The Dual Characteristics of Ten-eleven Translocation 1 in Cancer

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Abstract: DNA methylation abnormalities in tumors often manifest as an increase or decrease of 5-methylcytosine at the genomic level or individual promoter sites. The mechanism of DNA demethylation by ten-eleven translocation proteins (TET) in maintaining the stability of global genome methylation level has attracted extensive attention. The biological functions of TET1-mediated hydroxymethylation differ among cancers due to tumor heterogeneity. Herein, recent updates on the effects of TET1 on tumor proliferation, migration, and invasion by altering DNA methylation levels are reviewed, and the direct and indirect roles of TET1 in activating or suppressing tumor progression are also discussed. Besides, the potential uses of DNA methylation analysis in clinical diagnosis and research of tumor microenvironment in relation to epigenetics are prospected. In conclusion, further studies about the dual characteristics of TET1 in cancer diseases are warranted to expand our understanding of the effects of DNA methylation in tumor which could be instrumental in the development of tumor treatment.

Keywords: DNA methylation, Ten-eleven translocation proteins, Tumor

1 Introduction

The epigenetic modifications, including DNA methylation, histone modification, nucleosome remodeling, and RNA-mediated gene targeting, significantly vary among different cancers and developmental syndromes. DNA modification, such as DNA methylation and demethylation at the fifth position of cytosine, has gradually garnered attention in recent years because normal DNA methylation level could maintain genome stability, reduce chromosome deletions or rearrangements, and regulate the development of somatic cells. Aberrant DNA methylation level, such as hypermethylation and hypomethylation, would cause a series of severe pathological changes and diseases, especially cancers.

Nevertheless, the correlation between abnormal DNA methylation and pathogenesis remains ambiguous, and the proposed mechanisms underlying...
oncogenesis is also a subject of contention. Tumor-associated genes that are prone to frequent methylation could be characteristic markers of cancers. Demethylation is an important process that maintains the level of DNA methylation. Of note, dysregulations of methylation and demethylation have been noted in the process of tumor development.

According to the central dogma of molecular biology, modification of DNA would lead to the alterations of transcriptional gene expression, which would drive the development of cancers and other diseases. The cytosine base modification is the major DNA modification in mammals, and there are at least four modifications, including 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC). Modifications from cytosine to 5mC are dynamically driven by modifying enzymes called DNA methyltransferases (DNMTs), and hydroxymethylation from 5mC to 5hmC, 5fC, and 5caC is catalyzed by DNA demethylases such as ten-eleven translocation proteins (TET1, TET2, and TET3).

The underlying biological mechanism of TET protein family, especially TET1, in diverse cancer types is still a major unanswered question in the field. After analyzing whole-genome methylation profiles in cancer methylomes, Li et al. discovered frequent promoter methylation of TET1 in a large collection of tumor cell lines and primary tumors of multiple carcinomas and lymphomas, and eventually confirmed its tumor suppressive functions and demethylation activity. Concurrently, some adverse conclusions have been reported that TET1 exerts anti-tumor or pro-tumor effect in cancer still remains controversial, and this warrants the need to summarize the specific effects of TET1 in cancers.

Therefore, the objective of this review is to summarize the effect of TET1 in cancers and to investigate the possible applications in cancer treatment. In this review, we overview the dual roles of TET1 as tumor-promoting factor and tumor-suppressing factor and elucidate the relevant mechanisms in detail. From a clinical perspective, we also delineate the outlook and future directions of the application of DNA methylation in cancer treatment and research.

2 Discovery of TETs and its structure

Demethylation was first identified in 2000, when a genome-wide 5mC deletion that was not caused by replication dilution was found in mouse zygotes incidentally. In 2003, the TET (t[10;11](q22;q23)) on human chromosome 11 or TET1 which fuses with MLL1 gene was found in chromosome 11 in a rare case of acute myeloid and lymphocytic leukemia. In 2010, other members of the TET protein family, such as TET2 and TET3, were also discovered consecutively. TET proteins are known as the Fe(II) and α-ketoglutarate (α-KG)-dependent dioxygenases in mammals. The C-terminal catalytic domain is formed by a cysteine-rich domain winding a double-stranded β-helix domain. In this process, two zinc fingers, Fe(II), α-KG, and 5mC are required to form a TET-DNA complex.

TET protein family comprises three proteins with different structures in human and mouse. The ancestral gene TET underwent triplication leading to the generation of TET1, TET2, and TET3. Both TET1 and TET3 have CXXC domain in the amino terminal, which could bind the unmethylated cytosine. Chromosomal inversion caused the separation of CXXC domain from TET2 gene, and it has since become a neighboring gene which encodes IDAX protein (also known as CXXC4). At different developmental stages of different species, several isoforms of TET proteins had been identified. In mouse somatic tissues, an isoform of TET1 without CXXC domain (TET1s) is preferentially expressed in early embryos, embryonic stem (ES) cells, and primordial germ cells. A similar kind of N-terminal truncated TET1 that lacks CXXC, which is called TET1ALT, were found in human samples and overexpressed in breast cancer, glioblastoma, and uterine cancer. Less binding of CXXC domain to chromatin leads to reduced demethylation activity of TET1s and TET1ALT. Two TET3 isoforms without CXXC
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Domain, namely, the TET3o and TET3s, were discovered in mouse neural progenitor cells and embryonic brain-derived neurons successively. The catalytic activity of TET3o and TET3s is higher than that of full-length TET3. The results from the in vivo biological assays suggested that the CXXC domain of TET3 partly limits its genome-wide 5mC oxidation capacity\([25]\). Variants of TET1, TET2, and TET3 in human and mouse are shown in Figure 1.

3 Function of TETs

The function of TET1 has not been revealed until 2009 when two groundbreaking papers reported that 5hmC accumulates in brain and neuronal chromatin\([26]\], and the conversion from 5mC to 5hmC is catalyzed by TET1\([27]\). In fact, all three TET proteins could convert 5mC to 5hmC\([28]\). TET protein family could further oxidize 5hmC to 5fC and 5caC\([29]\), and the conversion from 5mC to 5hmC was the main catalytic reaction\([30]\). It has also been proven that TETs preferentially bind to 5mCpG, rather than 5mCpC and 5mCpA\([16]\).

TETs are primarily located at the gene bodies or the transcription start sites of CpG-rich gene promoters. Under the catalysis of DNMT, S-adenosylmethionine (SAMe) transfers a methyl group to the fifth position of cytosine, from which 5mC was formed, and almost 80-90% of DNA methylation modification happened on the fifth position of cytosine\([31]\). Moreover, mammalian TET1 proteins seem to catalyze the formation of 5hmC in RNA, suggesting a role of TET1 in RNA modification\([32]\). In addition to different enzymes (such as FTO and ALKBH5) that can demethylate the N6-methyladenosine (m6A), recent studies also found that TET could demethylate RNA. Further studies proposed that TET enzymes could catalyze the oxidation of 5-methylcytidine to 5-hydroxymethylcytidine\([32]\), and then to 5-formylcytidine and 5-carboxycytidine\([33]\). In different organs, 5mC could be actively removed from the genome through different mechanisms, including TET-mediated hydroxymethylation\([34]\). The cycle of DNA demethylation is shown in Figure 2.

![Figure 1](image.png)

**Figure 1.** Domain structure of ten-eleven translocation proteins (TET) in human and mouse. The full-length structure of three TET proteins (TET1FL, TET2FL, and TET3FL) and the structure of several major isoforms are shown in the figure. All TET proteins possessed a catalytic domain at the carboxyl terminus consists with a cysteine (Cys)-rich domain and a double-stranded \(\beta\) helix (DSBH). The N-terminal CXXC zinc finger domain is found in both full-length TET1 and TET3. The CXXC domain is separated from TET2 and becomes a neighboring gene, which encodes IDAX protein. TET1 lacking the CXXC domain in human and mouse encodes TET1ALT and TET1s, respectively. TET3o and TET3s are isoforms of TET3 without CXXC domain, which were discovered in the mouse.
Recently, many biological functions of TET1 have been uncovered in different diseases, and it is found to play a vital role in the erasure of genomic imprints. In mammals, the levels of DNA methylation and demethylation are at normal state in disease-free individuals due to multiple regulations. The role of TET family and the mechanism underlying TET-mediated DNA demethylation has been studied thoroughly. Since the methylation of CpG Island in promoters often leads to stable gene silencing, TET1 protein is regarded as the gene switch that regulates gene expression. Besides, TETs behave as transcriptional coactivators that promote the expression of hypoxia-responsive genes and induce epithelial-mesenchymal transitions (EMT) during oncogenesis. TET1 is also involved in the regulation of telomere length in TET triple-knockout mouse ES cells, resulting in an increased telomere length associated with a high frequency of telomere sister chromatid exchange. Furthermore, TET enzymes play an important role in embryonic development, cell lineage specification, neuronal function, and cancer. Scourzic et al. summarized the role of TET enzymes in hematologic cancers and solid tumors, but the functional consequences of TET1 in tumors remain to be elucidated.

4 Anti-tumor activities of TET1

The expression of TET1 is lower in many tumors than normal tissues. A line of evidence suggests the tumor suppressor role of TET1 as marked by its anti-proliferation and anti-metastasis effects, reversal of chemotherapy resistance, and dysregulation of signaling pathways. Pei et al. reported that TET1 suppressed tumor proliferation, invasion and migration in gastric cancer through downregulation of phosphatase and tensin homolog and activation of protein kinase B (Akt) and focal adhesion kinase pathways. TET1 was also found to suppress metastasis in lung cancer cell H460 by reducing the expression of E-cadherin, which is an epithelial marker. The previous studies reported that TET1 blocked EMT and metastasis, and reduced the expression of nuclear β-catenin along with its downstream target genes in the cell lines of pancreatic tumor, ovarian cancer, colon cancer, and prostate and breast cancer. It also inhibits Wnt/β-catenin signaling by demethylating and upregulating the upstream antagonists of the pathway, such as dishevelled-associated antagonist of β-catenin 2 (DACT2) and secreted frizzled related protein 2 (SFRP2). In addition to cell signaling pathway,
TET1 governs cell cycle as well. Fan et al. revealed that in nasopharyngeal carcinoma cell lines, TET1 inhibited cell migration and invasion by reducing apoptosis and blocking cell division in G0/G1 phase, suggesting TET1 as a tumor suppressor gene (TSG)\textsuperscript{52}.

Interestingly, some researchers illustrated that the catalytic domain of TET1 could also demethylate CpG Island of other TSG promoters, thus activating TSG expression and achieving anti-tumor effects. Similar conclusions have been verified in mouse models and cells. Forlomi et al. found out that the oncogenic activation of epidermal growth factor receptor could silence multitudinous TSGs by downregulating TET1 in lung cancer and glioblastoma multiforme cells\textsuperscript{53}. In human breast cancer cells and mouse breast tumors, deletion of high mobility group AT-hook 2 (HMGA2) resulted in a growth defect phenotype by upregulating the expression of TET1 and 5hmC. TET1 demethylated and activated its own promoter and the promoter of homeobox A9 (HOXA9), which is the downstream effector of TET1, thereby stimulating HMGA2–TET1–HOXA9 pathway to suppress breast tumor proliferation and metastasis\textsuperscript{54}. 

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**Figure 3.** Tumor promoting and suppressing roles of TET1. When the level of whole genome 5mC in tumor is higher than in normal tissue, TET1 exerts anti-tumor effects by regulating tumor suppressor genes and various signaling pathways. (A) Numerous genes and proteins are involved in anti-proliferation, anti-migration and anti-invasion activities of TET1, such as high mobility group AT-hook 2 (HMGA2), epidermal growth factor receptor (EGFR), and protein kinase B (Akt). (B) TET1 could alter the expression of DNA methyltransferase 1 (DNMT1) and peroxisome proliferator-activated receptor γ (PPARγ) when treated by small molecules. (C) TET1 is able to reverse chemotherapy resistance by regulating P-glycoprotein and (D) associated with immune modulation via through NF-κB pathway in immune cells. On the other hand, when the level of whole genome 5mC in tumor is at low level, TET1 manifests pro-tumor activity in cancers. (E) Proliferative effects of TET1 are mediated through phosphatidylinositol 3-kinase (PI3K) and Akt pathways. (F) TET1 could regulate the expression of p53 through microRNAs, and target friend leukemia virus integration 1 (FLI1) and DNMT1 through exonic circular RNA at post-transcriptional level, and (G) it is involved in chemotherapy resistance by regulating O-6-methylguanine-DNA methyltransferase (MGMT) and influencing epithelial–mesenchymal transition (EMT) pathways.
4.2 Reversal of chemotherapy resistance

Multidrug resistance of cancer cells is a principal factor of poor prognosis. In a recent study, Wang et al. found that TET1 overexpression could reverse gemcitabine-resistance to inhibit tumor proliferation in cholangiocarcinoma and decrease the expression of P-glycoprotein\cite{55}. The same study also suggested that the low expression of TET1 was associated with poor prognosis based on the analysis of the correlation between clinical outcomes and TET1 expression in 82 cholangiocarcinoma patients after chemotherapy. Therefore, TET1 may be a promising target to overcome chemoresistance of cholangiocarcinoma to improve prognosis. Hypothetically, TET1 could also counteract chemoresistance of other types of cancer.

4.3 Immune modulation

The study between DNA methylation and immune modulation has deepened our understanding of the contribution of TET1 to tumor progression. Experiments involving cell lines and mouse models conducted by Collignon et al. have shown that TET1 was downregulated in breast cancer cells on activation of nuclear factor kappa B (NF-kB) pathway\cite{56}, implying the potential role of TET1 in controlling tumor development. Binding of p65 to TET1 promoter stimulated high expression of immune markers and NF-κB in immune cells, leading to immune response and tumor suppression phenotype. Moreover, the study revealed that the immunity-driven repression of TET1 in NF-κB pathway was also common in melanoma, lung, thyroid, and ovarian cancers where the expression of TET1 and 5hmC level decreased compared to the normal tissues.

4.4 Targeted regulation by small molecules

A number of observations suggest that TET1 is a direct therapeutic target in tumor cells when treated by small molecules, which block relevant signaling pathways and tumor growth. Seo et al. proposed that the expression of TET1 in colon cancer cells was obviously increased, along with a simultaneous reduction in DNMT1 expression level, after hinokitiol treatment\cite{57}. Hore reported that the synergy of Vitamins A and C stimulated TET expression, enhanced TET catalytic activity, and reprogrammed differentiated cells to a naïve state\cite{58}. In addition, Vitamin C supplementation in melanoma cells activated TETs and adjusted 5hmC to normal levels, suggesting its medical value for cancer treatment\cite{59,60}. Eicosapentaenoic acid (EPA) is a DNA-demethylating agent that executes anti-cancer effects by increasing TET1 and 5hmC levels. In human hepatocellular carcinoma cell, EPA bound to the activated peroxisome proliferator-activated receptor γ to increase the levels of TET1 and 5hmC, which inhibit tumor progression\cite{61}. Besides, experiments in rat hepatoma cell lines showed that TET1 promoted the expression of p21 and suppressed tumor development by regulating cell cycle progression\cite{62}.

Taken together, the upregulation of TET1 levels mediated by small molecules or compounds, such as hinokitiol, Vitamin A, Vitamin C, and EPA, can repress tumor proliferation. Thus, these small molecules should be incorporated as part of the cancer treatment.

5 Pro-tumor activities of TET1

Unlike in some types of cancer where TET generally acts as a tumor suppressor, a body of evidence also indicated that TET1 may act as an oncogenic gene and its protein exerts pro-tumor activity in other types of cancer, as shown in Figure 3.

5.1 Resistance to chemotherapy

Recent evidence showed that TET1 modulated chemotherapy resistance. One study reported that TET1 was positively correlated with cisplatin resistance in ovarian cancer and TET1 could promote cisplatin resistance by demethylating the vimentin promoter and reversing the EMT process during drug resistance\cite{63}. Similar effect was also noted in oral squamous cell carcinoma (OSCC) stem cells where Wang et al. found that TET1 inhibited cisplatin sensitivity, along with increased cell viability, increased number of cells in the S phase and decreased apoptotic rate\cite{64}. In the OSCC stem cells, siRNA-induced TET1 knockdown ultimately enhanced the sensitivity...
of OSCC stem cells to chemotherapy by silencing mRNA expression of O-6-methylguanine-DNMT and stem cell markers, partly indicating that TET1 is associated with chemotherapy resistance. Therefore, TET1 is associated with pro-tumor effect and thus, it may be used as a therapeutic target.

5.2 Proliferation effects
TET1 plays an important carcinogenic effect which promotes tumor proliferation and progression in leukemia and solid tumors. Huang et al. found that TET1 in cooperation with MLL fusion proteins regulated the oncogenes and resulted in an overall increase in 5hmC levels, indicating an indispensable carcinogenic role of TET1 in leukemia. It was found that the level of TET1 upregulated specifically in triple negative breast cancer and by means of bioinformatics and in vitro experiments, TET1 may be a potential oncogene. To study the proliferative effects of TET1, CRISPR technology was used to generate a TET1 knockout cell lines which developed peculiar properties relative to the wild type cells, such as restrained cell proliferation, downregulation of genes in the phosphatidylinositol 3-kinase (PI3K) pathway, and upregulation of immune response genes. Moreover, both full length TET1 and its isoform TET1ALT caused genome-wide hypomethylation and gene activation in the PI3K-Akt-mTOR pathway. These results facilitated us to better understand the biological function of demethylation in the oncogenic pathway.

5.3 Post-transcriptional regulation
The targeted regulation of TET1 at post-transcriptional level by microRNA plays a key role in repressing the process of translation. For example, overexpression of miRNA-191 reduces the expression of TET1 in intrahepatic cholangiocarcinoma (ICC), maintains methylated level in CpG islands at the promoter of p53 gene, thereby downregulating the expression of p53 and abrogating its anti-cancer function. Besides, the study also indicated that the combination of miR-191 and TET1 may be a biomarker of ICC progression for predicting overall survival and disease-free survival. In breast, colon, prostate, and lung cancers, miRNA-29a was highly associated with the expression of epigenetic-related genes such as TET1, DNMT3A, DNMT3B, and thymine DNA glycosylase. As reported, the expression of TET1 increased significantly in the miRNA-29a knockdown cell lines, accompanied by the inhibition of tumor proliferation and migration. TET1 is also the direct target gene of miRNA-494, which is significantly correlated with vascular invasion in human hepatocellular carcinoma. Therefore, miR-191, miR-29a, and miR-494 could inhibit genomic DNA demethylation by downregulating TET1 expression, thereby promoting tumor development.

In addition, increasing evidence has shown the important role of circular RNAs at post-transcriptional level in the regulation of carcinogenesis. TET1 could regulate the expression of circular RNAs by regulating the methylation level of gene promoters. In addition, FECR1, an exonic circular RNA of friend leukemia virus integration 1 (FLI1), recruited TET1 while downregulated DNMT1 level. It also activated FLI1 by inducing hypomethylation of CpG Island DNA in the promoter region, and enhanced the metastasis and invasion of breast cancer cells, indicating the pro-tumor role of TET1 at post-transcriptional level and in tumor microenvironment.

6 Outlook and future directions
A thorough and comprehensive understanding on the mechanism of aberrant DNA methylation plays a significant part in the development of cancer treatment. Herein, a few research prospects and directions related to DNA methylation are illustrated.

6.1 DNA methylation profiling in cancer
Prediction based on methylation analysis could be applied to both early predictive diagnosis and prognosis monitoring of cancer. A growing number of studies found that abnormal hypermethylation or hypomethylation occurred in tumors, including excessive 5mC, 5hmC, and
5fC on the genome. It is important to note that the degree of tumor malignancy is closely related to the level of DNA methylation, especially in cancer with poor prognosis\textsuperscript{[31]}. Reduced 5hmC levels have been observed in many tumors with unfavorable clinical outcomes and high tumor recurrence rate\textsuperscript{[70]}. Besides, during the early stage of hepatocellular carcinoma, the global genome content of both 5hmC and 5fC was reduced in tumor tissues compared to that of the adjacent tissues\textsuperscript{[71]}. It has been indicated that the whole genome level of 5hmC and 5fC, which is regulated by TET1, is a potential biomarker for both early detection and determination of prognosis.

DNA methylation analysis has extensive applications and promising significance in predicting potential tumor cases. Based on the previous studies, a large collection of DNA methylation biomarkers has been applied in clinical diagnosis\textsuperscript{[72]}. Further understanding of the genomic location of DNA methylation and the clinical relevance of cancer is required for better healthcare of patients\textsuperscript{[73]}. Monitoring these biomarkers may also help assess the recovery rate and predict recurrence of cancer in the post-operative patients, suggesting their prospects in clinical applications.

6.2 Interaction with tumor microenvironment

Tumor microenvironment plays an indelible role in the process of tumor development, as well as the interaction with epigenetic regulation. The previous findings suggested that various molecular mechanisms and epigenetic dysregulations in tumor microenvironment contributed to the metastasis of tumor cells. Zhang \textit{et al.} found that pancreatic ductal adenocarcinoma cells were capable of inducing DNA methylation of cancer-associated fibroblasts, which helped promote tumor growth\textsuperscript{[74]}. Furthermore, a single-cell transcriptomic study illustrated the inter-tumor heterogeneity of human ICC and identified the important interactions between ICC cells and cancer-associated fibroblasts that induce epigenetic alterations to promote cancer progression\textsuperscript{[75]}

In addition, epigenome in immune cells that surround the tumor cells also plays a key role in the progression of epithelial ovarian cancer and its extracellular matrix by secreting proteins, growth factors, and cytokines into the extracellular space. Immune cells with DNA methylation changes in tumor microenvironment would trigger immune defense mechanisms, alter immune response, and stimulate immune cell-mediated cytotoxicity to further suppress tumor growth\textsuperscript{[76]}. In view of the above, a deeper understanding of the regulation of DNA methylation in tumor microenvironment and other cellular compartments is invaluable for delineating the pharmacological mechanisms of cancers.

7 Conclusion

In this review, both anti-tumor and pro-tumor activities of TET1 in tumorigenesis are concretely introduced in detail. TET1 regulates the promoter regions of diverse genes, resulting in either high or low gene expression in tumor which has a direct impact on tumor development and progression. Surprisingly, we also learned that the roles of TET1 could vary according to the tissue location and tumor types, even the roles could be different among subtypes of the same tumor.

TET1 could be the potential targets for medical diagnostics and drug development when tumor types, stages, and treatment methods are taken into consideration in a comprehensive manner. Nevertheless, further investigations are still required to corroborate the dual characteristics of TET1.

Acknowledgments

Our work was supported by grants from the National Natural Science Foundation of China (81772617), Great Wall Scholar Project (CIT&TCD20190311) and National Key Research and Development Program of China (2017YFC0908402).

Conflict of interest

The authors have no conflict of interest to disclose.

Author contributions

C.G. and X.Y. conceived the idea of this review. C.G., H.W. and H.C. were responsible for
literature search. C.G. prepared the figures and wrote the paper. W.Y. and X.Y. supervised and guided the literature search and writing process. All authors reviewed and approved the draft before submission.

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