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 α-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.

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.1 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
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...
mutations) in the coding region of TET2. 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. 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). 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%). 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. These findings suggest that disruption of TET2-mediated DNA demethylation processes constitutes a critical background for development of hematological malignancies. Most of the TET2 mutations are heterozygous, however, some mutations were found to be double heterozygous or homozygous in uniparental disomy of chromosome 4q24.

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. 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. Interestingly, mutations of IDH1/2 confer them with a gain-of-function activity to generate a novel oncometabolite, 2-hydroxyglutarate (2-HG). 2-Hydroxyglutarate inhibits the function of α-KG-dependent enzymes such as TET2 and histone demethylases like JMJD3 or FBXL11/KDM2A. IDH1/2*, mutant IDH1 and mutant IDH2; Kme, 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, 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. 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 were slightly similar 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. 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. 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.

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. 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. 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. 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. 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. 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. 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 (Tet2gt/gt) FL-CMPs possessed a higher in vitro self-renewal capacity to the same level as Tet2gt/gt FL-LSKs, although Tet2gt/gt FL-CMPs did not show enhanced self-renewal and LTR potential in vivo by serial transplantation assay. 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. OGT is a key
enzyme that transfers the O-GlcNAc group to serine (Ser) or threonine (Thr) residues of target proteins, such as histone 2B (H2B) or host cell factor 1 (HCF1). 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.

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, 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. 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. 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. Transient induction of TET2 by C/EBPα also facilitated reprogramming of B cells into induced pluripotent stem cells.

Assays for protein–protein interaction identified other potential interactors for TET2. These include early B cell factor1, AID, and ubiquitin ligase Uhrf2. 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.

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