TET Family of Dioxygenases: Crucial Roles and Underlying Mechanisms

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Abstract

DNA methylation plays an important role in the epigenetic regulation of mammalian gene expression. TET (ten-eleven translocation) proteins, newly discovered demethylases, have sparked great interest since their discovery. TET proteins catalyze 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine in 3 consecutive Fe(II)- and 2-oxoglutarate (2-OG)-dependent oxidation reactions. TET proteins dynamically regulate global or locus-specific 5-methylcytosine and/or 5-hydroxymethylcytosine levels by facilitating active DNA demethylation. In fact, in addition to their role as methylcytosine dioxygenases, TET proteins are closely related to histone modification, interact with metabolic enzymes as well as other proteins, and cooperate in transcriptional regulation. In this review, we summarize the recent progress in this exciting field, highlighting the molecular mechanism by which TET enzymes regulate gene expression and their functions in health and disease. We also discuss the therapeutic potential of targeting TET proteins and aberrant DNA modifications.

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DNA methylation has an essential role in regulating gene expression. The levels and patterns of DNA methylation are the result of the opposing actions of methylating and demethylating machineries. Although great progress has been made in clarifying the methylating machinery, including the identification and functional characterization of DNA methyltransferases, knowledge of the mechanisms of demethylation and the major players is relatively recent. In 2009, Rao and her colleagues first demonstrated that the human TET1 protein had the capacity to hydroxylate 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) [Tahiliani et al., 2009]. The other 2 members of the TET (ten-eleven translocation) family (TET2 and TET3) can also catalyze a similar reaction [Ito et al., 2010]. Moreover, TET proteins can catalyze the oxidation of 5hmC to 5-formylcytosine (5fC) and 5fC to 5-carboxylcytosine (5caC) [He et al., 2011; Ito et al., 2011]. TET proteins have gained much attention because of their key roles in the DNA demethylation pathway. Furthermore, subsequent studies have revealed that TET proteins are closely associated with other epigenetic modifications and cell metabolism. Indeed, TET proteins have important roles in embryogenesis, stem cell differentiation, development, and transformation. In this review, we detail the recent advances in this exciting field, focusing on the crucial roles and underlying mechanisms of TET proteins in epigenetic regulation.

The Family of TET Dioxygenases

The TET family has 3 members: TET1, TET2 and TET3. *TET1* is located on human chromosome 10q21.3, *TET2* on chromosome 4q24, and *TET3* on chromosome 2p13.1. All TET proteins contain a C-terminal catalytic domain that consists of a cysteine-rich region and a double-stranded β -helix fold characteristic of the Fe(II)- and 2-oxoglutarate (2-OG)-dependent dioxygenase superfamily. These enzymes require Fe(II) as a cofactor metal and 2-OG as a cosubstrate to catalyze their reactions. Mutation of the putative iron-binding sites of TET proteins can abolish their enzymatic activity, as can competitive inhibitors of 2-OG-dependent dioxygenases, such as 2-hydroxyglutarate [Tahiliani et al., 2009]. In the oxidation reaction, a TET protein coordinates one oxygen atom from molecular oxygen (O_2) to a hydroxyl group of the substrate (hydroxylation) and the other oxygen atom to 2-OG, leading to the decarboxylation of 2-OG and the subsequent release of carbon dioxide (CO_2) and succinate [Rose et al., 2011]. Both TET1 and TET3 contain an N-terminal CXXC zinc finger domain that has high affinity for clustered unmethylated CpG dinucleotides, whereas TET2 appears to have lost this motif during evolution. Interestingly, the TET2 CXXC domain exists as a separate gene, also called *IDAX* or *CXXC4*, which can negatively regulate TET2 via caspase-mediated cleavage [Ko et al., 2013; Liu N et al., 2013].

Moreover, the crystal structure of TET proteins provides good insight into understanding the mechanisms of TET-mediated 5mC oxidation. Two zinc fingers bring the Cys-rich and double-stranded β -helix domains together to form a compact catalytic domain in human TET2-DNA complex, with 5mC inserted into the catalytic cavity for the reaction [Hu et al., 2013]. Both the human TET2-DNA complex and *Naegleria* TET1-DNA complex utilize a base-flipping mechanism to target the methylated cytosine. The flipped 5mC is stabilized via hydrogen bonds formed with 3 residues, but the methyl group is not involved in any interactions. The catalytic cavity allows TETs to accommodate 5mC derivatives for further oxidation [Dong et al., 2014; Hashimoto et al., 2014].

TET Proteins-Mediated DNA Demethylation

TET proteins can iteratively oxidize the 5mC of DNA to generate 5hmC, 5fC, and 5caC. On one hand, 5fC and 5caC can be further processed by thymine-DNA glycosyl-

ase (TDG) followed by base excision repair, which are termed active DNA demethylation [He et al., 2011; Kohli and Zhang, 2013]. On the other hand, 5hmC cannot be recognized by DNA methyltransferase 1 (Dnmt1) during DNA replication [Valinluck and Sowers, 2007], and the conversion of 5mC to 5hmC will prohibit the maintenance of existing DNA methylation patterns and lead to passive DNA methylation dilution in proliferating cells. Thus, TET protein-mediated 5mC oxidation can regulate global or locus-specific 5mC and/or 5hmC levels dynamically by facilitating both active DNA demethylation and passive DNA methylation dilution, thereby modulating gene expression.

The Genomic Distributions of TET Proteins and 5mC Derivatives Mediated by TET Enzymes

It is important to explore the genomic distributions of TET proteins and 5mC derivatives to thoroughly understand their functions and mechanisms. TET1 is highly expressed in embryonic stem cells (ESCs) and strongly binds to CpG-rich DNA through its CXXC domain. ChIP-seq reveals that TET1 is preferentially associated with gene promoters and exons. TET1 is highly enriched at high-CpG-density promoters and positively correlated with histone 3 lysine 4 trimethylation (H3K4me3) at promoters in murine ESCs [Xu et al., 2011]. Similar to TET1 in mouse ESCs, both TET2 and TET3 localize primarily to CpG islands (CGI) and promoter regions in HEK293T cells [Deplus et al., 2013].

5hmC is the most abundant among the 5mC oxidants and is named as the sixth base. There are several studies regarding the genome-wide mapping of 5hmC in murine ESCs [Pastor et al., 2011; Wu and Zhang, 2011; Wu et al., 2011; Xu et al., 2011; Sun et al., 2015]. In brief, 5hmC is abundant in gene bodies (especially exons), promoter regions, and transcription start sites (TSSs). Moreover, many factors, such as the CpG content and histone modifications, can affect the distribution of 5hmC. For example, 5hmC is uniquely enriched within gene body CGIs but is in very low abundance in CGIs at promoters. Xu et al. [2011] found that 5hmC was abundant at 'univalent' histone 3 lysine 27 trimethylation (H3K27me3) promoters, whereas Pastor et al. [2011] showed that 5hmC was especially enriched at the start sites of genes with promoters bearing dual repressive H3K27me3 and active H3K4me3 marks. 5hmC is also abundant in some protein-DNA interaction sites, such as OCT4 and NANOG binding sites in human ESCs [Stroud et al., 2011].

Genome-wide mapping shows that 5fC is also enriched in CGIs of promoters and exons in murine ESCs [Raiber et al., 2012]. Moreover, 5fC preferentially occurs at poised enhancers among some gene regulatory elements [Song CX et al., 2013]. Single-base resolution mapping reveals the enrichments of 5fC and 5caC on hypomethylated promoters of highly expressed genes [Neri et al., 2015], and 5fC/5caC can be found at major satellite repeat loci. The downregulation of TDG can result in 5fC and 5caC accumulation [Shen et al., 2013]. Despite the differences among these studies, they all strongly suggest that TET proteins, 5hmC, and 5fC play key roles in the regulation of gene expression.

TET Proteins Modulate Gene Expression

TET proteins play crucial roles in controlling gene expression. It is widely accepted that TETs modulate gene transcription by dynamically regulating DNA methylation through their own methylcytosine dioxygenase activity. Nonetheless, it is clear that the underlying molecular mechanisms are complicated and remain largely unclear.

TET Proteins and DNA Methylation

The DNA methylation level is inversely correlated with gene transcription. As DNA demethylases, TET proteins downregulate the level of DNA methylation dynamically. On one hand, TETs regulate DNA methylation by binding to CpG-rich regions to prevent unwanted DNA methyltransferase activity, and on the other hand, TETs control DNA methylation by converting 5mC to 5hmC through their hydroxylase activity [Xu et al., 2011].

5hmC and Gene Transcription

Emerging evidences show that 5hmC, one of 5mC derivatives mediated by TETs, is an independent epigenetic mark that is distinct from 5mC [Doege et al., 2012; Hahn et al., 2013; Li and O'Neill, 2013] and can itself influence gene transcription [Williams et al., 2012]. Most studies support a positive correlation between the content of 5hmC and gene expression; nonetheless, opposite results have been reported [Xu et al., 2011; Dong et al., 2012].

Moreover, the relationship between the amount of 5hmC and gene expression depends on the location of the 5hmC marks. Although the presence of 5hmC in gene bodies is found to be positively correlated to gene expression levels, 5hmC peaks at transcription start sites do not correlate with gene expression levels [Jin et al., 2011]. Hahn et al. [2013] found that the gain of 5hmC was usu-

ally accompanied by the loss of H3K27me3, which may be another mechanism of active gene expression caused by 5hmC. Therefore, the regulation of gene transcription by 5hmC is complicated and affected by many factors.

TET Proteins and Histone Modification

As a key epigenetic mechanism, histone modification has important biological functions, and TET functioning far exceeds that of a methylcytosine dioxygenase. Indeed, TET proteins have a close relationship with histone modification and cooperate in transcriptional regulation.

TET2/3 Promote OGT-Mediated GlcNAcylation

O-GlcNAcylation is a reversible post-translational modification of proteins that plays a crucial role in cell metabolism and many pathophysiological processes. The level of O-GlcNAcylation is controlled mainly by O-linked β -N-acetylglucosamine transferase (OGT), the activity of which is essential for ESC viability and mouse development. OGT is present both in the cytoplasm and the nucleus of different cell types and catalyzes serine and threonine glycosylation. Recently, it has been reported that histones can also be modified by OGT at different sites [Fujiki et al., 2011; Zhang et al., 2011; Fong et al., 2012]. Furthermore, there are unexpected physical and functional interactions between TET2/3 and OGT. First, TET2 recognizes chromatin, recruits OGT to chromatin around the TSS, and promotes H2B Ser 112 GlcNAcylation. Such chromatin remodeling regulated by histone GlycNAcylation results in high levels of target gene transcription in ESCs [Chen et al., 2013]. Second, the interactions of TET2/3 with OGT upregulate H3K4me3 by promoting the GlcNAcylation of host cell factor 1 (HCF1), a component of the H3K4 methyltransferase SET1/COMPASS complex, to induce transcriptional activation [Deplus et al., 2013]. Third, the OGT-TET3 complex has a positive effect on transcription by facilitating the binding of transcription factor-like molecules to the promoter of associated genes. For example, chromatin immunoprecipitation (ChIP) analysis in combination with OGT or TET3 knockdown shows that the OGT-TET3 complex increases the efficient binding of NeuroD1, a potent transactivator, to the N-acetylglucosaminyltransferase-IX (*GnT-IX*) promoter [Kizuka et al., 2014]. The double epigenetic modifications on both DNA and histones by TET2 and OGT work together for the regulation of gene transcription [Vella et al., 2013] (fig. 1).

Histone Methylation

As mentioned above, TET2/3 can upregulate H3K4me3 level by interactions with OGT. TETs and 5mC de-

Fig. 1. TET2/3 promote OGT-mediated GlcNAcylation. TET2/3 interact with OGT and regulate gene transcription by the following 3 pathways: (1) TET2 recruits OGT to the chromatin around the transcription start site (TSS) and promotes H2B Ser 112 GlcNAcylation which activates transcription of the target gene; (2) the interactions of TET2/3 with OGT upregulate H3K4me3 through promoting the GlcNAcylation of host cell factor 1 (HCF1), a component of the H3K4 methyltransferase SET1/COMPASS complex, and induce transcriptional activation in the end; (3) OGT-TET3 complex facilitates the binding of transcription factor (TF)-like molecules to the promoter of the gene.

Fig. 2. The mechanisms of TET proteins regulating gene expression. TET proteins act as a crosstalk between DNA and histones. On one hand, TETs control gene transcription by regulating global or locus-specific 5mC and 5hmC levels dynamically. On the other hand, TETs regulate the transcription of genes through the close association with histone modifications such as H3K4me2/3, H3K27me2/3, and GlcNAcylation.

rivatives mediated by TETs are positively associated with H3K4me3 [Mikkelsen et al., 2007; Xu et al., 2011]. Genome-wide mapping of 5fC in mouse ESCs reveals that 5fC-rich promoters have elevated H3K4me3 levels, which is associated with active transcription [Raiber et al., 2012]. 5hmC accumulated at euchromatic chromosomal bands is found to be marked with di- and tri-methylated histone H3 at lysine 4 (H3K4me2/3) in mouse ESCs [Kubiura et al., 2012].

More excitingly, TET enzymes can participate in the crosstalk between DNA and histones in ESCs [Sui et al., 2012]. Polycomb Repressive Complex 2 (PRC2) di- and tri-methylates lysine 27 of histone 3 (H3K27me2/3), marking a gene for repression. TET1 can form a complex with PRC2 specifically in ESCs: PRC2 recruits TET1 to chromatin at H3K27me3-positive regions of the genome and contributes to epigenetic plasticity throughout cell differentiation [Neri et al., 2013]. Collectively, a complex epigenetic network formed by histone methylation/demethylation and DNA methylation/demethylation acts together to regulate stem cell self-renewal and differentiation [Coskun et al., 2012] (fig. 2).

TET Proteins and Their Co-Factors

TET proteins act in conjunction with many potential protein partners. For example, there are physical associations of NANOG with TET1 and TET2. In mouse ESCs, NANOG recruits TET1/TET2 to enhance the expression of a subset of key reprogramming target genes [Costa et al., 2013]. Moreover, Dppa3 is involved in the TET-mediated conversion of 5mC to 5hmC during the reprogramming of zygotes; Dppa3 protects the maternal genome from active Tet3-mediated demethylation by binding to dimethylated histone H3 lysine 9 (H3K9me2) in zygotes [Nakamura et al., 2012].

PRDM14, a PR domain-containing transcriptional regulator, physically interacts with TET1 and TET2, enhances the recruitment of TET1 and TET2 at target loci, and promotes active DNA demethylation in ESCs. Overexpression of *Prdm14* elevates 5hmC levels but reduces 5mC levels in pluripotency-associated genes. Furthermore, knockdown of *TET1* and *TET2* impair transcriptional regulation and DNA demethylation by PRDM14 [Okashita et al., 2014].

Vitamin C induces TET-dependent DNA demethylation by acting as a cofactor of TET enzymes in ESCs, and it enhances the activity of recombinant TET1 by interacting with the C-terminal catalytic domain of the TET en-

zyme, which likely promotes their folding and/or the recycling of the cofactor Fe(II). Furthermore, vitamin C-induced changes in 5hmC and 5mC are entirely suppressed in *TET1* and *TET2* double knock-out ESCs [Blaschke et al., 2013; Dickson et al., 2013; Minor et al., 2013; Yin et al., 2013].

The Upstream Regulatory Network of TET Proteins

Many molecules can control the expressions of TET proteins. As mentioned above, the IDAX CXXC domain binds to DNA sequences containing unmethylated CpG dinucleotides, localizes to promoters and CGIs in genomic DNA, recruits TET2, and interacts directly with the catalytic domain of TET2. IDAX ultimately results in caspase activation and the downregulation of the TET2 protein [Ko et al., 2013]. Furthermore, there is a strong association between cell metabolism and epigenetic regulation. Mutations in isocitrate dehydrogenase 1/2 (*IDH1/2*), a critical enzyme involved in cell metabolism, lead to the accumulation of the metabolite 2-hydroxyglutarate (2HG). 2HG can inhibit the enzymatic activity of 2-OG-dependent dioxygenases that mediate epigenetic events, such as TETs [Figuerola et al., 2010; Liu et al., 2012; Shim et al., 2014].

TET2 is a crucial downstream target of the pluripotency factor Oct4 which promotes TET2 transcription by binding to consensus sites in the *TET2* proximal promoter [Wu et al., 2013]. Moreover, activation-induced cytidine deaminase (*Aid*), a unique enzyme that deaminates cytosines in DNA, also acts as a regulator of the subcellular localization of TET proteins. TET proteins are gradually translocated from the nucleus to the cytoplasm when co-expressed with *Aid* [Arioka et al., 2012].

Several microRNAs can target *TET* genes. Oncogenic microRNA-22 targets the *TET2* tumor suppressor to promote hematopoietic stem cell self-renewal and transformation [Song SJ et al., 2013]. MicroRNA-29 can negatively regulate *TET1–3* and *TDG* mRNA levels by binding to their 3'-UTRs [Zhang et al., 2013]. Moreover, microRNA-26a negatively targets *TET* genes, especially *TET2* [Fu et al., 2013].

The Biological Functions of TET Proteins

It is clear that TET proteins play important roles in DNA demethylation and in regulating gene expression. Although recognized only a few years ago, increasing ev-

idence shows that TET proteins have crucial roles in epigenetic reprogramming in stem cell differentiation, embryogenesis, development, and tumorigenesis.

The Roles of TET Proteins in Differentiation and Development

The patterns of genomic methylation in mature somatic cells are usually stable and heritable. However, genome-wide DNA methylation reprogramming is indispensable in zygotes, PGCs (primordial germ cells), and early embryos. TET-mediated DNA demethylation plays crucial roles during methylation reprogramming. TETs prevent unwanted DNA methyltransferase activity by binding to CpG-rich regions. At the same time, TETs convert 5mC to 5hmC through their hydroxylase activity. Thus, TETs simultaneously modulate the levels of DNA methylation and DNA hydroxymethylation to some extent. The patterns of DNA methylation are closely associated with the expressions of some genes crucial for differentiation and development. In the end, TETs regulate development and guide differentiation.

Mammalian zygotes acquire a totipotent developmental potential through epigenetic reprogramming, and TET3 plays a key role in reprogramming of DNA methylation in the zygote. Knockdown of *TET3* simultaneously affects the patterns of 5hmC and 5mC in the paternal pronucleus [Wossidlo et al., 2011]. The conversion of 5mC to 5hmC in the paternal genome fails to occur, and the level of 5mC remains constant in *Tet3*-deficient zygotes from conditional knock-out mice. Oocytes lacking *Tet3* also appear to have a reduced ability to reprogram the injected nuclei from somatic cells. These data suggest an important role of 5hmC and TET3 in the epigenetic reprogramming of zygotic paternal DNA following natural fertilization [Gu et al., 2011].

The contents of 5mC and 5hmC change dynamically during PGC reprogramming and germ cell development. 5hmC levels increase, reaching a peak at embryonic day 11.5 (E11.5) and gradually decrease until E13.5 likely via replication-dependent dilution. In addition, the great majority of differentially expressed genes are upregulated from E9.5 to E13.5 in both male and female PGCs. Furthermore, global transcriptome analysis by RNA-seq reveals that a subset of meiosis-related and imprinted genes are also significantly increased at E13.5. The analysis of DNA methylation dynamics indicates that TET1 functions to eliminate a remaining methylation, including imprinted genes, at the late reprogramming stage in PGCs. This suggests that TET proteins not only have important roles during PGC reprogramming and germ cell develop-

ment but also play potential roles in the epigenetic reprogramming and transcriptional regulation of meiotic and imprinted genes [Yamaguchi et al., 2013a, b].

ESCs are pluripotent cells derived from the inner cell mass of blastocysts and possess the characteristics of self-renewal and pluripotency. Thus, these cells have the potential to differentiate into all cell types of the 3 germ layers. TET1 and TET2 are highly expressed in undifferentiated ESCs and have key roles in maintaining the pluripotency program in ESCs and in guiding the correct differentiation of the developing embryo. TET1 contributes to ESC self-renewal, differentiation, and the onset of embryonic development by regulating a comprehensive network of genes [Xu et al., 2011; Wang et al., 2013]. For instance, NANOG is one of the master transcription factors controlling the pluripotent state. To some extent, the important role of TET1 depends on maintaining the expression of NANOG in mouse ESCs. *Tet1* knockdown leads to the downregulation of NANOG, correlating with the methylation of its promoter, which supports a role of TET1 in regulating the DNA methylation status. Furthermore, knockdown of *Tet1* in pre-implantation embryos results in a self-renewal defect and a bias toward trophectoderm differentiation [Ito et al., 2010]. However, *Tet3* knock-out ESCs appear normal in self-renewal and maintenance but impaired in neuronal differentiation [Li et al., 2015]. Depleted 5hmC and impaired differentiation are observed in *Tet1/2/3* triple knock-out (TKO) mouse ESCs. Global gene expression and methylome analyses of TKO embryoid bodies have revealed promoter hypermethylation and the deregulation of genes implicated in embryonic development and differentiation, which suggests a role of TET-mediated DNA demethylation in the proper regulation of gene expression during ESC differentiation and development [Dawlaty et al., 2014].

In addition to ESCs, TETs also play important roles in somatic cell reprogramming and differentiation. Somatic cells can be reprogrammed into induced pluripotent stem cells. TET2 contributes to the early establishment of histone modifications that represent an activated chromatin state at pluripotency loci during induced pluripotent stem cell generation [Doege et al., 2012]. Moreover, TET2 is a novel and necessary master epigenetic regulator of smooth muscle cell (SMC) differentiation. *TET2* knockdown prevents rapamycin-induced SMC differentiation, whereas *TET2* overexpression is sufficient to induce a contractile phenotype [Liu R et al., 2013].

TET proteins and 5hmC are highly expressed in the brain, with both having important functions in the development of the nervous system. In neuronal cells, 5hmC is

not enriched at enhancers but is associated preferentially with the gene bodies of activated neuronal function-related genes. Functional deficiency in TET2 and TET3 leads to defects in neuronal differentiation [Hahn et al., 2013]. Moreover, TET1 is critical for neuronal activity-regulated gene expression and memory extinction. *Tet1* knock-out mice exhibit not only a downregulation of multiple neuronal activity-regulated genes, such as *Npas4*, *c-Fos*, and *Arc*, but also abnormal hippocampal long-term depression and impaired memory extinction [Rudenko et al., 2013].

5hmC, the most important 5mC derivative mediated by TETs, plays a crucial role in the development and function of the human liver. There are significant differences in the percentage and genomic distribution of 5hmC between fetal and adult human liver samples. Compared to fetal livers, the content of 5hmC increases, and 5hmC is enriched in genes involved in active catabolic and metabolic processes in adult liver samples [Ivanov et al., 2013].

Aging is generally characterized by the global decrease of DNA methylation [Johnson et al., 2012], and the expressions of Dnmt3a and Dnmt3b decline significantly with skin aging [Qian and Xu, 2014]. However, the global 5hmC content is increased with advanced age. Surprisingly, *Tet1–3* mRNAs do not change during the aging process; this phenomenon may be driven by altered activity and not by an increase in the amount of TET enzymes. From another point of view, this observation supports that 5hmC can act as an epigenetic marker and not merely serve as an intermediary in DNA demethylation [Chen et al., 2012].

The Roles of TET Proteins in Transformation

TET1 was first recognized as a fusion partner of the mixed-lineage leukemia (*MLL*) gene in acute myeloid leukemia. *TET2* was found to be mutated in patients with myeloproliferative neoplasms (MPN), myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and chronic myelomonocytic leukemia (CMML) [Abdel-Wahab et al., 2009; Delhommeau et al., 2009; Jankowska et al., 2009; Kosmider et al., 2009; Langemeijer et al., 2009]. The most common *TET2* mutations in myeloid tumors are observed within exons 3a and 10 [Delhommeau et al., 2009]. *TET2* targets both multipotent and committed progenitor cells in the hematopoietic lineage [Langemeijer et al., 2009]. *TET2* mutation is associated with reduced survival in AML compared to patients with wild-type *TET2*, and *TET2* mutation in MDS and MPN patients notably increase the possibility of progression to AML [Abdel-Wahab et al., 2009]. It has been strongly suggest-

ed that *TET2* is a tumor suppressor gene, especially in myeloid malignancies. *TET2* may regulate normal hematopoiesis to ensure proper lineage distribution and to control hematopoietic stem cell differentiation.

Mice with shRNA-based *Tet2* knockdown or targeted deletion of *Tet2* were used to clarify the functions of *TET2* in hematopoietic differentiation and homeostasis [Li et al., 2011; Moran-Crusio et al., 2011; Quivoron et al., 2011]. *TET1–3* are expressed in hematopoietic systems, but the deletion of *Tet2* alone is sufficient to cause a significant loss of 5hmC in genomic DNA. Moreover, an increasing number of myeloid stem/progenitor cells were observed, and all *Tet2*-null mouse models progressively developed myeloid neoplasms. The loss of 5hmC in hematopoietic malignancies suggests that impaired TET-mediated DNA demethylation plays a key role in tumorigenesis.

In addition to leukemia, *TET2* mutations are observed in lymphoma, causing the aberrant methylation of genes involved in hematopoietic development. It is reported that *TET2* is mutated in 12 of 100 diffuse large B-cell lymphomas, with 7% leading to loss-of-function and 5% being missense mutations [Asmar et al., 2013]. *TET2* mutations are detected in 47% of cases of angioimmunoblastic T-cell lymphoma and in 38% of peripheral T-cell lymphoma not otherwise specified, correlating with a shorter progression-free survival [Lemonnier et al., 2012].

It is reported that the levels of 5hmC are dramatically reduced in solid cancers such as human breast, liver, lung, pancreatic, prostate, colorectal, and gastric cancers when compared with matched surrounding normal tissues. The reduction in 5hmC is associated with the declining expression of TET proteins, which reveals a possible mechanism for the reduced level of 5hmC in cancer cells [Kudo et al., 2012; Yang H et al., 2013]. Moreover, decreased 5hmC is associated with the clinical pathology of gastric cancer and is an independent poor prognostic factor in gastric cancer patients [Yang Q et al., 2013]. These results suggest the critical roles of aberrant DNA demethylation in oncogenic processes in solid tissues [Kudo et al., 2012].

A decrease in 5hmC is also observed in glioma: the 5hmC level is high in low-grade glioma and low in those with high-grade disease. Additionally, there is a significant relationship between a low level of 5hmC and reduced survival in malignant glioma. These findings suggest that the 5hmC level in malignant glioma may represent an important determinant of tumor differentiation and aggressive behavior as well as serving as a prognostic indicator [Orr et al., 2012].

Overall, TET proteins and TET-mediated demethylation have important functions in cell transformation

and tumorigenesis. The reduction in 5hmC is closely associated with prognosis in some malignant tumors. However, the underlying mechanism remains unclear. Further studies will help improving the diagnosis, treatment and prediction of tumor prognosis.

The Roles of TETs in Other Diseases

The levels of *TET1*, 5mC, and 5hmC are increased in the hippocampus/parahippocampal gyrus of preclinical Alzheimer's disease, while the levels of 5fC and 5caC are significantly decreased. These data demonstrate altered methylation/demethylation patterns in vulnerable brain regions prior to the onset of the clinical symptoms in Alzheimer's disease, which suggests a role in the pathogenesis of the disease [Bradley-Whitman and Lovell, 2013].

Both the mRNA and protein expression of *TET1*, but not *TET2* or *TET3*, are increased (2- to 3-fold) in the inferior parietal lobule of psychotic patients compared with control subjects, highlighting the possible role of altered DNA demethylation mechanisms in the pathophysiology of psychosis [Dong et al., 2012; Gavin et al., 2013].

TET2 controls vascular SMC plasticity and the development of vascular disease. Loss of *TET2* and 5hmC positively correlates with the degree of injury in murine models of vascular injury and human atherosclerotic disease [Liu R et al., 2013; Prosdocimo et al., 2013].

Furthermore, 5hmC might be an important determinant for development of liver diseases as well as of inter-individual differences in liver functions such as protein synthesis, lipid and carbohydrate metabolism and drug metabolism and toxicity [Ivanov et al., 2013].

TET proteins are also closely associated with the development of autoimmune diseases. *TET2* promotes Th1 and Th17 cell differentiation and their signature cytokine expression in vitro and in vivo. Unexpectedly, *TET2* can relieve the disease severity of experimental autoimmune encephalomyelitis by increased IL-10 production in vivo [Ichiyama et al., 2015].

Epigenetic Therapy Targeting TET Proteins and DNA Demethylation

Recent advances have highlighted the central roles of TET-mediated DNA demethylation in many diseases, offering a novel strategy for epigenetic therapy. Several groups have focused on this field. Lian et al. [2012] found that rebuilding the 5hmC landscape in melanoma cells by reintroducing active *TET2* or *IDH2* not only suppressed melanoma growth but also prolonged tumor-free survival

al in animal models. Through epigenetic therapy targeting the active DNA demethylation pathway, previously pathological neurons in the brain may be reprogrammed to function more appropriately, resulting in great optimism in the field of psychiatry [Gavin et al., 2013].

Conclusions

Overall, TET proteins play important roles in regulating gene expression and in the onset and development of many diseases. Epigenetic therapy targeting TET pro-

teins and the DNA demethylation pathway in a cell type-specific manner would be a promising approach to combating many diseases. Further research on TET proteins and 5mC oxidants will advance our understanding of the underlying molecular mechanisms and provide new therapeutic strategies and broad prospects for epigenetic therapy.

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