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The TET2 interactors and their links to hematological malignancies

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Abstract

Ten-eleven translocation family proteins are dioxygenases that oxidize 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine in DNA, early steps of active DNA demethylation. TET2, the second member of TET protein family, is frequently mutated in patients with hematological malignancies, leading to aberrant DNA methylation profiling and decreased 5hmC levels. Located in the nucleus and acting as a DNA-modifying enzyme, TET2 is thought to exert its function via TET2-containing protein complexes. Identifying the interactome network of TET2 likely holds the key to uncover the mechanisms by which TET2 exerts its function in cells. Here, we review recent literature on TET2 interactors and discuss their possible roles in TET2 loss-mediated dysregulation of hematopoiesis and pathogenesis of hematological malignancies.

Introduction

The ten-eleven translocation (TET) family proteins TET1, TET2 and TET3 constitute a novel family of 2-oxoglutarate- (2-OG) and Fe (II)-dependent dioxygenases to convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) in a stepwise manner (1, 2). Besides serving as intermediates of DNA demethylation and being implicated in epigenetic and transcriptional regulation, 5hmC, 5fC and 5caC might also process their own specific biological functions such as proving docking sites for their respective reader proteins (3). All three family members of TET have both overlapping and unique expression patterns in different cell types, suggesting that they may have both redundant and non-redundant functions in diverse biological processes, such as gene transcription, embryonic development and cell differentiation (4).

TET2 is one of the most frequently mutated genes in myeloid malignancies such as chronic myelomonocytic leukemia (CMML), acute myeloid leukemia (AML) and myelodysplastic

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syndrome (MDS) (5). *TET2* mutations have also been found in lymphoid malignancies such as angioimmunoblastic T-cell lymphoma (6, 7). These mutations are missense, nonsense and frameshift mutations spanning the entire *TET2* coding sequences, suggesting loss-of-function and tumor suppressive function for *TET2* in hematopoiesis (8). A number of approaches have been employed to elucidate the interactome of TET2 that may facilitate the biological functions of TET2. This review summarizes the regulation and function of TET2 as an epigenetic regulator and tumor suppressor, and highlights the known TET2 interactome that may mediate/facilitate these activities. We also discuss the contribution of these TET2 interactors to *TET2* loss-mediated hematological malignancies.

5-hydroxymethylcytosine and Ten-Eleven Translocation Family Proteins

5hmC was first discovered in frog and rodent brain DNA in 1971, but the discovery did not draw much attention (9). In 2009, this mystery nucleotide, 5hmC, was reported to be present in mammalian DNA by two laboratories. Kriaucionis and Heintz (2009) detected an unusual nucleotide, 5hmC, in cerebellar Purkinje neurons and granule cells with an abundance of less than 1% of total nucleotides (10). Tahiliani et al. (2009) discovered that the TET family proteins catalyze the conversion of 5mC to 5hmC, suggesting a novel epigenetic regulation of 5hmC (11). 5hmC and TET family proteins have subsequently drawn a great deal of attention aiming to characterize their biological functions. 5hmC displays differential abundances and profiles between cell types, suggesting an important role in the balance between 5mC and 5hmC for maintaining the normal state of cells. For example, 5hmC accounts for ~1% of total 5mC in immune cells, 5–10% of total 5mC in embryonic stem cells (ESCs), and as high as 40% of total 5mC in neural cells (4). Whereas, 5fC and 5caC are far less abundant, representing only 0.03% and 0.01% of total 5mC in ESCs (1, 2). Besides serving as an intermediate during active DNA demethylation and being dynamic, some 5hmC marks are stable and may have other biological functions. Several techniques have been developed to map 5hmC in the genome, with some offering base-resolution. These techniques include cytosine-5-methylenesulfonate sequencing, oxidative bisulfite sequencing, hydroxymethyl DNA immunoprecipitation sequencing and Tet-assisted bisulfite sequencing (12–15). Neri et al. (2013) reported that Tet1 and Tet1-dependent 5hmC are enriched at transcription start sites (TSS) along with repressive histone modifications in mouse ESCs (16). However, 5hmC is also shown to be enriched at enhancers, promoters and exons to regulate active transcription (17, 18). These discrepancies could be due to different cell systems and techniques utilized for 5hmC signal capture, and due to dynamics of 5hmC in cells. Indeed, 5hmC abundance changes dynamically during human $CD34^+$ and erythroid cell differentiation with increased 5hmC at erythroid-specific transcription factor binding sites and decreased 5hmC at repressive genes (19, 20), while accompanied by rapid DNA demethylation (19). Therefore, 5hmC abundance and 5hmC dynamics likely play important roles in gene transcription regulation and cell-fate decision.

Role of *TET2* in normal hematopoiesis and hematological malignancies

To elucidate the role of *TET2* in normal hematopoiesis and hematological malignancies, numerous studies have been performed *in vitro* and *in vivo*. The *TET2* gene is highly expressed in hematopoietic stem and progenitor cells (HSC/HPC), and is downregulated

during differentiation (21, 22). RNAi-mediated Tet2 knockdown in mouse HPCs and human cord blood $CD34^+$ cells yields decreased 5hmC levels, and promotes expansion of monocytic lineage (23, 24). Tet2 deletion in mouse bone marrow cells leads to an elevated percentage of $Lin^-c\text{-}Kit^+$ HPCs with increased replating ability *in vitro* (25). Several *Tet2* knockout mouse models have been generated using different targeting strategies. All of these *Tet2*-null mice have a similar hematopoietic phenotype which includes an elevated HSC pool and a skewed differentiation toward myelomonocytic lineage (23, 25–27). *Tet2*-null mice also exhibit altered B- and T-cell development, with a decrease of B-cell lineages in the bone marrow and an increase of $CD4^-CD8^-$ T-cell progenitors in the thymus (27). Furthermore, with its increased self-renewal and proliferating capacity, $Tet2^{-/-}$ HSCs has a competitive advantage over wild type HSCs for repopulating hematopoietic lineages. Importantly, $Tet2^{-/-}$ mice develop CMML- and myeloproliferative neoplasm (MPN)-like diseases as they age. The onset of these malignancies varies between 2–12 months, suggesting that additional genetic lesions may be acquired, which cooperate with the Tet2 loss to promote diverse hematological malignancies (23, 25–27). TET2, therefore, plays an important role in the maintenance of normal hematopoiesis, including HSC self-renewal, lineage commitment and specific terminal differentiation of lineages (22). Tet2 depletion is sufficient to cause myeloid malignancies in mice, implying a tumor suppressive function for Tet2 in myelopoiesis.

TET2 mutations in human hematological malignancies

Somatic *TET2* mutations were first identified in patients with MPN and MDS (28, 29). These *TET2* gene alterations resulted in a significant reduction in global levels of 5hmC, suggesting that the enzymatic activity of TET2 is affected (21). *TET2* mutations have subsequently been screened in a series of patients with subtypes of myeloid malignancies. The frequencies of *TET2* mutations are ~13% in classic MPNs, (including polycythemia vera, essential thrombocytosis and primary myelofibrosis) (30), 25–35% in MDS (31, 32), 50% in CMML (a MDS/MPN) (33), and 12–34% in AML (8, 34, 35). *TET2* mutations are believed to be an early genetic event in MDS and MPN, and are shown to have no obvious prognostic impact (36). While, *TET2* mutation is an unfavorable prognostic marker in CMML (37).

Somatic *TET2* mutations also occur in human B-cell malignancies (such as mantle cell lymphomas, ~4%; and diffuse large B-cell lymphoma, ~12%) (38, 39) and T-cell malignancies (such as angioimmunoblastic T lymphoma, ~47%) (6, 40). Interestingly, some patients with *TET2*-mutated myeloid malignancies develop lymphoma (22); and AML/MDS arising secondary to lymphoma harbor the same *TET2* mutation as the previous lymphoma, suggesting a common cell origin (22). Indeed, some $Tet2^{-/-}$ mice develop both myeloid and lymphoid malignancies (Xu M et al., unpublished data). More recently, *TET2* mutations have been observed in patients with blastic plasmacytoid dendritic cell neoplasm (41).

TET2 mutations often co-exist with other gene mutations in human hematological malignancies. Mutations in serine/arginine-rich splicing factor 2 (*SRSF2*), enhancer of zeste 2 polycomb repressive complex 2 (*EZH2*) and additional sex combs like transcriptional regulator 1 (*ASXL1*) have been shown to co-occur with *TET2* mutations in MDS (42–44). In

patients with angioimmunoblastic T lymphoma, *TET2* mutations accompany a recurrent mutation in ras homolog family member A (*RHOAG17V*) and/or DNA (cytosine-5-)-methyltransferase 3 alpha (*DNMT3A*) mutations (40, 45). In addition, TET2 mutations often co-occur with oncogenic V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog (*KITD816V*) mutation in mastocytosis (46). Taken together, *TET2* mutations appear to be an early event in the pathogenesis of hematological malignancies, and may co-operate with other gene mutations to promote the development/transformation of various types of hematological malignancies.

The TET2 interactome

Numerous studies have established a role for TET2 in regulating gene expression, presumably through its function in active DNA demethylation (18, 47, 48). For example, deletion of Tet2 in mouse ESCs causes extensive loss of 5hmC at enhancers, accompanied by reduction of enhancer activity and delayed gene induction in the early steps of differentiation (18). As well, TET2 interacting proteins have been surveyed in several cell types such as mESCs, mouse embryonic fibroblasts (MEFs) and human cell lines. These studies typically involve expression of Tagged-Tet2 and affinity purification, coupled with mass spectrometry. As well, some TET2 interactors have been evaluated for their role in regulating TET2 function. Known TET2 interactomes include small molecules, microRNAs, transcription factors and other proteins as summarized in TABLE 1.

Small molecules

TET2 is a 2-OG-dependent dioxygenase, which requires cofactors Fe (II) and O_2 in addition to 2-OG (11). The latter metabolite is decarboxylated to succinate during the oxidation reaction. TET2 is also sensitive to reactive oxygen species and TET2 activity is enhanced in the presence of ascorbic acid (49). Direct regulation of cofactors for TET2 could be a straightforward and effective strategy to modulate TET2's enzymatic activity. TET2 contains a Cys-rich region and double-stranded β-helix (DSBH) (forming a conserved C-terminal catalytic domain) which binds to Fe(II) and N-oxalylglycine. The intramolecular interaction of Cys-rich and DSBH regions includes an unexpected domain swap, which helps create a unique holder for DNA substrate recognition. Mutated structural analysis indicates that TET2 seems unlikely to discriminate among 5hmC, 5fC, and 5caC being in the active site as long as the 5mC derivatives are positioned correctly in the catalytic pocket next to Fe(II) and 2-OG (50). Therefore, small molecules such as 2-HG, H_2O_2 and other reductors can either promote or suppress this dioxygenase (51, 52).

Vitamin C

Vitamin C is another small molecule that can stimulate and enhance the activity of TET2 (49, 53, 54). Vitamin C interacts with the C-terminal catalytic domain of TET2, which probably promotes folding and/or recycling of the Fe(II). The supplement of Vitamin C to both mouse ESCs and MEFs leads to rapid and global increase of 5hmC with concomitant decrease in 5mC (53, 54), including DNA demethylation at the promoter regions of germline genes. Vitamin C-induced changes in 5hmC and 5mC profiling are Tet1 and/or Tet2-dependent, as such demethylation processes are specifically absent in Tet1/2 double

knockout ES cells (53). Genomic regions that are resistant to Vitamin C-mediated DNA demethylation exhibit higher levels of H3K9me3 (53), suggesting that this histone mark may prevent Tet2-mediated DNA demethylation pathways. Thus, Vitamin C is a direct regulator of Tet2 activity and DNA methylation fidelity, which provides new mechanistic insights into the biochemical role of Vitamin C in DNA methylation. Severe deficiency of Vitamin C results in scurvy, a human disease characterized by hemorrhaging and abnormal bone formation. Recently, Park (2013) showed that Vitamin C at certain concentrations, induces a dose- and time-dependent inhibition in the proliferation of cells in AML cell lines and primary AML cells (55). In fact, earlier studies have reported that the growth of progenitor cells from patients with AML and MDS are significantly modulated by Vitamin C (56, 57). The ability and specificity of Vitamin C to modulate TET2 activity and DNA methylation *in vivo* in HSC/HPCs need to be carefully determined. Our laboratory is currently evaluating the effect of long term Vitamin C treatment on the initiation/progression of hematological malignancies in *Tet2*$^{+/-}$ mice.

MicroRNAs

MicroRNAs are small noncoding RNAs, which are frequently deregulated in hematological malignancies, such as AML and MDS (58, 59). A recent study has identified miR-22 as an oncogene that directly targets TET2 transcripts (60). MiR-22 is remarkably upregulated in patients with MDS. Conditionally overexpressed miR-22 in mouse hematopoietic compartment leads to reduced global 5hmC along with increased self-renewal of HSCs and impaired differentiation ability (60). Furthermore, miR-22 overexpression augments the proliferative capacity of HSC/HPCs, causing them to progressively overcompete their wild type counterparts in competitive transplantation assays (60). Over time, recipient mice transplanted with LSK cells from overexpressed miR-22 donor give rise to an MDS-like syndrome (60). The phenotype displayed in miR-22 transgenic mice is reminiscent of that observed in *Tet2*$^{-/-}$ mice. Bioinformatics analysis has revealed direct interaction of miR-22 with 3′UTR of *TET2* gene (60). Therefore, miR-22 is an epigenetic modifier and key oncogenic determinant for the pathogenesis of MDS *in vivo* likely by regulating TET2 protein levels. These findings imply that the contribution of TET2 loss to the pathogenesis of myeloid malignancies can also be related to deregulation of miR-22-TET2 pathway. Importantly, miR-22 decoying could be a therapeutic strategy that impacts TET2 levels.

Besides miR-22, several TET2-targeting miRNAs have been identified using high-throughput 3′UTR activity screening including miR-29, miR-125 and miR-101 (61). Overexpression of miR-125b, miR-29b, miR-29c and miR-101 is more frequently observed in *TET2*-WT than *TET2*-mutant AML patients (61). Expression of these TET2-targeting miRNAs decreases TET2 expression and aberrant 5hmC levels in hematopoietic cells, leading to traits associated with malignant hematopoiesis such as myeloid bias and HPC expansion (61). Therefore, TET2 expression is regulated by a network of miRNAs, dysregulation of which could subsequently lead to TET2/5hmC alteration and hematological malignancies (61). The identification of a group of TET2-targeting miRNAs with oncogenic potentials in malignant hematopoiesis further supports a role for miRNA-TET2 pathway in the pathogenesis of human hematological malignancies. *TET2*-targeting miRNAs may represent a target for therapeutic intervention.

Early B-Cell Factor 1 (EBF1)

EBF1 has been identified as a potential candidate TET2 interactor through motif analysis of hypermethylated regions in cancer cases with impaired TET2 function (by increased levels of 2-HG) (62). The physical interaction of EBF1 and TET2 is supported by a proportional enrichment of EBF1 and TET2 at selected loci and confirmed by co-immunoprecipitation of endogenous EBF1 and TET2 (62). Interacting with EBF1, TET2 may have a sequence-specific mechanism for regulating DNA demethylation in B-cells (62). It remains unclear as to what extent the EBF1-TET2 interaction is attributed to TET2 inactivation-induced 5hmC alteration in B-cells development dysregulation and pathogenesis of B-cell malignancies.

Wilms Tumor 1(WT1)

The enzymatic activity of TET2 can be catalytically inactivated by 2-HG, an intermediate metabolite generated by isocitrate dehydrogenase 1/2 (IDH1/2). The mutations in TET2 and IDH1/2 occur in a mutually exclusive manner with WT1 mutations, suggesting a common pathway for TET2, IDH1/2 and WT1 (63, 64). As a sequence-specific transcription factor, WT1 protein is composed of a proline/glutamine-rich DNA binding domain at N-terminal and zinc-finger motifs at C-terminal. WT1 is required for cell development and survival through regulating its target gene expression. Overexpression of WT1 has been documented in AML, ALL, CML and MDS (65). Recently, two groups independently reported that WT1 physically interacts with TET2 in AML cells, and the formed WT1-TET2 complex is directed to transcription start sites of WT1 target genes, resulting in active DNA demethylation at the promoter regions and increase of WT1 target gene transcription (63, 64). The association between TET2 and WT1 occurs mainly through the catalytic domain of TET2 and the zinc-finger domain of WT1. In addition, TET2 inhibits leukemia cell proliferation and colony formation in a WT1-dependent manner as WT1 knockdown completely abolishes such inhibitory effect (64). Double knockdown of both TET2 and WT1 is unable to promote cell proliferation and colony formation (64), suggesting TET2 and WT1 function in the same pathway. Furthermore, in *TET2* mutated AML (D1242V, G1256D, R1302G, A1505T, P1617H, G1861R, and T1884A), the interaction between TET2 and WT1 is interrupted, altering the expression of WT1 target gene (64).

Through integrated analyses of the mutational signatures, gene expression and DNA methylation profiles on a cohort of AML patients, Rampal et al. (2014) identified an anti-correlation between WT1 mutations and TET2 mutations, indicating a shared regulatory pathway by these mutations in AML (63). Similar to TET2 mutations, AML patients harboring WT1 mutations also display reduced 5hmC levels (63), which predominantly occurs at enhancers and distal regulatory elements, leading to altered gene expression. Between WT1 mutant and TET2 mutant samples, hypermethylation at specific loci largely overlap (63). Furthermore, loss of WT1 significantly decreases 5hmC levels in murine bone marrow cells and leads to a similar hematopoietic phenotype as does TET2-deletion (63). The physical interaction of WT1 and TET2 has been verified by co-IP in HEK293T cells and hematopoietic cells (63). These results demonstrate that WT1 regulates 5mC hydroxylation by recruiting TET2 to specific genomic loci, providing a novel insight into the contribution of WT1 to TET2-loss mediated hematological malignancies.

O-Linked N-Acetylglucosamine (GlcNAc) Transferase (OGT)

TET2 is physically associated with OGT, an O-linked N-acetylglucosamine transferase adding a GlcNAc to Ser/Thr residues of target proteins (47, 66, 67). OGT binds to the C-terminal catalytic domain of TET2, and TET2 and OGT have shown genome-wide co-localization, especially at transcription start sites. Loss of TET2 in ESCs prevents the association of OGT with chromatin, and overexpression of TET2 increases the level of chromatin-bound OGT (47, 66, 67). However, deletion of OGT does not affect TET2-chromatin association and its enzymatic activity (47). Interestingly, the interaction between TET2 and OGT promotes H2B Ser112 O-GlcNAcylation. The genes that are associated with TET2 and OGT exhibit H2B Ser112 O-GlcNAcylation and enhanced transcription (47). TET2 may facilitate OGT's ability to recognize chromatin substrate and mediate H2B O-GlcAcylation, which triggers TET2-dependent epigenetic regulation of gene expression (47). In addition, O-GlcNAcylation regulates site-specific proteolytic maturation of HCF1, a critical component of SET1/COMPASS complex that mediates H3K4 methylation (66, 67). Increased levels of H3K4me3 have been observed at the TSS of TET2/OGT target genes, while depletion of TET2 or OGT decreases the level of H3K4me3 with concomitantly decreased transcription (66). It has been reported that both global GlcNAcylation and H3K4me3 are reduced in bone marrow cells of *Tet2*$^{-/-}$ mice, notably at several critical regulators of hematopoiesis (66). However, the significance of OGT-TET2 interaction to TET2 loss-mediated hematopoiesis dysregulation and hematological malignancies remains to be explored.

Vpr (HIV-1) Binding Protein (VprBP)

Mass spectrometric analysis using VprBP as bait, has identified TET2 as one of the VprBP interacting proteins (68). VprBP belongs to the recognition component of an E3 ubiquitin ligase complex. The endogenous interaction between TET2 and VprBP has been confirmed in MEFs, ESCs and human monocytes. In vitro binding assays have demonstrated that VprBP directly binds to the catalytic domain of TET2 (68). In addition, deletion of VprBP causes a substantial reduction of 5hmC, suggesting that VprBP is important for optimal TET activity (68). An *in vivo* ubiquitylation assay has been used to gain mechanistic insight into VprBP-TET2 interaction, and results suggest that TET2 is monoubiquitylated by CRL4-VprBP at conserved lysine residues (human K1299 and mouse K1212) (68). Mutation of this conserved lysine residue abolishes the monoubiquitylation of TET2 (68). The VprBP-mediated monoubiquitylation of TET2 promotes DNA binding ability of TET2, but does not promote catalytic activity. Such binding could be disrupted by mutant TET2 proteins at K1299 (68). Recurrent AML-derived mutations in TET2 (K1299N and K1299E) disrupted VprBP-TET2 binding and CRL4-VprBP-mediated TET2 monoubiquitylation without affecting hydroxylase activity (68). Dysregulation of ubiquitination has been implicated in the pathogenesis of hematological malignancies (69). Further studies are warranted to determine if the monoubiquitylation of TET2 is critical for its tumor suppressive function *in vivo* and therefore, plays a key role in TET2 loss-mediated hematological malignancies.

CXXC Finger Protein 4 (IDAX)

In jawed vertebrates, the ancestor *TET* gene underwent triplication, giving rise to *TET1*, *TET2* and *TET3*. *TET2* underwent a chromosomal inversion event, which resulted in the detachment of the CXXC domain from the gene, which encodes a separate protein IDAX (also known as CXXC4) (4). The IDAX CXXC domain preferentially binds to unmethylated CpG-rich sequences. Ko et al. (2013) showed that IDAX interacts directly with the catalytic domain of TET2, resulting in TET2 protein degradation in a caspase-dependent mechanism (70). The loss of IDAX in ESCs prevents TET2 from being degraded during ESC differentiation, while depletion of IDAX in human myeloid cell line upregulates TET2 and increases 5hmC levels (70). When CXXC domain of IDAX is mutated, IDAX loses its binding to TET2 and effects on 5hmC, suggesting that IDAX recruits TET2 to chromatin through its CXXC domain (70). This is a unique case of epigenetic marking as IDAX recruits TET2 to modify DNA and subsequently, likely degraded. The role of IDAX-TET2 interaction in hematopoietic cell differentiation and neoplastic initiation currently remains unknown.

Ubiquitin-Like With PHD And Ring Finger Domains 2, E3 Ubiquitin Protein Ligase (UHRF2)

The identification of 5hmC and evidence of its abundance in DNA have led to the search for 5hmC “readers”. Spruijt et al. (2013) reported that UHRF2 specifically binds to 5hmC in neural progenitor cells and HEK293T cells (71). UHRF2 encodes a nuclear E3 ubiquitin ligase and is implicated in cell-cycle regulation (72). Overexpression of UHRF2 leads to increased 5hmC levels, while co-expression of UHRF2 and TET1 catalytic domain strikingly elevates 5fC and 5caC levels. Thus, the binding of UHRF2 and 5hmC may enhance the accessibility of TET proteins to further oxidize 5hmC. Note that UHRF2 is frequently lost in chronic lymphocytic leukemia, which might be attributed to impaired binding of UHRF2-5hmC, and further oxidation (72). Nevertheless, the importance of UHRF2-5hmC in TET2 loss-mediated hematological malignancies remains to be elucidated.

Concluding remarks

TET2’s prominent role in the pathogenesis of hematological malignancies makes TET2 and its pathways ideal therapeutic targets. Since the majority of cases of hematological malignancies harboring *TET2* mutations are heterozygous (hyploinsufficient in TET2), an obvious therapeutic strategy is to increase/modulate the expression and/or enzymatic activity of the TET2 protein resulting from the remaining wild type *TET2* allele. In addition, the functionally significant TET2 interactors for TET2 loss-mediated hematological malignances represent additional therapeutic targets. This review centers on a collection of interactors that participate in biological processes that involve TET2. However, many TET2 interactors remain to be identified and their biological relevance clarified. Ongoing investigations using rigorous genomic, proteomic and structural approaches will provide valuable insights into the mechanisms by which TET2 loss dysregulates hematopoiesis and leads to hematological malignancies.

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Table 1

Summary of current TET2 Interactors

Interactors	Cell resource	Function	Study
2-HG	Human cell line	Competitively inhibits 2-OG	Xu *et al.*(51)
Vitamin C	Mouse ESCs	Enhances TET2 enzymatic activity	Blaschke *et al.* Minor *et al.* Yin *et al.*(49, 53, 54)
miR-22	Mouse hematopoietic cells	Inhibits TET2 gene expression	Song *et al.*(60)
miR-29, miR-125 and miR-101	Human and mouse hematopoietic cell lines	Inhibits TET2 gene expression	Cheng *et al.*(61)
EBF1	Human cell line	Regulates DNA methylation by binding to TET2	Guilhamon *et al.*(62)
WT1	Human and mouse hematopoietic cell lines	Recruits TET2 to specific genomic loci for 5mC hydroxylation	Wang *et al.* Rampal *et al.*(63, 64)
OGT	Human cell line and mouse ESCs	Modifies histones and transcribes genes through recruitment to chromatin	Chen *et al.* Vella *et al.* Deplus *et al.*(47, 66, 67)
VprBP	Human cell line and mouse ESCs, MEFs	Promotes TET2 binding to DNA through monoubiquitylation	Nakagawa *et al.*(68)
IDAX	Human cell line and mouse ESCs	Promotes TET2 protein degradation in a caspase-dependent mechanism	Ko *et al.*(70)
UHRF2	Mouse ESCs and NPCs	Enhances the accessibility of TETs to hydroxymethylated DNA for further oxidation	Spruijt *et al.*(71)