uniparental disomy of chromosome 4q24.[24]

Mutation of Metabolic Pathways Converge on TET2-Mediated DNA Demethylation

Other recurrent genetic mutations in hematological malignancies include key enzymes of tricarboxylic acid (TCA) cycle, isocitrate dehydrogenase 1 (*IDH1*) and *IDH2*.[2,34–37] *IDH1* and *IDH2* (*IDH1/2*) catalyze the conversion of isocitrate to α-KG, an essential cofactor for *TET2*. Their mutations, mostly heterozygous somatic mutations at R132 of *IDH1* and at R140 or R172 of *IDH2*, were originally described in approximately 70% of low-grade glioma or secondary glioblastoma multiforme, and subsequently in up to 10% of cytogenetically normal AML.[38–40] Interestingly, mutations of *IDH1/2* confer them with a gain-of-function activity to generate a novel oncometabolite, 2-hydroxyglutarate (2-HG).[41–43] 2-Hydroxyglutarate inhibits the function of α-KG-dependent enzymes such as TET2 or Jumonji D3 by competing with α-KG (Fig. 4).[44] Of note, mutant *IDH1/2* does not act in a dominant negative manner to its wild-type counterparts.[45] It is therefore suggested that perturbation of *TET2* function by 2-HG is a primary mechanism for oncogenesis mediated by mutant *IDH1/2*. This notion is supported by the observation that mutations of *IDH1/2* and *TET2* are mutually exclusive in most hematological malignancies.[2,46] It is note-
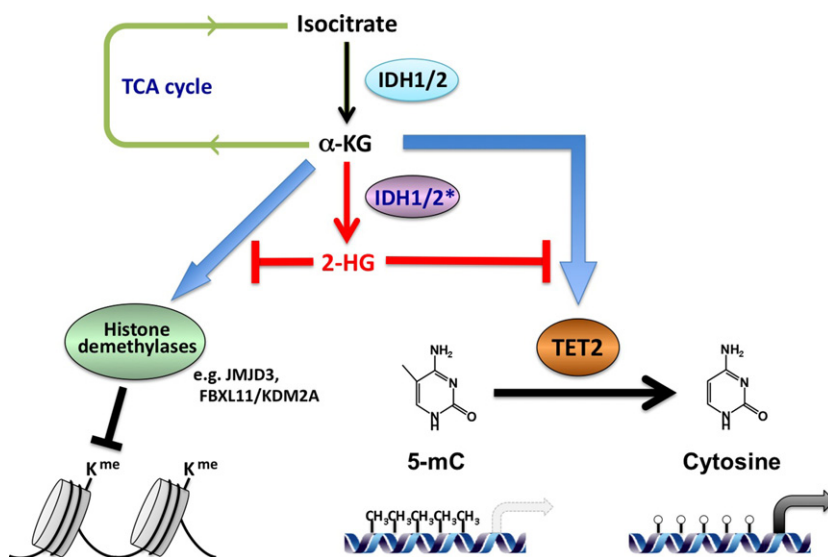
Fig. 4. Inhibition of ten-eleven translocation (TET)-mediated DNA demethylation processes by mutant isocitrate dehydrogenase (IDH)1/2. 2-Hydroxyglutarate (2-HG) competitively inhibits the function of α-ketoglutarate (α-KG)-dependent enzymes such as TET2 and histone demethylases like JMJD3 or FBXL11/KDM2A. IDH1/2*, mutant IDH1 and mutant IDH2; K^me, methylated lysine; 5-mC, 5-methylcytosine; TCA, tricarboxylic acid.

worthy that frequent coexistence of *TET2* and *IDH2* mutations was recently described in T-cell lymphomas,[30,47] whose pathological implication in lymphomagenesis is to be elucidated in future studies. Taken together, these findings underscore the importance of *TET2*-mediated epigenetic regulation, plausibly DNA demethylation processes, in the development of hematological malignancies.

Regulation of Hematopoietic Stem Cell Homeostasis by *TET2*

Frequent mutation of *TET2* in hematological malignancies strongly suggests that *TET2* plays a critical role in the regulation of normal hematopoiesis as well. Several groups, including ours, have tried to answer this question using *Tet2*-mutant mice generated by different strategies.[27,48–52] These included simple knockout, conditional knockout, or gene-trap, which targeted distinct regions of *Tet2*. Strikingly, these mice all presented highly similar phenotypes in the hematopoietic system, supporting the hypothesis that *TET2* is a critical regulator for HSC homeostasis.

In adult bone marrow (BM) of *Tet2* homozygously mutant mice, lineage negative Sca-1^+ c-Kit$^+$ (LSK) fraction containing hematopoietic stem cells (HSCs) and multipotent progenitors was expanded as compared to wild-type (WT), while frequencies of highly purified HSC fractions (CD34$^-$LSK, CD150$^+$LSK) were not affected. Common myeloid progenitors (CMPs) were also amplified in *Tet2* homozygous mice. Surprisingly, *Tet2*-mutated HSCs showed enhanced self-renewal and long-term repopulation (LTR) capacities in serial transplantation assays as compared to WT. *Tet2*-mutated HSCs are extremely competitive over WT counterparts *in vivo*, as peripheral engraftment of the former became increasingly dominant when cells were transplanted serially into the secondary and tertiary recipients.[51] In fact, *Tet2*-mutated HSCs were amplified in the BM of the recipient animals. We have shown that these HSC phenotypes were observed not only with BM HSCs, but also with HSCs from fetal livers (FL) of *Tet2* homozygous mice, suggesting that the critical role of *Tet2* in HSC homeostasis is conserved throughout development.[51] It is interesting to note that heterozygous disruption of *Tet2* resulted in the enhanced HSC capacity and myeloid expansion phenotype similar to those of *Tet2* homozygous mice, indicating haploinsufficiency of *Tet2* is sufficient to impair HSC homeostasis.[48,51]

These observations strongly suggest that, in steady-state hematopoiesis, *TET2* limits the function of HSCs such as self-renewal and LTR capacities, and functional disruption of *TET2* either by gene-targeting or disease-associated mutations would lead to aberrantly elevated HSC capacities and subsequent amplification of multipotent progenitor/CMP fraction.

TET2 is a Tumor Suppressor For Hematological Malignancies In Mice

Compatible with the finding of frequent *TET2* mutations in hematological malignancies, most *Tet2*-mutant strains develop CMML-like myeloid disease with splenomegaly, a sign of extramedullary hematopoiesis, or T-cell lymphoma after a long latency, usually more than several months.[27,48,53] However, penetrance of the disease was only 20–40%, and some mutant strains did not even show a sign of peripheral myeloid expansion during the observation period up to 2 years.[51] Taken together, these observations suggest that *TET2* mutation *per se*

is not sufficient for disease formation and requires additional mutations for developing full-blown disease. Genome studies on human patient samples have confirmed this speculation and led to the discovery of candidate mutations plausibly cooperating with *TET2*.[54–56] Future studies will focus on prospective validation of minimal genetic events collaborating with *TET2* mutation to initiate hematological malignancies.

Role for *TET2* Mutation in Human Hematological Diseases

Molecular processes leading to hematological malignancies in human patients with *TET2* mutation are considered to be quite similar to those in *Tet2*-mutant mice, as most *TET2* mutations in human disease are of the loss-of-function type, as described above. In fact, it was reported that hematopoietic progenitor cells (HPCs) (CD34$^+$ cells) from patients with *TET2* mutation showed expansion when transplanted into immune-deficient mice and knockdown of *TET2* in cord blood HPCs led to aberrant myeloid differentiation.[25] Importantly, transformation of *TET2*-mutated cells was not observed in these assays, confirming that *TET2*-mutated HSCs/HPCs are not fully transformed, but rather behave as premalignant cells. In support of this notion, it was recently shown that *TET2* was mutated in 5–6% of elderly individuals with myeloid-skewed clonal hematopoiesis.[57] Surprisingly, these individuals were normal in peripheral complete blood cell count and showed no sign of either myelodysplasia or leukemia. These data strongly suggest that, similar to the knockout models, mutation of *TET2* leads to a generation of premalignant clones, setting a critical basis for malignant transformation.
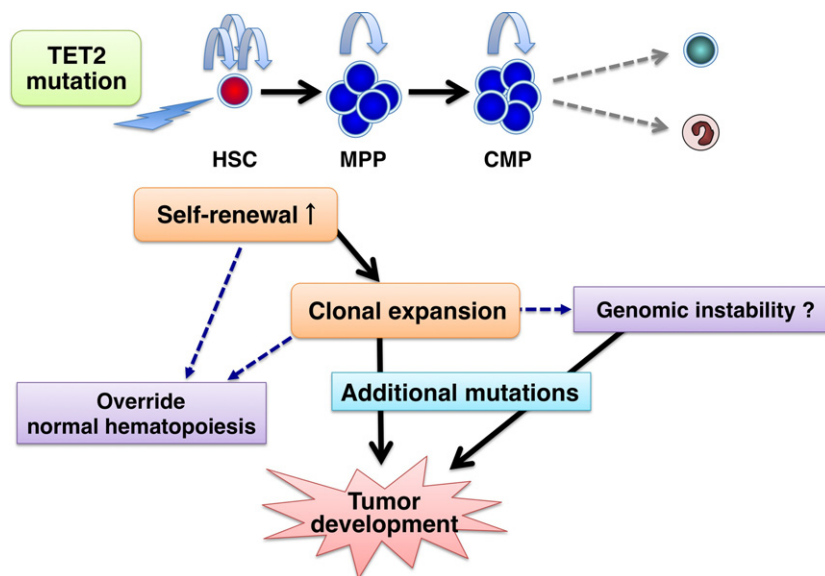
Cell of Origin For Hematological Malignancies with *TET2* Mutation

One of the common features in *Tet2*-mutant mice is the expansion of LSK and CMP fractions both in the BM and the FL, leaving the frequency of the long-term HSC fraction unaffected. As described earlier, *Tet2*-mutated long-term HSCs possess enhanced self-renewal and LTR potential.[51] However, as LSKs and CMPs are amplified in *Tet2*-mutant mice, it is also possible that *Tet2* mutation confers aberrant self-renewal and LTR capacity to multipotent or myeloid progenitors, which may lead to the development of leukemic stem cells in collaboration with their inherently high proliferative capacity. Indeed, our data showed that Tet2 homozygous gene-trap (*Tet2*$^{gt/gt}$) FL-CMPs possessed a higher *in vitro* self-renewal capacity to the same level as *Tet2*$^{gt/gt}$ FL-LSKs, although *Tet2*$^{gt/gt}$ FL-CMPs did not show enhanced self-renewal and LTR potential *in vivo* by serial transplantation assays.[58] These results seem to support the hypothesis that abnormal HSCs with *TET2* mutation are the cell of origin for myeloid malignancies. However, it is also possible that *TET2*-mutated myeloid progenitors act as premalignant cells by attracting additional mutations through their enhanced *in vitro* self-renewal capacity and *in vivo* clonal expansion.

Roles of *TET2* In Chromatin Modification

Recent studies have unveiled a novel role of TET proteins in another aspect of epigenetics, chromatin modifications. In a search for TET-binding proteins, several groups have identified *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) transferase (OGT) as a binding partner of TET.[59–61] OGT is a key

Fig. 5. Schematic model for development of hematological malignancies induced by *TET2* mutation. CMP, common myeloid progenitor; HSC, hematopoietic stem cell; MPP, multipotent progenitor; TET, ten-eleven translocation.

enzyme that transfers the *O*-GlcNAc group to serine (Ser) or threonine (Thr) residues of target proteins, such as histone 2B (H2B)[62] or host cell factor 1 (HCF1).[61] *O*-GlcNAcylation of Ser112 residue of H2B is one of the critical histone marks activating gene transcription. In addition, OGT-mediated *O*-GlcNAcylation of HCF1 may affect methylation of histone 3 lysine 4 residue (H3K4), as HCF1 is an integral component of SET1/COMPASS, a major H3K4 methyltransferase complex.[63]

Interestingly, interaction of OGT with TET was shown to augment catalytic activity of OGT, resulting in enhanced *O*-GlcNAcylation of H2B and HCF1. Furthermore, OGT is recruited to DNA, especially on the transcriptional start sites of the target genes, through interaction with TET protein. Of note, although OGT interacts with the catalytic domain of TET2, it does not interfere with the dioxygenase activity of TET2.

In summary, TET regulates gene transcription not only through DNA demethylation, but also through chromatin modification such as H2B *O*-GlcNAcylation or H3K4 trimethylation by tethering OGT to the target gene promoters. As *O*-GlcNAcylation is critically involved in a variety of cellular processes by modifying cellular signaling or transcriptional components,[64] association with OGT allows TET other ways to regulate critical biological functions.

Regulators of *TET2*

Several regulators for the function of TET2 protein or the expression of *TET2* mRNA have been reported. The former includes CXXC-containing protein IDAX. As described earlier, IDAX negatively regulates *TET2* by facilitating its degradation.[6] The latter includes microRNA-22 and myeloid transcription factor, C/EBPα. MicroRNA-22 promotes degradation of *TET2* mRNA, and enhances self-renewal of HSCs and their transformation.[65] C/EBPα was shown to directly activate transcription of *TET2*, which facilitates transdifferentiation of pre-B cells to the myeloid lineage through derepression of myeloid target genes.[66] Transient induction of *TET2* by C/EBPα also facilitated reprogramming of B cells into induced pluripotent stem cells.[67]

Assays for protein–protein interaction identified other potential interactors for TET2. These include early B cell factor1,[68] AID,[69] and ubiquitin ligase Uhrf2.[70] The functional significance of these interactions is unclear, and remains to be elucidated. Further discovery of interacting partners for TET proteins and revealing their functional relevance would lead to better understanding of *TET*-mediated cellular processes.

Conclusion

One of the remaining questions regarding TET function is that *TET2* is the only mutated gene among *TET* family members in hematological malignancies, although *TET1* and *TET3* is expressed in hematopoietic cells and considered functionally redundant to *TET2*. This suggests that *TET2* has, as yet, unknown functions unique to blood homeostasis. The current scheme showing how *TET2* mutation leads to hematological malignancies is depicted in Figure 5. Dysregulated HSC function by *TET2* mutation induces expansion of self-renewing premalignant clones, and these clones may be prone to additional mutations due to their enhanced self-renewal and possible genomic instability, which may be incurred by *TET2* mutation. *TET2* mutation is definitely a driver for MDS or myeloproliferative neoplasm, in which a primary role of disease-initiating mutation is to expand premalignant clones in the BM overriding normal hematopoiesis.

Future investigations will focus on the emerging roles of TET proteins in a variety of epigenetic as well as signaling processes. These studies will lead to a better understanding of normal and malignant hematopoiesis controlled by *TET2*, which will then help in developing ways to intervene in cellular pathways dysregulated by *TET2* mutations.

Disclosure Statement

The authors have no conflict of interest.

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