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REVIEW

The TET enzymes

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Abstract
During the past decade, we have learnt that the most common DNA modification, 5-methylcytosine (5mC), playing crucial roles in development and disease, is not stable but can be actively reversed to its unmodified form via enzymatic catalysis involving the TET enzymes. These ground-breaking discoveries have been achieved thanks to technological advances in the detection of the oxidized forms of 5mC and to the boldness of individual scientists. The TET enzymes require molecular oxygen for their catalysis, making them important targets for hypoxia research. They also require special cofactors which enable additional levels of regulation. Moreover, mutations and other genetic alterations in *TETs* are found, especially in myeloid malignances. This review focuses on the kinetic and inhibitory properties of the TET enzymes and the role of TETs in cellular differentiation and transformation and in cancer.

Keywords Cancer · DNA methylation · EMT · Gene regulation · 5hmC · Hypoxia

Introduction

The enzyme family of 2-oxoglutarate-dependent dioxygenases (2-OGDDs) gained a new family member in 2009 when the conversion of 5mC to 5-hydroxymethyl cytosine (5hmC) in DNA was found to be associated with catalysis by TET1 [1]. *TET1* in 10q22 had actually been cloned a few years earlier as a leukemia-associated protein with a CXXC domain (*LCX*) as a fusion partner of *mixed-lineage leukemia* (*MLL*) in 11q23, and was suggested as playing a role in the pathogenesis of 11q23-associated leukemia [2]. The first acute myeloid leukemia (AML) patient with this *TET1* translocation was subsequently characterized and the name Ten-Eleven Translocation was suggested [3]. Based on sequence homology, the existence of three isoenzymes in human and mouse was recognized, although no function could yet be associated with these proteins [3]. An attempt to identify mammalian enzymes that would modify 5mC by means of structurally informed iterative sequence profile searches using the oxygenase domains of Trypanosomal JBP1 and JBP2, which are 2-OGDDs and catalyze the first step in β-D-glucosyl hydroxymethyluracil (base J) synthesis, revealed homology with the TETs [1]. Further experimental data then showed that TET1 overexpression resulted in a reduction in 5mC levels in genomic DNA and the appearance of a novel species, identified as 5hmC [1]. Human embryonic stem cells (ES) and Purkinje cells were the first cell types reported to contain 5hmC [1, 4]. Subsequently two isoenzymes, TET2 and TET3, were shown to possess similar catalytic activity [5].

The 2-OGDD family has ~ 70 members in mammals. The others in addition to the TETs are, for example, numerous histone lysine demethylases (KDMs), the prolyl 4-hydroxylases that modify the hypoxia-inducible factor (HIF-P4Hs) or collagens (collagen P4Hs), the hypoxia-inducible factor asparagine hydroxylase FIH and the obesity-associated FTO, the first identified RNA demethylase (for a complete list see [6]). The 2-OGDDs share the same reaction mechanism and cofactors, but their substrates vary from DNA to RNA, proteins and fatty acids. 2-OGDDs require Fe^{2+}, 2-oxoglutarate (2-OG/α-ketoglutarate), molecular oxygen [6] and many require a reducing agent, typically vitamin C (ascorbate) to support the reaction [6, 7]. The cofactors are coordinated at the active site by conserved residues, iron by two histidines and an aspartate and 2-oxoglutarate by a positively charged residue, an arginine or a lysine. In TETs this latter is an arginine. The catalytic domains possess a double-stranded β-helix (DSBH) structure known as a jelly roll. Following

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cofactor and substrate binding, the molecular oxygen oxidizes Fe^{2+}, inducing substrate oxidation and decarboxylation of 2-OG to succinate and CO_2 (Fig. 1). In the case of TETs, hydroxylation of the 5mC substrate in the DNA CpG dinucleotides to 5hmC can be followed by further oxidation of 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) catalyzed by the TETs themselves (Fig. 1) [5, 8].

The human TETs are large proteins, full-length TET1 being composed of 2039 amino acids (aa), TET2 of 1921 aa and TET3 of 1803 aa [5], although shorter variants of TET1 and TET3 are also found [9–11]. The aminoterminal parts of TET1 and TET3 contain a DNA-binding CXXC domain, whereas this is lacking in TET2 but is coded by its neighboring gene *IDAX* [12]. The catalytic domains are situated in the C-termini and contain a cysteine-rich domain and a DSBH domain. A large low-complexity insert is found within the DSHB domain that may have regulatory roles via post-translational modifications [13, 14]. According to mRNA and protein expression databases, the human TETs are widely expressed (https://www.ebi.ac.uk/gxa/home, http://www.proteinatlas.org/), while experimental data suggest that *Tet1* is preferentially expressed in ES cells (ESCs) whereas *Tet2* and *Tet3* are expressed in many tissues and have overlapping expression profiles [5].

The catalytic activity of the TETs is strongly dependent on Fe^{2+} and 2-OG [1, 5, 15], whereas the omission of ascorbate did not significantly reduce TET1 catalytic activity [1]. The reported K_m values for Fe^{2+} and 2-OG of TET1 and TET2 are similar to those for the collagen P4H-I (Table 1). Mutations of the iron-binding residues of TET1 and TET2 impair their catalytic activity and increase the K_m value for Fe^{2+} up to ~ 60-fold while reducing the V_{max} values by half [1, 15]. Divalent metals other than Fe^{2+} can typically act as competitive inhibitors for 2-OGDDs [16, 17]. The reported K_m values of TET1 and TET2 for molecular oxygen are 0.3–30 µM [15, 18] (Table 1), suggesting that they can retain their catalytic activity under low oxygen tension and do not act as cellular oxygen sensors, unlike the HIF-P4Hs, with K_m values of ≥ 70 µM for oxygen (Table 1). In vitro, TETs possess a nanomolar affinity for a substrate with 5mC (Table 1), but their affinity for substrates containing 5hmC and 5fC appears to be weaker than that for 5mC [5, 19]. However, in a situation of high TET catalytic activity or overexpression, the levels of both the 5mC substrate and 5hmC are likely to reduce because of substrate exhaustion [20, 21].

Table 1 K_m values of TETs and several other 2-OGDDs for cosubstrates and substrates

	Fe^{2+} (µM)	2-Oxoglutarate (µM)	Oxygen (µM)	Substrate (µM)
TET1	5[a]	55[a]	0.3–30[a,b]	0.075[a]
TET2	4[a]	60[a]	0.5–30[a,b]	0.125[a]
HIF-P4H-1	0.05[c]	2[d]	230[e]	0.01–0.02[f]
HIF-P4H-2	0.05[c]	1[d]	67–250[e,f,g]	0.14[f]
HIF-P4H-3	0.1[c]	12[d]	230[e]	0.07[f]
Collagen P4H-I	2[h]	20[h]	40[h]	0.2[i]
FIH	0.5[j]	25[j]	90–240[j,k]	100–220[i,j,k]
KDM4A	ND	23[l]	173[l]	23–656[l,m]
ABH2	ND	4[n]	ND	0.08–4[n,o]

ND not determined

[a][15]
[b][18]
[c][17]
[d][83]
[e][65]
[f][84]
[g][85]
[h][86]
[i][87]
[j][88]
[k][85]
[l][89]
[m][90]
[n][59]
[o][91]

Diverse roles of oxidized 5mC bases

DNA methyltransferases (DNMTs) convert unmethylated cytosine to 5mC, which has a well-established role as a transcriptional repressor and therefore a regulator of gene expression [22]. The methylation of cytosine was for a long time assumed to be permanent, but the discovery of the ability of the TET enzymes to convert 5mC to 5hmC, and further to 5fC and 5caC has suggested a new mechanism by which DNA can be demethylated [1, 23]. In 2011,

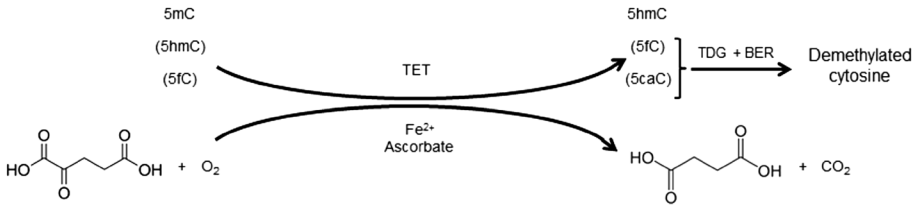

Fig. 1 Schematic representation of the enzymatic reactions catalyzed by the TET enzymes resulting in DNA demethylation. *TDG* thymine DNA glycosylase, *BER* base excision repair

He et al. [8] showed how 5mC converted to 5caC by the TET enzymes can be demethylated via excision by thymine DNA glycosylase (TDG) and base excision repair (BER), demonstrating a role for the TET enzymes in DNA demethylation (Fig. 1) [8]. Nevertheless, it has also been suggested recently that the oxidized forms of 5mC have their own functions independent to that of DNA demethylation. Many endogenous proteins have been identified as potential reader proteins for 5hmC, 5fC and 5caC [24], and 5fC, for example, has been associated with tissue development [25].

5hmC is the first and most abundant form of the oxidized 5mCs (Fig. 1) [22] and the one that has been studied the most. 5hmC has many important functions in embryonic development, hematopoiesis, hematological malignancies and other cancers, and high levels are found in many types of stem cells and also in neural cell lines. Since TET enzymes are also highly expressed in these cell types [1], it has been suggested that the TETs and 5hmC both control pluripotency and cell differentiation, and lack of 5hmC is also associated with the malignant progression of cancer cells [26, 27].

CpG islands are short regions rich of CpG dinucleotides usually found in promoter regions of many genes. In non-cancer cells these regions are mostly nonmethylated [28, 29]. Methylated status of CpG islands near promoter regions is associated with transcriptional repression [28, 29]. Aberrant methylation of CpG islands near promoter regions of tumor suppressor genes has been a major focus of DNA methylation research since discovery of CpG island methylator phenotype (CIMP) in colorectal cancer [30]. Since TET enzymes convert 5mC to 5hmC, reduced 5hmC levels resulting from abnormal TET function, can be associated with dysregulated DNA demethylation which can result in increased 5mC levels, CIMP and hypermethylator phenotype [18, 31]. Reduced global 5hmC levels, possibly associated with a hypermethylator phenotype of chromatin or CpG islands, are found in hematological malignancies as well as in several solid tumors [9, 26, 32–36]. However, in contrast to CIMP and the hypermethylator phenotype, recent findings suggest that in human seminomas TET1 is overexpressed and both 5mC and 5hmC levels are decreased probably reflecting the germ cell origin of these tumors [20, 21]. Due to reduced 5hmC levels being an epigenetic hallmark of a number of cancers, it has been suggested that TETs and 5hmC levels may control cell differentiation and epithelial-to-mesenchymal transition (EMT) [27, 37], and low 5hmC has been associated with a poorer prognosis [33].

TET mutants in cancers

TET2 appears to be the most frequently mutated of the *TET1-3* genes, its mutations being found in ~ 20% cases of myelodysplastic syndrome (MDS), ~ 20% myeloproliferative neoplasms (MPN), ~ 20% AML and ~ 45% chronic myelomonocytic leukemia (CMML) [38–40]. *TET2* mutations have also been found in ~ 15% T cell lymphomas, often appearing in these together with a *DNMT3A* mutation [41]. The majority of the mutations are somatic heterozygous missense mutations that do not cluster to a certain site in the *TET2* gene [38–40]. The mutations residing in the C-terminal Cys-rich domain or the DSBH domain have been shown to impair hydroxylation of 5mC [42]. Some mutations that target the Fe^{2+}-binding histidine or the 2-OG-coordinating arginine have been shown to increase the K_m value for these cofactors by at least 50- to 80-fold and reduce the V_{max} value, indicating significant loss of function [15]. As the *TET2* mutants studied in detail do not show complete loss of function, increasing the local concentration of iron or 2-OG in the bone marrow might, for example, be a mechanism for restoring its catalytic activity and reversing the oncogenic properties of *TET2* mutants [15, 42]. Altogether, these data suggest that *TET2* is a critical tumor suppressor, especially in myeloid tissue.

TET1 has frequently been found in the *MLL*-containing chromosome translocations in AML, T-cell lymphoma and B-cell acute lymphoblastic leukemia (B-ALL) [3, 43]. In AML, it is significantly upregulated, being a direct target gene of the *MLL* fusion proteins and resulting in increased 5hmC levels [43]. Somatic splice site, missense or nonsense/frameshift mutations in *TET1* have been found at low frequencies (~ 1%) in AML and in ~ 6% of T-cell acute lymphoblastic leukemia (T-ALL) [44]. No mutations in *TET3* have been characterized, suggesting that they are not tolerated, which is supported by the lethal nature of its knockout in mouse embryos [45] whereas $Tet1^{-/-}$ and $Tet2^{-/-}$ mice largely develop normally, although TET2 deficiency leads to myeloid malignances later [46–49].

TETs and hypoxia

As the TETs require oxygen for their catalysis, it is of interest to study how hypoxia affects the catalytic activity of TETs and global 5hmC levels. As many tumors are known to be hypoxic [50], a connection between TETs and tumorigenesis has been hypothesized. Moreover, the effects of hypoxia on the expression of *TET* mRNAs and transcription of the genes regulated by TETs have been examined. The response of TETs to hypoxia appears to be cell-type specific. In neuroblastoma cells, hypoxia increases the global 5hmC levels accumulating in canonical hypoxia response genes and induces *TET1* mRNA levels via hypoxia-inducible factor (HIF)1 [51]. The full induction of the hypoxia response in these cells is nevertheless dependent on TET1 [51]. Interestingly, in the neuroblastoma cells hypoxia-inducible genes appear to be regulated in a multilayered

manner including HIF stabilization and epigenetic regulation via TETs and 5hmC level, and not all HIF target genes are regulated equally via TETs [15]. In human retinal pigment epithelial (RPE) cells, chemical hypoxia induced by cobalt chloride increased *TET1* and *TET2* mRNA levels but reduced the methylation status of the promoter regions of the corresponding genes [52], while in several cancer cells and murine ES cells hypoxia specifically reduces 5hmC levels in gene promoters and causes either a modest or no effect on *TETs 1-3* mRNA levels [18]. In hypermethylated cancer patient samples, a reduction in 5hmC levels was seen especially in tumor-suppressor gene promoters [18]. Data have been presented to suggest that these reductions are specifically due to severe pathophysiological tumor hypoxia that impairs the TET catalytic activity [18]. Modest hypoxia (2–5% O_2) did not inactivate the TETs [18], an observation which is in line with the reported K_m values for TET1 and TET2 (Table 1).

TETs and 2-OG analogues

The catalytic activity of the TET enzymes can be inhibited by natural or synthetic 2-OG analogues that act as competitive inhibitors with respect to the cofactor (Figs. 1, 2). Of the Krebs cycle intermediates, succinate and fumarate, which accumulate in succinate dehydrogenase (SDH) and fumarate hydratase (FH) mutant tumors, respectively [53], have been shown to impair TET catalytic activity (Fig. 2) [15, 54]. The IC_{50} values of TET1 and TET2 for fumarate and succinate were about 400–500 μM, and they reduced global 5hmC levels in neuroblastoma cells (Table 2) [15]. As these oncometabolites can accumulate to high millimolar

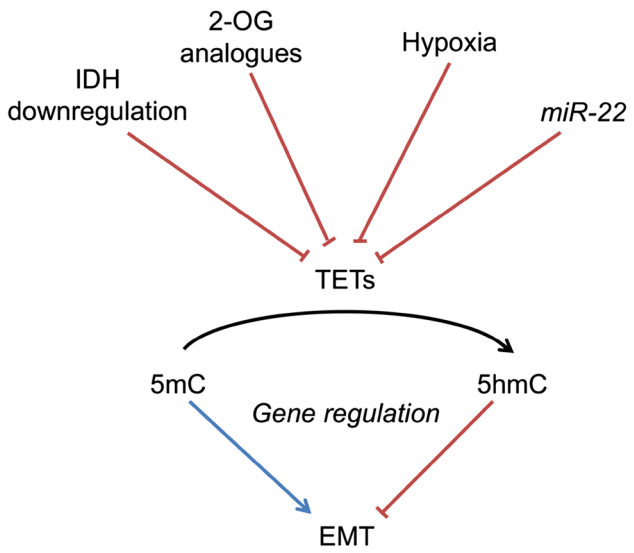

Fig. 2 Factors influencing gene regulation via TET enzymes

levels in FH- and SDH-mutant tumor samples [53], inhibition of TETs is likely to be involved in their pathogenesis, although fumarate and succinate are also potent inhibitors of the HIF-P4Hs and collagen P4H-I, and succinate additionally of KDM4E histone lysine demethylase (Table 2). Further support for the role of TET inhibition by succinate in tumorigenesis is provided by the evidence on SDH-mutant paragangliomas, which present a hypermethylator phenotype [55]. In mouse ES cells, a high 2-OG/succinate ratio is found to contribute to pluripotency-associated gene expression via TETs [56]. Dimethylfumarate (DMF), a cell-permeable form of fumarate, has been used for a few years now as an efficient immunomodulatory agent in the treatment of relapsing–remitting multiple sclerosis [57]. Though not studied in detail, treatment with DMF is likely to signal via several 2-OGDDs, including the TETs (Table 2). *R*-2-hydroxyglutarate (R-2HG), generated by isocitrate dehydrogenase (IDH) mutations in glioblastoma, AML, cholangiocarcinoma or chondrosarcoma, impairs TET activity [58] even though the IC_{50} values of TET1 and TET2 for R-2HG are at a high millimolar level (Table 2). Within the 2-OGDD family, several KDMs, FIH, the alkylation damage-correcting DNA demethylase ABH2 and collagen P4H-I are more sensitive than TETs to inhibition by R-2HG, whereas this does not efficiently inhibit the HIF-P4Hs, but rather can support their activity (Table 2) [58–60]. However, as R-2HG can accumulate to 5–35 mM levels in IDH-mutant tumors [61] inhibition of TET catalytic activity is also likely to occur in them. *S*-2-hydroxyglutarate (S-2HG), an enantiomer of R-2HG that has been found to accumulate in a rare metabolic disease caused by a defect in S-2HG dehydrogenase [62, 63], inhibits TET1 and TET2 with about fourfold lower IC_{50} values than R-2HG (Table 2) [15, 58]. Citrate, a key regulator of lipid metabolism and inhibitor of HIF-P4H-3, FIH and collagen P4H-I, was not an effective inhibitor of TET1 or TET2 (Table 2). Like other 2-OGDDs, TETs can be targeted with synthetic 2-OG analogue inhibitors such as dimethyloxalyl glycine (DMOG) [64] that have shown a potential for treating ischemic, anemic or inflammatory conditions by being efficient inhibitors of the HIF-P4Hs [65–68]. However, in the case of the TETs such inhibitors of catalytic activity may only possess therapeutic potential for the treatment of *MLL*-fusion AMLs, in which TET1 overexpression is involved in the disease mechanism [43].

TET enzymes contribute to cell differentiation and transformation

Altered activity of the TET enzymes and the concomitant changes in 5hmC levels and DNA methylation have recently been associated with pathological changes in cell differentiation and transformation. First, reduced global 5hmC levels

Table 2 IC_{50} values of TETs and several other 2-OGDDs for 2-oxoglutarate analogues

	Fumarate (μM)	Succinate (μM)	*R*-2-hydroxyglutarate (μM)	*S*-2-hydroxyglutarate (μM)	Citrate (μM)
TET1	390[a]	540[a]	4000[b]	1000[b]	> 5000[a]
TET2	400[a]	570[a]	5000[b]	1600[b]	> 5000[a]
HIF-P4H-1	120[c]	830[c]	Not an inhibitor[b]	630[b,d]	6300[c]
HIF-P4H-2	80[c]	510[c]	Not an inhibitor[b]/7300[e]	1150[b,d]	4800[c]
HIF-P4H-3	60[c]	570[c]	Not an inhibitor[b]	90[b,d]	550[c]
Collagen P4H-I	190[c,d]	400[d,f]	1800[b]	310[b]	450[d,g]
FIH	> 10,000[c]	> 10,000[c]	1100–1500[b,e]	190–300[b,e]	850[c]
KDM4A	1500[h]	800[h]	20[e]	30[e]	ND
KDM4C	ND	ND	80[e]	100[e]	ND
KDM4E	2300[i]	320[i]	ND	ND	ND
ABH2	ND	ND	420[e]	150[e]	ND

ND not determined

[a][15]

[b][60]

[c][83]

[d]K_i value

[e][59]

[f][92]

[g][93]

[h][54]

[i][94]

were found in many human cancers, e.g. melanoma, glioblastoma, seminoma, breast, prostate, urothelial, gastric and renal cancers [20, 21, 26, 27, 32, 35, 69–72], while later, loss of 5hmC was found to be an epigenetic hallmark of many cancers and silencing of the TET enzymes was found to be a key mediator not only of reduced 5hmC levels but also of EMT in cancer cells [26, 27].

Loss of 5hmC has been shown to be an epigenetic hallmark of human melanoma [26, 73]. Low 5hmC levels have been shown to be a marker of melanoma progression and to correlate negatively with Breslow scores and mitotic rate, well-established markers used for melanoma staging. Loss of 5hmC is present in both primary melanomas and metastases and appears to be mediated by downregulation of IDH2 and TET expression [26]. The most dramatic decrease was seen in the *TET2* mRNA levels and, interestingly, modeling the overexpression of IDH2 or TET2 increased the previously lost 5hmC levels and suppressed tumor invasion and growth. Later article paper by Gong et al. shed light on the mechanism linking loss of 5hmC to melanoma progression. It was reported that TGF-β1-induced downregulation of *TET2* and *TET3* was responsible for EMT in melanoma cells [74]. TGF-β1 increased the methylation of *TET2* and *TET3* promoters through recruitment of DNMT3A methyltransferase, and a compound inhibiting gene methylation, 5-aza-2′-deoxycytidine, was able to reverse the downregulation of TET2 and TET3 and furthermore inhibit EMT [74]. Also, overexpression of TET2 was able to inhibit the EMT process and suppress tumor growth and metastasis. In addition to melanoma, *TET1* expression is downregulated in non-Hodgkin B-cell lymphoma and acute B-lymphocytic leukemia [75], and also in prostate cancer [71]. TET1 downregulation was also linked to lower expression of tissue inhibitors of metalloproteinase (TIMPs), which in turn contributes to tumor invasion [71].

Interestingly, oncogenic micro-RNAs have also been shown to downregulate TETs, and TETs themselves have been shown to regulate the expression of tumor-suppressor micro-RNAs [27]. It was found during an attempt to explain the connection between TETs and EMT that TETs and TDG-mediated DNA demethylation are essential for mesenchymal epithelial transition and somatic cell differentiation [37]. TET-mediated demethylation of a tumor-suppressor micro-RNA family called *miR-200*, which is responsible for modulating the expression of EMT transcription factors such as Zeb1 and Zeb2, was reported to be essential for cell differentiation [37]. Song et al. [27] in turn showed that oncogenic *miR-22* is able to downregulate TETs and, therefore, inhibit expression of the tumor-suppressive *miR-200* family.

Since the cancer-associated 2-OG analogues such as fumarate, succinate, R-2HG and S-2HG have been shown to inhibit the TETs (Table 2) [15, 54], it is intriguing to

hypothesize that, in addition to downregulation of TETs, inhibition of the catalytic activity of TETs by these compounds could be responsible for the low 5hmC levels and EMT in cancers with *IDH1* or *2*, *FH* or *SDH* loss-of-function mutations [53, 55, 58]. Interestingly, it has recently been shown that fumarate is also able to promote EMT in $FH^{-/-}$ cells by inhibiting the TET-mediated demethylation of the antimetastatic miRNA cluster *mir-200ba429* [76].

Taken together, the TET enzymes have been consistently shown to be key mediators in cell differentiation and transformation, most notably in EMT. The DNA demethylation controlled by TETs appears to be a dynamic process that can control the state of cell differentiation. In cancers, pathological states resulting in hypoxia, the accumulation of 2-OG analogues, a scarcity of 2-OG, e.g. via *IDH* downregulation, or micro-RNAs, such as *miR22*, can affect the activity or expression of the TET enzymes and, therefore, result in pathological cell differentiation and transition, leading to increased tumor aggressiveness and invasion (Fig. 2).

The diagnostic and prognostic value of 5hmC levels in cancer

As low 5hmC levels have been found in many cancers, several evaluations of its value as a diagnostic or prognostic marker have been published. Promising suggestions that 5hmC levels could be used as a diagnostic or prognostic tool in many cancers have emerged.

Since Lian et al. [26] showed that loss of 5hmC is an epigenetic hallmark of melanoma, it has been reported that 5hmC expression is not only associated with the prognosis for melanoma but could also be used for microstaging the disease and more notably for differentiation between benign congenital nevi and malignant melanoma [73, 77]. In these studies, malignant melanomas showed an almost complete loss of 5hmC, whereas benign proliferative nodules displayed high 5hmC expression [77]. Low 5hmC levels were also associated with a high mitotic rate and high Breslow scores [73]. Besides melanoma, reduced 5hmC levels are found in laryngeal and esophageal squamous cell carcinomas and are associated with a higher TNM score and lower overall survival [32, 78, 79]. In non-small cell lung cancer, 5hmC expression correlated negatively with a higher tumor stage, lymph node metastasis and primary tumor size [34]. Orr et al. [35] associated low 5hmC levels in cases of malignant human glioma with the neural progenitor phenotype and shorter survival. In additional, low 5hmC levels have been associated with increased aggressiveness and a poorer prognosis in adult T-cell leukemia and chronic lymphocytic leukemia [36, 80]. Interestingly, low 5hmC levels in these leukemias were linked to low levels of *TET2* and *IDH* expression, which was also correlated significantly with the prognosis, underlying the fact that TETs mediate these effects in cancers.

A recent meta-analysis combining data from numerous cancers of various types reports that decreased 5hmC levels correlate with overall cancer progression and poor survival [33]. In general, these new studies show that determining the 5hmC levels in various tumors and cancers could be a valuable diagnostic tool and an aid to cancer staging and prediction of the prognosis for the disease. In addition to the use of 5hmC as a diagnostic and prognostic tool, cancers with low 5hmC levels might be more susceptible to treatment with hypomethylating agents. One promising aspect is that previous studies have shown AML and MDS patients with *TET2* mutations and low 5hmC levels to have a higher response rate to these agents [81, 82].

Conclusions

The TET enzymes play central roles in regulating gene expression via DNA demethylation in myeloid tissue, ES cells and several other tissues. Dysregulation of TET function and imbalance in genomic 5mC/5hmC levels are associated with oncogenic transformation. Although kinetic analyses suggest that the TET enzymes do not act as cellular oxygen sensors, pathological tumor hypoxia is thought to impair their catalytic activity. The 2-OG analogue oncometabolites such as fumarate, succinate, R-2HG and S-2HG have been shown to signal via TETs. However, we do not currently have any precise knowledge of their potency, or that of oxygen deprivation, for inhibiting all other 2-OGDDs, including several KDMs that can be considered equally credible to the TETs as potential regulators of gene expression and oncogenic transformation. The overall contribution of TET inhibition to carcinogenesis, therefore, remains to be resolved.

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