

Roles of ten-eleven translocation family proteins and their O-linked β -N-acetylglucosaminylated forms in cancer development (Review)

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Abstract. Members of the ten-eleven translocation (TET) protein family of which three mammalian TET proteins have been discovered so far, catalyze the sequential oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine which serve an important role in embryonic development and tumor progression. O-GlcNAcylation (O-linked β -N-acetylglucosaminylation) is a reversible post-translational modification known to serve important roles in tumorigenesis and metastasis especially in hematopoietic malignancies such as myelodysplastic syndromes, chronic myelomonocytic leukemia and acute myeloid leukemia. O-GlcNAcylation activity requires only two enzymes: O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). OGT catalyzes attachment of GlcNAc sugar to serine, threonine and cytosine residues in proteins, while OGA hydrolyzes O-GlcNAc attached to proteins. Numerous recent studies have

demonstrated that TETs can be O-GlcNAcylated by OGT, with consequent alteration of TET activity and stability. The present review focuses on the cellular, biological and biochemical functions of TET and its O-GlcNAcylated form and proposes a model of the role of TET/OGT complex in regulation of target proteins during cancer development. In addition, the present review provides directions for future research in this area.

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Abbreviations: TET protein, ten-eleven translocation family protein; OGT, O-GlcNAc transferase; OGA, O-GlcNAcase; PTM, post-translational modification; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; MDS, myelodysplastic syndromes; AML, acute myeloid leukemia; ESC, embryonic stem cell; IL-6, interleukin-6; Ser, serine; Thr, threonine; O-GlcNAcylation, O-linked β -N-acetylglucosaminylation; EMT, epithelial-mesenchymal transition; CLL, chronic lymphocytic leukemia

Key words: TET, O-GlcNAcylation

1. Introduction

Methylation of cytosines, a common epigenetic modification in eukaryotic cells, serves an important role in a variety of genetic processes, including gene stability and expression, chromosome accessibility and inactivation, and nucleosome positioning (1). 5-methylcytosine (5mC) is produced by DNA methyltransferase activity and is located in CG dinucleotides in DNA (2). Ten-eleven translocation (TET) family proteins participate in oxidation reactions of 5mC to 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine (5hmC, 5fC, 5caC), which further decrease DNA methylation patterns (2).

Post-translational modifications (PTMs) of proteins facilitate immediate responses of cells to intracellular or extracellular environmental stimuli by modifying functions of targeted proteins (3). PTMs are involved in various pathological processes such as proliferation, apoptosis and migration in tumors (3). O-linked β -N-acetylglucosaminylation (O-GlcNAcylation) is an atypical, dynamic and reversible PTM consisting of addition of N-acetyl-D-glucosamine, a

unique non-elongated monosaccharide on proteins (4). Unlike classical glycosylation present in the endoplasmic reticulum and Golgi apparatus, O-GlcNAcylation takes place in the cytoplasm, nucleus and mitochondria and is implicated in a wide range of effects on cellular function and signaling in metabolic diseases and cancer (5). Compared to complex glycosyltransferase and glycosidase system of classical glycosylation, O-GlcNAcylation is only regulated by two enzymes: The glycosyltransferase OGT (O-GlcNAc transferase) and the glycoside hydrolase OGA (O-GlcNAcase) (3). Numerous recent studies indicate a close connection between OGT and TET (5-7). OGT can catalyze TET to form O-GlcNAcyated TET and can also interact with TET to form a complex with the ability to further modify chromatin which participated in regulating embryonic development and cancer progression (6,7). The present review focuses on the functional roles of TET family proteins and O-GlcNAcylation in cancer progression, with focus on the connection between TET proteins and OGT to clarify the effects of these proteins on cancer development.

2. TET family proteins

Epigenetic modifications, which include DNA methylation and histone modifications can alter gene expression but cannot change the primary sequence of DNA (8) Epigenetic modifications has been proven to be widely involved in tumor development (9-11). DNA hypermethylation is observed in myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), colorectal cancer, hepatocellular carcinoma and ovarian cancer (12-14). TET family proteins function as DNA hydroxymethylases in vertebrates and can catalyze conversion of 5mC to 5hmC, and subsequently to 5fC and 5caC (15) (Fig. 1). These three versions of oxidized methylcytosines are all associated with DNA demethylation (16,17).

TET proteins have a common cysteine-rich dioxygenase region and C-terminal region which binds to ferrous iron and α -ketoglutarate and catalyzes an oxidation reaction which involves hydroxylation of 5mC to 5hmC and further to 5fC and 5caC (12). The three TET proteins (TET1, TET2, TET3) have differing N-terminal regions (18). TET1 and TET3 have a CXXC-type zinc finger domain (19). TET2 has no CXXC DNA-binding domain, but can interact with a CXXC domain protein, inhibitor of disheveled Dvl and Axin complex (IDAX) (18).

TET proteins are highly expressed in embryonic stem cells (ESCs) (20). They are essential for ESC differentiation during embryogenesis and help regulate homeostasis of hematopoietic stem cells, mesenchymal stem cells and progenitor cells (21). TET1 and TET2 are upregulated in ESCs and TET3 in oocytes (22). Expression of TET proteins is closely related to tumor malignancy (23-25); and their expression is significantly lower in tumor tissues compared with normal tissues (24,25). TET2 mutation is often observed in hematopoietic neoplasms including myelodysplastic syndromes and chronic myelomonocytic leukemia (26-28). TET2 expression enhances self-renewal, proliferation potential, osteoblast differentiation and hematopoietic supportive capacity of bone marrow stem cells in humans and mice (29). Li *et al* reported somatic mutation frequencies of the *TET2* gene as 30% in MDS, 20% in myeloproliferative neoplasms, 42% in chronic

myelomonocytic leukemia and 20% in AML (30). In breast cancer cells, TET2 occupies active enhancers and facilitates proper recruitment of estrogen receptor α , which then transcriptionally activates TET2 expression to establish a positive feedback loop between TET2 and estrogen signaling (31). TET2 also exerts tumor-promoting effects in melanoma and osteosarcoma cells (32,33). TET2 expression is enhanced in tumor-associated macrophages and myeloid-derived suppressor cells, and TET2 deletion in myeloid cells results in inhibition of melanoma growth (32). TET2 can target the promoter of interleukin-6 (IL-6) to increase its expression, and elevated IL-6 may promote lung cancer cell metastasis (33).

Studies on aberrant expression of the three types of TET proteins in various types of cancer are summarized in Table I.

3. O-GlcNAcylation

O-GlcNAcylation is a reversible PTM that typically targets proteins in the cytoplasm, cell nuclei (34), or mitochondria (35). It can regulate cellular processes at various levels, such as transcription, translation, signal transduction or cell metabolism (36). In general, proteins modified by O-GlcNAcylation are phosphoproteins or parts of macromolecular complexes (phosphoglycerate kinase 1), transcription complexes (p53, c-myc), or nucleopores (transmembrane nucleoporin Pom121, nucleoporin 155) (37). O-GlcNAcylation has also been reported for numerous functional proteins, including epigenetic regulation factors including the TET proteins, the SIN3 transcription regulator family member A-histone deacetylases and the Polycomb group proteins that regulate DNA methylation, chromatin accessibility and chromatin modification (38).

O-GlcNAcylation often affects subcellular localization, stability and function of target proteins (7,39), and in some cases helps modulate protein phosphorylation status, protein stability, enzymatic activity, protein aggregation and interactions with other proteins or DNA (5,36,40). O-GlcNAc activity requires two enzymes: OGT and OGA (41). OGT catalyzes attachment of GlcNAc to serine (Ser), threonine (Thr) and cysteine residues in proteins (40,42).

OGT activity is highly sensitive to the uridine diphosphate GlcNAc level, and is altered by variations of glucose, glutamate or free fatty acid levels in cells (43). OGT activity is associated with epithelial-mesenchymal transition (EMT), p53, Wnt and TGF- β signaling pathways, inflammatory responses and apoptosis in cervical cancer cells (44). Knockdown of OGT in colon cells results in upregulation and altered glycosylation of E-cadherin, an important factor in EMT progression and may disrupt biosynthesis of glycosphingolipids (lactosylceramide, gangliosides and globosides), with consequent reduction of gangliosides (ganglioside 3 and ganglioside 2) but increase of globosides (globoside 3 and globoside 4) (45). Chronic lymphocytic leukemia (CLL) cells demonstrate high expression of O-GlcNAcyated proteins, including p53, c-Myc, and Akt and enhanced protein glycosylation alters intracellular signaling processes (p53 and PI3K/AKT/mTOR signaling pathways) in these cells (46). O-GlcNAcylation increases downstream signaling of toll-like receptors following cytokine stimulation in CLL cells (46). On the other hand, high baseline O-GlcNAc levels inhibit responses to such stimulation, resulting in increased resistance to TLR agonists, chemotherapeutic agents,

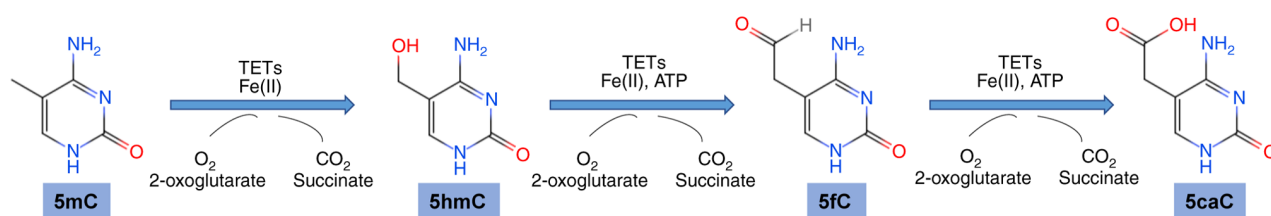


Figure 1. TET proteins catalyze conversion of 5mC to 5hmC, 5fC and 5caC. Fe, ferrous; ATP, adenosine triphosphate; TETs, ten-eleven translocation family proteins; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; 5-fc, 5-formylcytosine; 5-caC, 5-carboxylcytosine.

B cell receptor crosslinking and mitogens (46). Hart *et al* (36) reported that increased O-GlcNAcylation of Thr58 on c-Myc inhibited c-Myc activity and reduced transformation of non-Hodgkin's lymphoma cells.

OGA has O-GlcNAc hydrolase and associated enzymatic activity of lysine acetyltransferase (47-49) and can therefore hydrolyze O-GlcNAc residues from attached proteins (41). Inhibition of OGA expression in rats and mice resulted in increased O-GlcNAcylation of all tissues (50). OGA shows high mRNA expression in lung, colon and breast cancers (49). In colon cell lines, O-GlcNAcylation level was increased by inhibition of OGA but decreased by inhibition of OGT (51). OGA serves an essential role in differentiation of ESCs (52,53). Blocking of O-GlcNAc cycling in mice by OGA knockdown resulted in anatomical defects and notable changes in expression of pluripotency markers such as Nanog, Sox2 and Orthodenticle homeobox 2 (53). OGA knockdown in mouse hematopoietic stem cells reduced progenitor pools, reduced cell stemness of cells, altered transcription of several crucial genes such as hypoxia inducible factor-1 α and cyclin dependent kinase inhibitor 1C and increased apoptotic cell number in bone marrow (54).

4. O-GlcNAcylation of TETs

TET proteins mediate DNA demethylation, while OGT mediates protein O-GlcNAcylation (39,55). These two enzymatic activities may seem to be independent of each other. However, several recent studies have revealed the physical and functional interactions between TETs and OGT.

Firstly, TETs can be O-GlcNAcylated by OGT (6,56-58). Addition of a GlcNAc group to Ser and Thr residues of TET proteins inhibits TET phosphorylation, since Ser and Thr are potential phosphorylation sites (59). Cross-talk between modified Ser and Thr residues facilitates rapid adaptation of TET protein localization, activity, or targeting in response to altered environmental conditions or other external stimuli (6,59). Secondly, TETs preferentially associate with or bind to OGT in certain gene promoters located close to CpG-rich transcription start sites, hence regulating transcriptional levels of these genes through epigenetic modification (6). A large proportion of nuclear OGT is complexed with TETs (60). Such TET/OGT-occupied promoter regions are characterized by low levels of DNA modification, suggesting that TET demethylation activity serves a role in regulation of CpG island methylation (6). OGT in TET/OGT complexes also mediates O-GlcNAcylation of nearby histone H2B at Ser112, thereby facilitating lysine120 ubiquitination of H2B and transcriptional activation (61), particularly near transcription start

sites (62). Thirdly, the TET/OGT complex can serve as a scaffold for epigenetic complexes, in addition to its own demethylation and O-GlcNAcylation activities (57,63). Host Cell Factor 1, a component of the H3K4 methyltransferase SET1/COMPASS complex (63), can be O-GlcNAcylated by OGT and bind further to the TET2/3/OGT complex to mediate transcriptional activation through methylation on histone 3 lysine 4 (6). TET/OGT complex can also interact with chromatin regulator SIN3 transcription regulator family member A and with several components of the nucleosome remodeling and deacetylase complex, hence enhancing expression of downstream genes, such as single stranded DNA binding protein 2 and LIM homeobox 2 regulated by TET and maintaining ESC pluripotency (57).

Although TET proteins and OGT have been hot topics of research in recent years, very limited knowledge of TET function and TET O-GlcNAcylation in cancer development and progression exists. TET/OGT complex contributes to certain epigenetic modifications, such as DNA demethylation, histone O-GlcNAcylation, histone methylation associated with positive regulation of gene expression, hence providing a direct link between epigenetics and cellular metabolism (62). Hsu *et al* (64) reported that TET1 demonstrated reduced expression in prostate and breast cancers, and suppresses cancer cell invasion by promoting expression of tissue inhibitors of metalloproteinases. In a study of cervical cancer cells, Guan *et al* (65) observed that nuclear localization and O-GlcNAcylation of TET3 were modulated by glucose metabolism, and that gene expression was regulated through TET/OGT-mediated epigenetic changes in response to nutrient availability. The role of O-GlcNAcylated TET proteins in cancer progression is an exciting topic for future study. Based on current finding in the field, a working model of the role of TET/OGT complex in regulation of target proteins during cancer development may be proposed (Fig. 2).

5. Discussion

The TET proteins (TET1, TET2, TET3) catalyze conversion of 5mC to 5hmC. O-GlcNAcylation is a reversible PTM and it can O-GlcNAcylate TETs (59). OGT and O-GlcNAcylation have been clearly demonstrated to serve an important role in tumorigenesis and metastasis (66). TET proteins can recruit the OGT to chromatin, which promotes post-transcriptional modifications of histones and facilitates gene expression (40). It was reported that TET2 mediates OGT modification on H2B Ser112 and is associated with highly transcribed genes (62). In addition, TET/OGT complex can serve as the scaffold for epigenetic complexes (7,63).

Table I. Aberrant expression of TET proteins in various cancers.

Name of protein	Cancer types	Alteration	Consequent effects	(Refs.)
TET1	Breast cancer, colon cancer, liver cancer	Decreased expression	Advanced cancer stage, nodal metastases and poor overall survival	(64,72)
TET2	Leukemia, lymphoma	Mutation	Aberrant self-renewal and advanced-stage disease	(73-76)
TET3	Ovarian cancer	Increased expression	Unfavorable prognosis	(77)
TET3	Glioblastoma	Decreased expression	Promoted tumorigenesis	(78)

TETs, ten-eleven translocation family proteins.

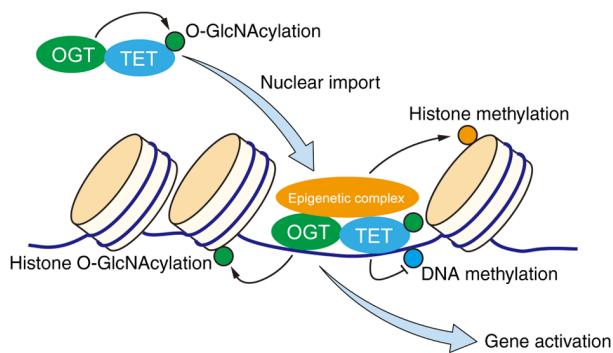


Figure 2. Proposed working model of the role of TET/OGT complex in regulation of target proteins during cancer development. TET/OGT complex can work as an epigenetic complex participating in the progress of DNA demethylation. Meanwhile, TET also facilitates the O-GlcNAc modification of histones regulated by OGT. The black arrow represents the product synthesized by the OGT, TET or TET/OGT complex. The blue arrow above represents nuclear import. The blue arrow below represents gene activation. TETs, ten-eleven translocation family proteins; OGT, O-GlcNAc transferase; O-GlcNAcylation, O-linked β -N-acetylglucosaminylation.

However, the present review had some limitations, such as the research of TET/OGT complex mainly focused on the function during embryonic development (6,7,55). The role of TETs and their O-GlcNAcylation in cancer development is largely unknown (60). The essential characteristic of cancer is uncontrolled cell proliferation resulting from accumulated alterations of cell metabolism and signaling pathways (67). One trait of cancer initiation is the dynamics of O-GlcNAcylation are highly sensitive to availability of nutrients and oxygen, determined by the cellular microenvironment (68). Aberrant glucose metabolism in cancer cells may alter O-GlcNAcylation of TET proteins and therefore affect their stability; conversely, TET loss-of function in cancer may influence the nuclear and/or cytoplasmic distribution of OGT, which in turn may affect the stability of tumor suppressors and oncogenes such as p53 (69), MYC (70), and β -catenin (71). The dysregulated expression and loss-of-function mutation of TET family proteins participated in the progress of a variety of cancers especially hematopoietic malignancies (29). Hence, it is logical to raise the question about whether TET/OGT is involved in cancer development and how they get involved. TETs can be post-translationally modified by the nutrient-sensing enzyme OGT, also suggesting a connection between metabolism and

the epigenome (6,62). In addition to suggesting a broader role for the TET/OGT complex, the present review provides information about the interaction between OGT and TET proteins, which may provide new insights into the development of cancer.

6. Conclusions

TET proteins can interact with and undergo O-GlcNAcylation by OGT and O-GlcNAcylation can alter properties of TET enzymes (62). TET/OGT complexes are primarily targeted to promoter regions through interaction of TET with DNA, and TET-linked OGT can O-GlcNAcylate a wide variety of proteins (58). Relationships between OGT and TETs during cancer pathological processes remain to be elucidated. Identification of modified proteins present upstream and downstream of TET/OGT complex will be useful in this regard. The functions of TETs and their O-GlcNAcylation in cancer development is an important topic for future studies.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XL, XCP, HJL, and BXL designed the study and co-wrote the manuscript. YW, FG, and YY were involved in study

conception and design, and revised the manuscript for important intellectual content. All authors have read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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