Seminars in Hematology 58 (2021) 27–34

Contents lists available at ScienceDirect

Seminars in Hematology

journal homepage: www.elsevier.com/locate/seminhematol

TET-dioxygenase deficiency in oncogenesis and its targeting for tumor-selective therapeutics ☆

Yihong Guan, Metis Hasipek, Anand D. Tiwari, Jaroslaw P. Maciejewski, Babal K. Jha*

Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH

ARTICLE INFO

Keywords:
TET-dioxygenases
MDS
Therapeutic targeting
Ascorbic Acid
IDH1/2

ABSTRACT

TET2 is one of the most frequently mutated genes in myeloid neoplasms. TET2 loss-of-function perturbs myeloid differentiation and causes clonal expansion. Despite extensive knowledge regarding biochemical mechanisms underlying distorted myeloid differentiation, targeted therapies are lagging. Here we review known biochemical mechanisms and candidate therapies that emerge from this. Specifically, we discuss the potential utility of vitamin C to compensate for TET-dioxygenase deficiency, to thereby restore the biochemical function. An alternative approach exploits the TET-deficient state for synthetic lethality, exploiting the fact that a minimum level of TET-dioxygenase activity is required for cell survival, rendering *TET2*-mutant malignant cells selectively vulnerable to inhibitors of TET-function.

Cytosine, one of the 4 nucleotides in our DNA, when present in the genome in a CpG configuration (C preceding G in cis-), is often modified by methylation of its C-5 position. This modification of cytosine contributes critically to the structure and assembly of chromatin (DNA and the histone proteins around which it is organized) and to transcription regulation [1]. The cytosine methylation is catalyzed by DNA cytosine-5-methyltransferases (DNMTs) which transfer a methyl group from S-adenosyl methionine (SAM) to the cytosine to form methyl-cytosine (5mC). Methylation of DNA in this way is associated with silencing of gene transcription. Thus, DNA methylation is an epigenetic process central to gene regulation. In humans, 60% to 80% of CpGs are methylated in somatic cells as a default state [2]. What about demethylation? It was recognized several decades ago that removal of methyl groups from cytosines in DNA was a passive process, that is, demethylation was by failure to recapitulate methylation marks on a parental DNA strand onto the newly synthesized DNA strand during S-phase. Only last decade, however, the mechanism was identified by which specific cytosine residues were identified for demethylation [3,4]. Ten eleven translocation genes *TET1*, *TET2*, and *TET3* encode for iron(II) and α-ketoglutarate (α-KG) dependent DNA dioxygenases that sequentially oxidize 5mC → 5 hydroxy methyl cytosine (5hmC)→ 5-formyl cytosine (5fC)→ 5-carboxyl cytosine (5caC), and facilitate the removal either via thymine DNA glycosylase (TDG)-mediated base excision repair of 5fC and 5caC, or inability of DNMTs to recognize 5hmC during replication. In this way, therefore, TET-dioxygenase activity mediates mCpG demethylation. Loss-of-function mutations in *TET2* are amongst the most frequently observed lesions in hematologic malignancies, including myelodysplastic syndromes and acute myeloid leukemia [5], and as expected, the TET2 loss-of-function is associated with CpG hypermethylation compared to normal myeloid precursors [6].

Structure-Function of TET-dioxygenases

Each member of the TET family possesses a highly conserved and well characterized C-terminal catalytic domain. The catalytic domain consists of a conserved cysteine-rich and a double-stranded β-helix (DSBH) regions along with a stretch of so called low complexity structure dispensable for catalytic activity [4,7-13]. The iron(II), α-KG and substrate binding sites are imbedded in these cysteine-rich and DSBH regions and are critical for dioxygenase function [8-10,14]. Although the enzymatic domain is highly conserved across species, including human, mouse, *Xenopus*, and zebrafish [13,15,16], the N-terminal regulatory domains of TET1, TET2, and TET3 greatly differ between one another and between species, with a maximum amino acid similarity of less than 13% among TET-dioxygenases (Table 1). TET1 and TET3 have a cysteine-X-X-cysteine (CXXC) zinc finger domain at the N-termini, however, this zinc finger motif is absent in TET2 [16-18]. The CXXC motif has been shown to be critical for binding to DNA segments

☆ This work was supported in parts, by grants from the NIH/NHLBI (R35HL135795-01 and R01132071-01), Leukemia and Lymphoma Society (LLS) TRP to BKJ, and LLS SCOR and Taub Foundation to JPM and BKJ.

* Corresponding author. Babal K. Jha, Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, 9500 Euclid Avenue, NE6, Cleveland, OH 44195.

E-mail address: jhab@ccf.org (B.K. Jha).

https://doi.org/10.1053/j.seminhematol.2020.12.002
0037-1963/

Table 1
Similarity among TET Proteins compared to human TET2.

Proteins*	Full length	N-terminus	C-terminus
Homo Sapiens	100	100	100
Pan troglodytes	99.2	99.1	99.3
Macaca mulatta	96.8	95.4	98.4
Bos Taurus	84.6	79.4	91.5
Gallus gallus	64.4	49.9	82.7
Rattus norvegicus	63.7	60.6	67
Mus musculus	62.7	60.4	65.3
Xenopus tropicalis	51	31.7	70
Danio rerio	38.5	21.7	55.7
Homo Sapiens TET3	29.9	12.7	47
Homo Sapiens TET1	25	9.8	46.4

* The protein sequences were obtained from the NCBI database with protein accessions: NP_001120680.1, XP_003310448.3, XP_014994413.2, XP_010804239.1, XP_015131709.1, XP_006224326.1, NP_001035490.2, XP_002934823.2, XP_005159960.1, NP_001274420.1 and NP_085128.2 for indicated species. The sequences were aligned against human TET2 using COBALT (https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi) to identify the corresponding N-terminus and C-terminus for each protein. Identity of fragments were calculated by DNASTAR MegAlign Version 7.1 using ClustlaW2.

Table 2
Residues essential for substrate, metal and the cofactor αKG binding in the catalytic domain.

Protein*	Substrate	α-KG binding site		Metal binding site		
TET2	5mC	R1896		H1382	H1881	D1384
PHD2	Proline	R383		H313	H374	D315
P4H	Proline	R192	Y141	H124	H183	D126
JMJD2A	Lysine$(Me)_3$	Y132	K206	H188	H276	E190
BBOX1	Small Molecule	R360		H202	D204	

* The crystal structure of proteins with cofactors were obtained from the RCSB protein data bank (https://www.rcsb.org/) with ID 4NM6 (TET2), 5L9B(PHD2), 4IW3 (P4H), 2OQ6 (JMJD2A) and 3O2G (BBOX1).

containing modified (e.g., 5mC, 5hmC, 5fC, and 5caC) as well as unmodified cytosines [16,18]. Interestingly, due to the lack of the CXXC domain, it has been reported that TET2 may target DNA via other DNA binding adaptor proteins, such as SNIP1 [19,20]. In short, how TET2 is targeted to specific genetic loci is not fully understood, and likely involves adaptor proteins.

Structural determinants of TET-dioxygenase activity have been extensively studied via several high resolution crystal structures [7,9,10]. Studies of the structure of the engineered catalytic domain of human TET2 showed that the cysteine-rich and the DSBH regions are stabilized by 3 zinc atoms that allow these structures to form a compact fold in which DNA substrate is embedded above the DSBH. TET2 structures in complex with DNA substrate demonstrated that the methylated cytosine is located in the catalytic cavity with orientation towards catalytic iron(II) and pseudo substrate N-Oxalylglycine (Fig. 1) [9,10]. TET-dioxygenase activity is a two-step reaction that utilizes iron(II) and α-KG. The first step is the activation of molecular oxygen that involves iron(II) and α-KG to convert the dioxygen into a highly active iron(IV)-oxo intermediate transition state complex which then oxidizes the C-H bond to C-OH in the second step [9,17,21]. A key aspect of all dioxygenases is the conserved nature of residues involved in binding to the α-KG cofactor and to the metal ion (Table 2). A detailed ligand plot analysis of different classes of DNA [9], RNA [22], protein [23], and small molecule [24] dioxygenases demonstrate that cofactor binding sites are similar in disparate classes of α-KG/iron(II) dependent enzymes (Fig. 1 and Table 2). Interestingly, 2 histidine, 1 arginine, or lysine and 1 glutamic acid or aspartic acid are always conserved and seem essential for binding of α-KG and the divalent metal ions. However, the mechanism of substrate recruitment and binding are significantly different from one class of dioxygenase, for example, TET-dioxygenases, to another, like, histone demethylases [21].

Studies using knock-out models have demonstrated that TET-dioxygenase actions on CpG methylation control patterns and efficiencies of gene transcription in a manner that dictates lineage-commitment, maturation within a lineage, and accordingly, also proliferation [25-27]. Specifically TET2 haploinsufficiency or knock-out from the myeloid compartment did create mutator phenotype and accelerated neoplastic evolution [5,28-30].

Configuration of TET2 mutations

Myeloid oncogenesis selects for truncating frame shift deletions or stop gain missense mutations in *TET2*, that account for more than 80% of all *TET2* mutations ($TET2^{MT}$) [28,31]. Most missense mutations are localized in or near the catalytic site (Fig. 2A). *TET2* mutations are found in myeloid malignancies in all possible configurations, mono allelic, bi-allelic, heterozygous, hemizygous, and homozygous.

Consistent with the experimentally demonstrated role of TET2 in hematopoiesis, the loss-of-function mutations in myeloid malignancies are linked with a differentiation block and proliferative advantage, hence increasing fitness of mutant over normal cells [5,6,32-37]. Studies of variant allele frequencies in patient bone marrow and blood indicate that *TET2* loss-of-function mutations increase ectopic replications in several hematopoietic lineages and at different maturation stages within these lineages [6,28,31,38-43]. Diminished TET activity has also been reported in melanoma, glioblastoma, and breast cancer, wherein, it seems to correlate with advanced disease and poor survival [44]. However, the frequency of loss-of-function truncating mutations in solid tumors is far less than in hematologic malignancies (Fig. 2B). In chronic myelomonocytic leukemia (CMML), the prevalence of $TET2^{MT}$ approaches 50% with almost half being biallelic alterations [28,31,35]. $TET2^{MT}$ are ancestral (the first driver mutation in the malignant clone) in nearly 50% of cases and their incidence increases with age. $TET2^{MT}$ prevalence is $>$70% in MDS patients over the age of 80 [28]. $TET2^{MT}$ are also found in aging “healthy controls” in whom their detection in otherwise asymptomatic patients is referred to as “clonal hematopoiesis of indeterminate potential .” The prevalence of clonal hematopoiesis of indeterminate potential in healthy adults strongly associates with an increased risk of subsequently developing hematological malignancies [28,45,46]. Altogether, these findings demonstrate that TET2 is a key player in myeloid oncogenesis [47].

Although the TET2 gene has been widely sequenced in large cohorts of patients in leukemia and somatic mutations frequently associated with disease occurrence and severity, evidence of inherited *TET2* mutations have only been reported recently [48-50]. A Finnish family with multiple cases of lymphoma segregating with a heterozygous germline TET2 frameshift variants were reported [49,50]. In these case reports, neither unusual predisposition to atherosclerosis nor abnormal pro-inflammatory cytokine or chemokine expressions were observed in these patients. These observations were in cells from three additional unrelated TET2 germline mutation carriers. The TET2 defect elevates blood DNA methylation levels, especially at active enhancers and cell-type specific regulatory regions with binding sequences of master transcription factors involved in hematopoiesis. Interestingly, a recent case report of a French pedigree harboring germline TET2 frameshift variant found that the 3 siblings developed myeloid malignancies at early age [48].

Post-translational modifications in TET2

Enzyme activity depends on the three dimensional structure of the catalytic site, with contributions also from noncatalytic domains that maintain stability in a cellular milieu. Apart from

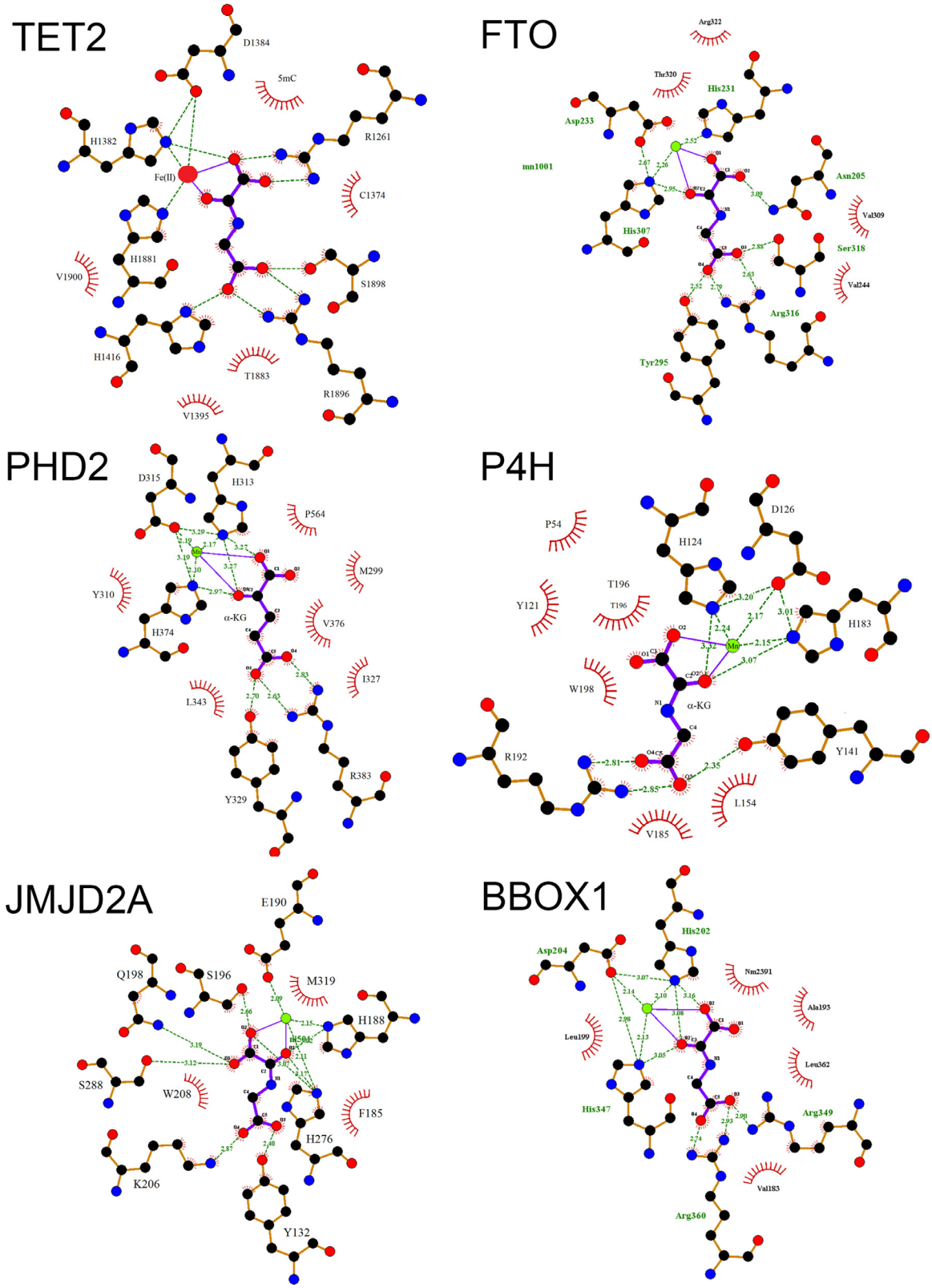

Fig. 1. The catalytic site residues in α–KG/iron(II) dependent dioxygenases are conserved. Ligand plot of the catalytic sites of DNA dioxygenase TET2, RNA dioxygenase FTO, protein dioxygenases PHD2, P4H and JMJD2A and small molecule dioxygenase BBOX1. Analysis was performed using LIGPLOT v.4.5.3 (https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/). Three dimensional structures with PDB ID 4NM6 (TET2), 5L9B(PHD2), 4IW3 (P4H), 2OQ6 (JMJD2A) and 3O2G (BBOX1) were used for the analysis.

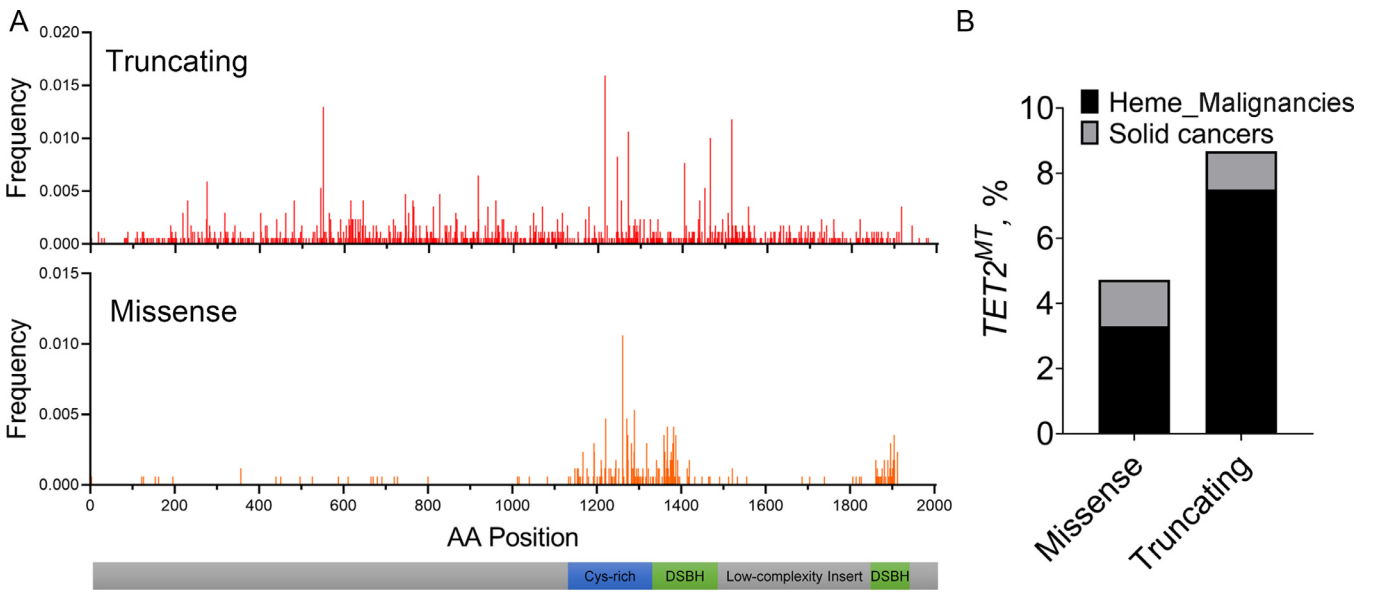

Fig. 2. Distribution of missense and truncating mutations in TET2**.** A. Configuration of truncating and missense mutation in TET2 among 2000 myeloid neoplasia patients (data source: Hirsch C., et al 2018). B. Distribution missense and truncating mutations among 11664 patients across 28 studies in hematologic malignancies and 67743 patients across 206 studies of solid tumors (data source: www.cbioportal.org). Percentage of different mutations were plotted.

mutations, post-translational modifications, such as phosphorylation [51-58] and acetylation, have been reported to impact TET2 stability and activity [59-69]. The consequences of post-translational modifications depend on the type of modification and the residue modified [52,59,63]. Acetylation of the C-terminal catalytic domain lysine residues cause loss of TET-dioxygenase activity [59,61,62], due to the changes in the conformation of the catalytic site that prevent substrate recognition or cofactor binding [10,11,59]. N-terminal regulatory domain lysine acetylation prevents ubiquitination and protects TET2 from proteasome mediated degradation and increases its half-life in cells [59,63]. Interestingly, several oncogenic mutations target the post-translationally modified residues to structurally mimic function suppressing acetylation, for example, by causing lysine to glutamic acid or glutamine substitution [59] (Fig. 3).

Similar to N-terminal lysine acetylation, phosphorylation of N-terminal serine (S99) by AMP-activated kinase (AMPK), or phosphorylation of tyrosines (Y1939 and Y1964) by cytokine receptor-associated kinase JAK2, prolong TET2 protein half-life and in this way increase dioxygenase activity [53,58]. AMPK is a nutrient sensor usually inactivated in diabetic patients due to high glucose levels. The phosphorylation of S99 in TET2 by AMPK inversely correlated with glucose concentration [58]. Accordingly, activation of AMPK by the anti-diabetic drug metformin augmented TET2 half-life resulting in genome wide increased 5hmC levels even under high glucose conditions [58]. These findings indicated that low levels of 5hmC observed in high glucose conditions are a consequence of the inhibition of AMPK-mediated TET2 phosphorylation and hence TET2 degradation [58].

Similarly, it is reported that hematopoietic cytokines can phosphorylate TET2, leading to its activation in erythroid progenitors *in vitro*. Specifically, cytokine receptor-associated kinase JAK2 phosphorylated TET2 tyrosines (Y1939 and Y1964), which promoted the interaction between TET2 and the erythroid lineage master transcription factor KLF1 [53]. This interaction between TET2 and KLF1 was further increased by exposure to erythropoietin. Contrary to the loss-of-function mutations in *TET2* that reduce the global 5hmC in myeloid malignancies, JAK2V617F mutation seen in myeloproliferative neoplasms increases TET-activity and decreases genome-wide cytosine methylation [53]. These findings suggest avenues, that is, kinases, by which TET-dioxygenase function may be regulated [53].

Therapeutic targeting of TET-dioxygenases in myeloid neoplasia

Thus, *TET2* is a *bona fide* tumor suppressor gene (TSG), with its partial or complete loss-of-function playing a driver role in myeloid oncogenesis [70-72]. In considering how to turn this understanding into therapy, it is worth bearing in mind that the malignant cells, despite partial or complete loss-of-function of TET2, contain and express two other TET-dioxygenases, TET1 and TET3, that provide residual TET-dioxygenase activity likely critical to cell survival [73]. Thus, two approaches for preferential targeting of TET-dioxygenase deficient cells can be conceptualized. The first, to compensate for the TET2 loss by augmenting the activity of remaining TETs, by utilizing either a “super” substrate or co-substrate, for example, vitamin C and/or α-KG analogs that are known to increase TET-dioxygenase activity. The second, to drive already reduced net TET-dioxygenase activity below minimum thresholds needed for cell survival (“synthetic lethality”).

Augmenting the activity of residual TET-dioxygenase activity

Ascorbic acid (AA, vitamin C) is a water-soluble ketolactone with 2 ionizable hydroxyl groups. It has 2 pKa values, pK1 is 4.2 and pK2 is 11.6; thus, the ascorbate monoanion is the dominant form at physiological pH. Ascorbate is an excellent reducing agent and readily undergoes 2 consecutive, 1-electron oxidations to form ascorbate radical and dehydroascorbic acid (DHA). The ascorbate is relatively unreactive due to resonance stabilization of the unpaired electron; it readily dismutes to ascorbate and DHA ($k_{obs} = 2 \times 10^5$ M^{-1} s^{-1}, pH 7.0) [74]. One of the main physiological function of AA is to maintain physiological redox balance [75]. Thus, AA acts as a specific co-factor for a large family of enzymes known as the iron(II)- and 2-oxoglutarate-dependent dioxygenases [76]. In recent years, several high profile studies reported that known as vitamin C enhances TET-dioxygenase activity by reducing the iron moiety in the catalytic site from iron(III) to iron(II) [14,59,77]. Several studies suggested that vitamin C acts as a broad spectrum augmenter of the activity of all α-KG/iron(II)-dependent dioxyge-

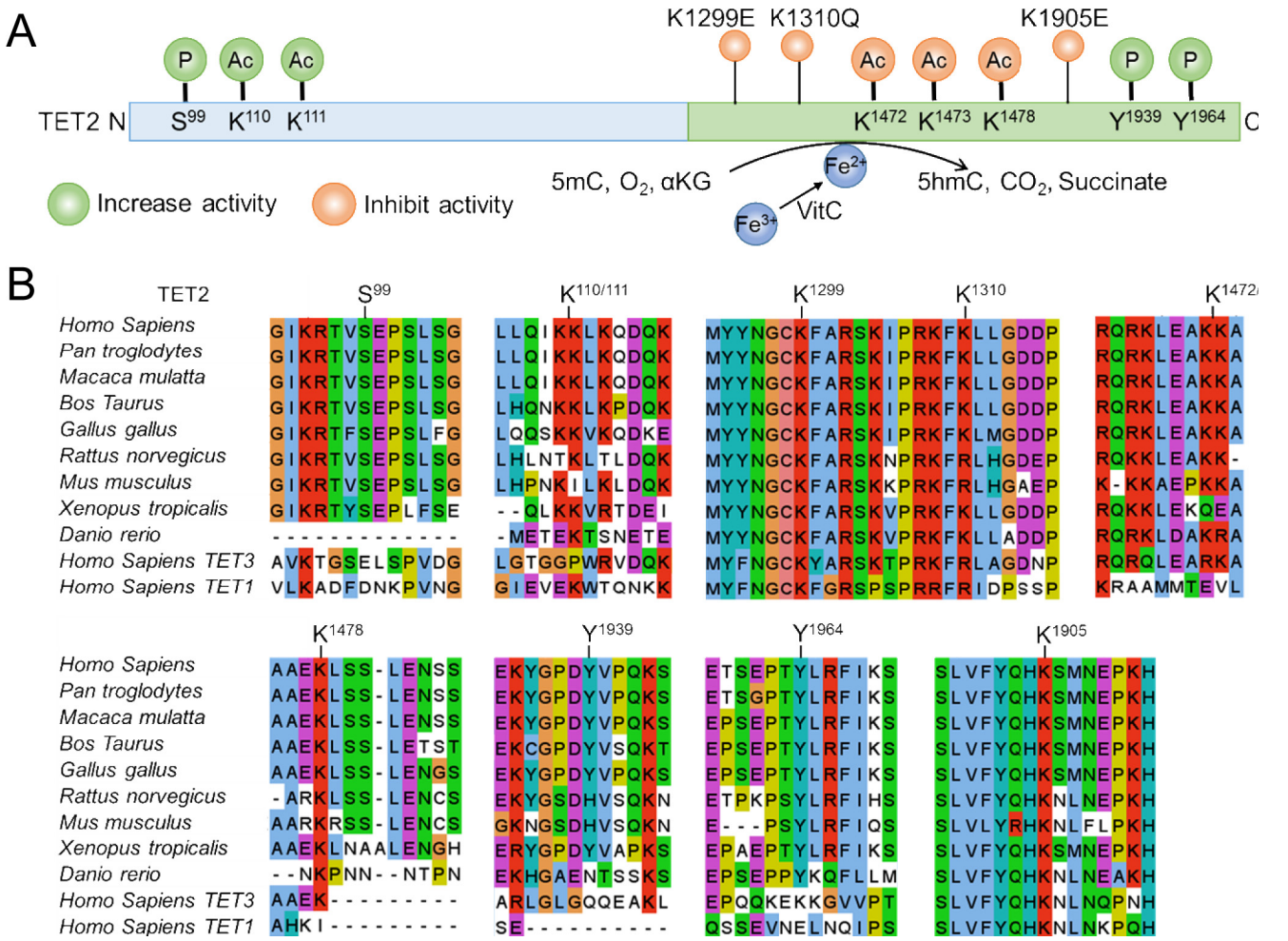

Fig. 3. Target residues for post translational modifications. A. Schematics of human TET2 and residues target for post translational modification and their effect on the activity. B. Sequence alignment of TET dioxygenases showing the conserved nature of these residues.

Table 3
Intracellular concentration of ascorbic acid (AA) in circulating cells achieve at 500 mg daily supplement.

Circulating cells	Ascorbic acid (mM)
Lymphocytes	4.0
Monocytes	3.0
Platelets	3.5
Neutrophils	1.2

EC50 for majority of aKG and Iron (II) dependent dioxygenases are < 0.1 mM [75,76]

nases, including TETs, and therefore may have therapeutic utility in *TET2*-mutated neoplasms [75,76]. Recent reports using *in vitro* cell culture and *in vivo* murine model systems have postulated that the treatment of TET2 deficient cells with vitamin C can restore TET-dioxygenase activity, block aberrant self-renewal, and prevent neoplastic evolution [14,59,78-80]. In interpreting these studies, there are two important considerations, first, that vitamin C has anticancer properties independent of its effects on TET-dioxygenase, for example, by perturbing the redox balance in cancer cells [73-76]. The second is that vitamin C mediated augmentation of TET-dioxygenase activity (EC50 ~14μM) is saturated at vitamin C concentrations of >100 μM [14,59]. However, the intracellular levels of vitamin C remains above 1 mM in most of the circulating cells (Table 3). For example, ~500 mg/daily dietary ascorbic acid supplementation routinely achieve 3.5 mM in monocytes and 4.0 mM in lymphocytes [81]. Therefore, the antileukemic effect of the supra physiological doses of vitamin C in humans [82] and in mice leukemia model [78] remain unclear. A comprehensive analysis of the cellular concentration of vitamin C suggested that the intracellular levels of ascorbic acid remains in the millimolar range [81], well above the saturating concentration required for activation of majority of αKG/iron(II) dependent dioxygenases [21,76]. The most recent National Health and Nutrition Examination Survey (NHANES) [83] of healthy adults living in the United States suggested that the overall age-adjusted mean from the square-root transformed (SM) concentration was 51.4 μM (95% CI: 48.4, 54.6). Interestingly, the highest concentrations were found in children (age <18) and older persons (age >60) [83] (Table 4). Taking these two factors together, it is possible that anti-cancer effects observed in mice and humans, using very high doses of vitamin C, may not actually be mediated via augmentation of TET-dioxygenase activity. Interestingly, mice manufacture vitamin C endogenously (vitamin C is not a 'vitamin' for mice), in this way differing from humans. A recent case report [82] indicated that an acute supraphysiological dose of vitamin C, as a single agent, benefitted a patient with chemorefractory *TET2* mutant AML. In this case report the intravenous vitamin C dose was gradually increased from 35 g/day to 95 g/day 2X/week over a period of ~4 weeks. However, for the reasons mentioned above, it cannot necessarily be concluded that the mechanism of action was by augmentation of TET-dioxygenase activity, and

Table 4
Serum vitamin C concentration in healthy individuals from 2003-2004 National Health and Nutrition Examination Survey [83].

Age, year	Ascorbic acid, median, (95% CI), μM	
	Male	Female
>6	48.0 (44.9, 51.2)	54.8 (51.6 58.0)
6-11	73.5 (69.8, 77.3)	68.9 (64.7, 73.3)
12-19	50.7 (47.2, 54.2)	54.88 (50.6, 59.2)
20-39	42.0 (37.6, 46.6)	48.88 (44.5, 53.3)
40-59	43.2 (38.9, 47.6)	52.08 (47.5, 56.7)
>60	52.7 (48.8, 56.4)	62.98 (60.5, 65.4)

TET independent effects of vitamin C may have also been in play [82].

The ability of vitamin C to augment TET-dioxygenase activity also depends on the acetylation status of catalytic domain lysine residues [61-63]. That is, the suppression of TET-dioxygenase activity that is produced by acetylation of catalytic domain lysines is not restored by increasing vitamin C concentrations [59,62]. From a translational perspective, these observations are important because several TET2 mutations mimic these post-translational modifications, and vitamin C is not expected to increase the TET-dioxygenase activity of these mutants [59,62]. The conditions or triggers for TET family acetylation or deacetylation have not as yet been characterized, although in our studies of leukemia cell lines, we found that acetylation appears to be the default setting.

Thus, the effect of vitamin C is dependent on whether baseline concentrations are below levels that saturate TET-dioxygenase activity, and the complex interplay of different types of post translational modifications that impact dioxygenase activity and the ability of vitamin C to affect this. One implication is that pharmacologic modulation of acetyltransferases and histone deacetylases may regulate TET dioxygenase-dependent effects of vitamin C, creating a rationale for vitamin C combinations with class I/II HDAC inhibitors [59,63] or sirtuin activators [59,62]. Since the overall pharmacodynamic goal is to decrease aberrant DNA methylation, combinations with DNA hypomethylating agents, for example, azacytidine [64,84], and decitabine [85], can also be proposed.

Inhibiting TET-dioxygenases in TET deficient cells

Basal TET-dioxygenase activity is essential for the activation of several 5mC-sensitive transcription factors (reviewed in [86]), including *Myc [20], and Runx1* [87]. Thus, *TET2*-deficient leukemia cells rely on the remaining TET-dioxygenase activity provided by TET1 and TET3, as evidenced by the persistence of hydroxymethylation in cells with biallelic inactivation of *TET2* in human leukemias and in *Tet2*$^{-/-}$ mice [88]. In addition, TET3 has been shown to have overlapping function with TET2 in hematopoietic progenitors [73]. Knockout of all three *Tet* genes is embryonically lethal in mice [73,89-91]. In zebrafish, conditional knockout of all three TET-dioxygenases in HSC abrogated hematopoiesis [89]. In humans with myeloid malignancies, mutations in *IDH1* or *IDH2*, that produce the oncometabolite R-2-hydroxyglutarate (R-2HG) that antagonizes α-KG, are notably mutually exclusive with *TET2* mutations. There are two potential reasons for this. One is that by inhibiting TET-dioxygenase activity (via the oncometabolite that competes with α-KG that is a mandatory cofactor for TET-dioxygenases), IDH mutations and TET mutations are in the same pathway and thus redundant. Another potential reason is that 2HG producing neomorphic IDH mutations and TET mutations are synthetic lethal, by reducing TET-dioxygenase activity beneath minimum thresholds needed for cell survival [92]. Our own data supports the latter reason, since we have found that introducing neomorphic IDH mutations into TET2-mutant cells causes cell death [93]. Altogether, these observations suggest that *TET2* mutant malignant cells may be more vulnerable to inhibition of residual TET-dioxygenase activity, essential for cell survival, than normal dividing cells containing the usual TET family complement (therapeutic index). We accordingly are developing TET-specific inhibitors (Fig. 1, Table 2) - an extensive analysis of the structural elements of the substrate recognition motif demonstrate a large structural diversity among different class of dioxygenases that can be exploited to developed inhibitors specific for TET-dioxygenases [21], with potential application to simultaneously expand normal hematopoietic progenitors and suppress *TET2*-mutant malignant cells [94].

Conclusion and future prospect

A central challenge in developing novel strategy for oncotherapy is to terminate malignant but not normal replication needed for health and life. Increasingly detailed understanding of TET-dioxygenase biochemistry and roles in cell physiology has created opportunities to meet this challenge for *TET2*-mutated myeloid malignancies. TET2 mutation in many healthy elderly significantly increase the risk of developing myeloid neoplasms. The strategies that either circumvent the loss in neoplastic cells and induce terminal differentiation as well as the one that terminate the deficient clone without impacting normal hematopoiesis can be employed for a successful development of novel therapeutics [93]. For example, naturally existing neomorphic mutations in IDH1/2 causes TET-dioxygenase inhibition which in turn restrict the growth of *TET2*^{MT} hematopoietic clones in human body. Hence, establishing TET-dioxygenase deficient cells can be therapeutically targeted.

Authors contributions

YG, MH and ADT analyzed data and read and edited the manuscript. JPM help in the conceptual development of the studies, edited the manuscript. BKJ conceived the idea, designed and supervised the studies, analyzed data, provided resources, and wrote as well as edited the manuscript.

Conflict of interest

Authors have no conflict of interests.

References

[1] Ren R, Horton JR, Zhang X, Blumenthal RM, Cheng X. Detecting and interpreting DNA methylation marks. Curr Opin Struct Biol 2018;53:88–99.
[2] Strichman-Almashanu LZ, Lee RS, Onyango PO, et al. A genome-wide screen for normally methylated human CpG islands that can identify novel imprinted genes. Genome Res 2002;12:543–54.
[3] Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 2009;324:929–30.
[4] Tahiliani M, Koh KP, Shen Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 2009;324:930–5.
[5] Jankowska AM, Szpurka H, Tiu RV, et al. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. Blood 2009;113:6403–10.
[6] Ko M, Huang Y, Jankowska AM, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature 2010;468:839–43.
[7] Hashimoto H, Pais JE, Dai N, et al. Structure of Naegleria Tet-like dioxygenase (NgTet1) in complexes with a reaction intermediate 5-hydroxymethylcytosine DNA. Nucleic Acids Res 2015;43:10713–21.
[8] Hashimoto H, Pais JE, Zhang X, et al. Structure of a Naegleria Tet-like dioxygenase in complex with 5-methylcytosine DNA. Nature 2014;506:391–5.
[9] Hu L, Li Z, Cheng J, et al. Crystal structure of TET2-DNA complex: insight into TET-mediated 5mC oxidation. Cell 2013;155:1545–55.
[10] Hu L, Lu J, Cheng J, et al. Structural insight into substrate preference for TET-mediated oxidation. Nature 2015;527:118–22.
[11] Tsai CL, Tainer JA. Probing DNA by 2-OG-dependent dioxygenase. Cell 2013;155:1448–50.

[12] Xu S, Li W, Zhu J, et al. Crystal structures of isoorotate decarboxylases reveal a novel catalytic mechanism of 5-carboxyl-uracil decarboxylation and shed light on the search for DNA decarboxylase. Cell Res 2013;23:1296–309.
[13] Iyer LM, Tahiliani M, Rao A, Aravind L. Prediction of novel families of enzymes involved in oxidative and other complex modifications of bases in nucleic acids 2009;8: 1698-1710.
[14] Yin R, Mao SQ, Zhao B, et al. Ascorbic acid enhances Tet-mediated 5-methylcytosine oxidation and promotes DNA demethylation in mammals. J Am Chem Soc 2013;135:10396–403.
[15] Chavez L, Huang Y, Luong K, et al. Simultaneous sequencing of oxidized methylcytosines produced by TET/JBP dioxygenases in Coprinopsis cinerea. Proc Natl Acad Sci U S A 2014;111:E5149–58.
[16] Xu Y, Xu C, Kato A, et al. Tet3 CXXC domain and dioxygenase activity cooperatively regulate key genes for Xenopus eye and neural development. Cell 2012;151:1200–13.
[17] Kohli RM, Zhang Y. TET enzymes, TDG and the dynamics of DNA demethylation. Nature 2013;502:472–9.
[18] Jin SG, Zhang ZM, Dunwell TL, et al. Tet3 Reads 5-Carboxylcytosine through Its CXXC Domain and Is a Potential Guardian against Neurodegeneration. Cell Rep 2016;14:493–505.
[19] Ko M, An J, Bandukwala HS, et al. Modulation of TET2 expression and 5-methylcytosine oxidation by the CXXC domain protein IDAX. Nature 2013;497:122–6.
[20] Chen LL, Lin HP, Zhou WJ, et al. SNIP1 Recruits TET2 to Regulate c-MYC Target Genes and Cellular DNA Damage Response. Cell Rep 2018;25 1485-1500 e1484.
[21] Islam MS, Leissing TM, Chowdhury R, Hopkinson RJ, Schofield CJ. 2-Oxoglutarate-Dependent Oxygenases. Annu Rev Biochem 2018;87:585–620.
[22] Han Z, Niu T, Chang J, et al. Crystal structure of the FTO protein reveals basis for its substrate specificity. Nature 2010;464:1205–9.
[23] Chen Z, Zang J, Kappler J, et al. Structural basis of the recognition of a methylated histone tail by JMJD2A. Proc Natl Acad Sci U S A. 2007;104:10818–23.
[24] Leung IK, Krojer TJ, Kochan GT, et al. Structural and mechanistic studies on gamma-butyrobetaine hydroxylase. Chem Biol 2010;17:1316–24.
[25] Yamazaki J, Jelinek J, Lu Y, et al. TET2 Mutations Affect Non-CpG Island DNA Methylation at Enhancers and Transcription Factor-Binding Sites in Chronic Myelomonocytic Leukemia. Cancer Res 2015;75:2833–43.
[26] Wang L, Ozark PA, Smith ER, et al. TET2 coactivates gene expression through demethylation of enhancers. Sci Adv 2018;4:eaau6986.
[27] Lou H, Li H, Ho KJ, et al. The Human TET2 Gene Contains Three Distinct Promoter Regions With Differing Tissue and Developmental Specificities. Front Cell Dev Biol 2019;7:99.
[28] Hirsch CM, Nazha A, Kneen K, et al. Consequences of mutant TET2 on clonality and subclonal hierarchy. Leukemia 2018;32:1751–61.
[29] Meggendorfer M, Haferlach C, Kern W, Haferlach T. Molecular analysis of myelodysplastic syndrome with isolated deletion of the long arm of chromosome 5 reveals a specific spectrum of molecular mutations with prognostic impact: a study on 123 patients and 27 genes. Haematologica 2017;102:1502–10.
[30] Pan F, Wingo TS, Zhao Z, et al. Tet2 loss leads to hypermutagenicity in haematopoietic stem/progenitor cells. Nat Commun 2017;8:15102.
[31] Awada H, Nagata Y, Goyal A, et al. Invariant phenotype and molecular association of biallelic TET2 mutant myeloid neoplasia. Blood Adv 2019;3:339–49.
[32] Kishtagari A, Jha BK, Maciejewski JP. TET2 mutations and clonal dynamics. Oncotarget 2019;10:2010–11.
[33] Smith AE, Mohamedali AM, Kulasekararaj A, et al. Next-generation sequencing of the TET2 gene in 355 MDS and CMML patients reveals low-abundance mutant clones with early origins, but indicates no definite prognostic value. Blood 2010;116:3923–32.
[34] Abdel-Wahab O, Mullally A, Hedvat C, et al. Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood 2009;114:144–7.
[35] Patel BJ, Przychodzen B, Thota S, et al. Genomic determinants of chronic myelomonocytic leukemia. Leukemia 2017;31:2815–23.
[36] Tefferi A, Lim KH, Abdel-Wahab O, et al. Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML. Leukemia 2009;23:1343–5.
[37] Palomo L, Meggendorfer M, Hutter S, et al. Molecular landscape and clonal architecture of adult myelodysplastic/myeloproliferative neoplasms. Blood 2020.
[38] Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 2014;28:241–7.
[39] Jankowska AM, Makishima H, Tiu RV, et al. Mutational spectrum analysis of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2, and DNMT3A. Blood 2011;118:3932–41.
[40] Nagata Y, Zhao R, Awada H, et al. Machine learning demonstrates that somatic mutations imprint invariant morphologic features in myelodysplastic syndromes. Blood 2020.
[41] Tiu RV, Visconte V, Traina F, Schwandt A, Maciejewski JP. Updates in cytogenetics and molecular markers in MDS. Curr Hematol Malig Rep 2011;6:126–35.
[42] Rothenberg-Thurley M, Amler S, Goerlich D, et al. Persistence of pre-leukemic clones during first remission and risk of relapse in acute myeloid leukemia. Leukemia 2017.
[43] Koeffler HP, Leong G. Preleukemia: one name, many meanings. Leukemia 2017;31:534–42.
[44] Huang Y, Rao A. Connections between TET proteins and aberrant DNA modification in cancer. Trends Genet 2014;30:464–74.
[45] Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371:2477–87.
[46] Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371:2488–98.
[47] Park SJ, Bejar R. Clonal hematopoiesis in cancer. Exp Hematol 2020;83:105–12.
[48] Duployez N, Goursaud L, Fenwarth L, et al. Familial myeloid malignancies with germline TET2 mutation. Leukemia 2020;34:1450–3.
[49] Stremenova Spegarova J, Lawless D, Mohamad SMB, et al. Germline TET2 loss of function causes childhood immunodeficiency and lymphoma. Blood 2020;136:1055–66.
[50] Kaasinen E, Kuismin O, Rajamaki K, et al. Impact of constitutional TET2 haploinsufficiency on molecular and clinical phenotype in humans. Nat Commun 2019;10:1252.
[51] Kundu A, Shelar S, Ghosh AP, et al. 14-3-3 proteins protect AMPK-phosphorylated ten-eleven translocation-2 (TET2) from PP2A-mediated dephosphorylation. J Biol Chem 2020;295:1754–66.
[52] Zhang T, Guan X, Choi UL, et al. Phosphorylation of TET2 by AMPK is indispensable in myogenic differentiation. Epigenetics Chromatin 2019;12:32.
[53] Jeong JJ, Gu X, Nie J, et al. Cytokine-Regulated Phosphorylation and Activation of TET2 by JAK2 in Hematopoiesis. Cancer Discov 2019;9:778–95.
[54] Chen H, Yu D, Fang R, et al. TET2 stabilization by 14-3-3 binding to the phosphorylated Serine 99 is deregulated by mutations in cancer. Cell Res 2019;29:248–50.
[55] Fiedler EC, Shaw RJ. AMPK Regulates the Epigenome through Phosphorylation of TET2. Cell Metab 2018;28:534–6.
[56] Qu X, Zhang S, Wang S, et al. TET2 deficiency leads to stem cell factor-dependent clonal expansion of dysfunctional erythroid progenitors. Blood 2018;132:2406–17.
[57] Strzyz P. A sugar rush of DNA methylation. Nat Rev Mol Cell Biol 2018;19:617.
[58] Wu D, Hu D, Chen H, et al. Glucose-regulated phosphorylation of TET2 by AMPK reveals a pathway linking diabetes to cancer. Nature 2018;559:637–41.
[59] Guan Y, Greenberg EF, Hasipek M, et al. Context dependent effects of ascorbic acid treatment in TET2 mutant myeloid neoplasia. Commun Biol 2020;3:493.
[60] Yuan EF, Yang Y, Cheng L, et al. Hyperglycemia affects global 5-methylcytosine and 5-hydroxymethylcytosine in blood genomic DNA through upregulation of SIRT6 and TETs. Clin Epigenetics 2019;11:63.
[61] Simeoni F, Somervaille TCP. Revert the SIRT: Normalizing SIRT1 Activity in Myelodysplastic Stem Cells. Cell Stem Cell 2018;23:315–17.
[62] Sun J, He X, Zhu Y, et al. SIRT1 Activation Disrupts Maintenance of Myelodysplastic Syndrome Stem and Progenitor Cells by Restoring TET2 Function. Cell Stem Cell 2018;23 355-369 e359.
[63] Zhang YW, Wang Z, Xie W, et al. Acetylation Enhances TET2 Function in Protecting against Abnormal DNA Methylation during Oxidative Stress. Mol Cell 2017;65:323–35.
[64] Manzoni EF, Pennarossa G, deEguileor M, Tettamanti G, Gandolfi F, Brevini TA. 5-azacytidine affects TET2 and histone transcription and reshapes morphology of human skin fibroblasts. Sci Rep 2016;6:37017.
[65] Zhang Q, Zhao K, Shen Q, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. Nature 2015;525:389–93.
[66] Etchegaray JP, Mostoslavsky R. Cell Fate by SIRT6 and TETs. Cell Cycle 2015;14:2187–8.
[67] Etchegaray JP, Chavez L, Huang Y, et al. The histone deacetylase SIRT6 controls embryonic stem cell fate via TET-mediated production of 5-hydroxymethylcytosine. Nat Cell Biol 2015;17:545–57.
[68] Salminen A, Kaarniranta K, Hiltunen M, Kauppinen A. Krebs cycle dysfunction shapes epigenetic landscape of chromatin: novel insights into mitochondrial regulation of aging process. Cell Signal 2014;26:1598–603.
[69] Wright J. Epigenetics: reversible tags. Nature 2013;498:S10–11.
[70] Solary E, Bernard OA, Tefferi A, Fuks F, Vainchenker W. The Ten-Eleven Translocation-2 (TET2) gene in hematopoiesis and hematopoietic diseases. Leukemia 2014;28:485–96.
[71] Mercher T, Quivoron C, Couronne L, Bastard C, Vainchenker W, Bernard OA. TET2, a tumor suppressor in hematological disorders. Biochim Biophys Acta 2012;1825:173–7.
[72] O'Keefe C, McDevitt MA, Maciejewski JP. Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies. Blood 2010;115:2731–9.
[73] Li C, Lan Y, Schwartz-Orbach L, et al. Overlapping Requirements for Tet2 and Tet3 in Normal Development and Hematopoietic Stem Cell Emergence. Cell Rep 2015;12:1133–43.
[74] Bielski BHJ. Chemistry of Ascorbic Acid Radicals. Ascorbic Acid: Chemistry, Metabolism, and Uses. AMERICAN CHEMICAL SOCIETY; 1982. p. 81–100.
[75] Du J, Cullen JJ, Buettner GR. Ascorbic acid: chemistry, biology and the treatment of cancer. Biochim Biophys Acta 2012;1826:443–57.
[76] Kuiper C, Vissers MC. Ascorbate as a co-factor for fe- and 2-oxoglutarate dependent dioxygenases: physiological activity in tumor growth and progression. Front Oncol 2014;4:359.
[77] Hore TA, von Meyenn F, Ravichandran M, et al. Retinol and ascorbate drive erasure of epigenetic memory and enhance reprogramming to naive pluripotency by complementary mechanisms. Proc Natl Acad Sci U S A 2016;113:12202–7.
[78] Agathocleous M, Meacham CE, Burgess RJ, et al. Ascorbate regulates haematopoietic stem cell function and leukaemogenesis. Nature 2017;549:476–81.
[79] Minor EA, Court BL, Young JI, Wang G. Ascorbate induces ten-eleven translocation (Tet) methylcytosine dioxygenase-mediated generation of 5-hydroxymethylcytosine. J Biol Chem 2013;288:13669–74.

[80] Monfort A, Wutz A. Breathing-in epigenetic change with vitamin C. EMBO Rep 2013;14:337–46.
[81] Levine M, Wang Y, Padayatty SJ, Morrow J. A new recommended dietary allowance of vitamin C for healthy young women 2001;98: 9842-9846.
[82] Das AB, Kakadia PM, Wojcik D, et al. Clinical remission following ascorbate treatment in a case of acute myeloid leukemia with mutations in TET2 and WT1. Blood Cancer J 2019;9:82.
[83] Schleicher RL, Carroll MD, Ford ES, Lacher DA. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003-2004 National Health and Nutrition Examination Survey (NHANES). Am J Clin Nutr 2009;90:1252–63.
[84] Sajadian SO, Ehnert S, Vakilian H, et al. Induction of active demethylation and 5hmC formation by 5-azacytidine is TET2 dependent and suggests new treatment strategies against hepatocellular carcinoma. Clin Epigenetics 2015;7:98.
[85] Zhao H, Zhu H, Huang J, et al. The synergy of Vitamin C with decitabine activates TET2 in leukemic cells and significantly improves overall survival in elderly patients with acute myeloid leukemia. Leuk Res 2018;66:1–7.
[86] Heberle E, Bardet AF. Sensitivity of transcription factors to DNA methylation. Essays Biochem 2019;63:727–41.
[87] Chu Y, Zhao Z, Sant DW, et al. Tet2 Regulates Osteoclast Differentiation by Interacting with Runx1 and Maintaining Genomic 5-Hydroxymethylcytosine (5hmC). Genomics Proteomics Bioinformatics 2018;16:172–86.
[88] Li Z, Cai X, Cai CL, et al. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. Blood 2011;118:4509–18.
[89] Dawlaty MM, Breiling A, Le T, et al. Loss of Tet enzymes compromises proper differentiation of embryonic stem cells. Dev Cell 2014;29:102–11.
[90] Verma N, Pan H, Dore LC, et al. TET proteins safeguard bivalent promoters from de novo methylation in human embryonic stem cells. Nat Genet 2018;50:83–95.
[91] Dai HQ, Wang BA, Yang L, et al. TET-mediated DNA demethylation controls gastrulation by regulating Lefty-Nodal signalling. Nature 2016;538:528–32.
[92] Guan Y, Tiwari AD, Phillips JG, et al. TET Dioxygenase Inhibition As a Therapeutic Strategy in TET2 Mutant Myeloid Neoplasia. Blood 2019;134: 880–880.
[93] Guan Y, Tiwari AD, Phillips JG, et al. A Therapeutic Strategy for Preferential Targeting of TET2 Mutant and TET-dioxygenase Deficient Cells in Myeloid Neoplasms 2020: bloodcandisc.0173.2020.
[94] Li X, Yue X, Pastor WA, et al. Tet proteins influence the balance between neuroectodermal and mesodermal fate choice by inhibiting Wnt signaling. Proc Natl Acad Sci U S A 2016;113:E8267–76.