Ten-Eleven Translocation 1 (Tet1) Is Regulated by O-Linked N-Acetylglucosamine Transferase (Ogt) for Target Gene Repression in Mouse Embryonic Stem Cells*§

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As a member of the Tet (Ten-eleven translocation) family proteins that can convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), Tet1 has been implicated in regulating global DNA demethylation and gene expression. Tet1 is highly expressed in embryonic stem (ES) cells and appears primarily to repress developmental genes for maintaining pluripotency. To understand how Tet1 may regulate gene expression, we conducted large scale immunoprecipitation followed by mass spectrometry of endogenous Tet1 in mouse ES cells. We found that Tet1 could interact with multiple chromatin regulators, including Sin3A and NuRD complexes. In addition, we showed that Tet1 could also interact with the O-GlcNac transferase (Ogt) and be O-GlcNAcylated. Depletion of Ogt led to reduced Tet1 and 5hmC levels on Tet1-target genes, whereas ectopic expression of wild-type but not enzymatically inactive Ogt increased Tet1 levels. Mutation of the putative O-GlcNAcylation site on Tet1 led to decreased O-GlcNAcylation and level of the Tet1 protein. Our results suggest that O-GlcNAcylation can positively regulate Tet1 protein concentration and indicate that Tet1-mediated 5hmC modification and target repression is controlled by Ogt.

Tet1 belongs to the Tet4 (Ten-eleven translocation) family of proteins that comprises Tet1, Tet2, and Tet3 and catalyzes the hydrolysis of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), a reaction that can lead to active DNA demethylation (1–5). Tet proteins have been implicated in genome-wide DNA methylation control, gene expression regulation, cell fate determination, and cancer development (1, 2, 6–12). Numerous studies have demonstrated that Tet1 is highly expressed in embryonic stem (ES) cells and certain neuronal cells, and is required for maintaining pluripotency (1, 2, 7, 8). Depletion of Tet1 in mouse ES cells led to reduced global 5mC levels and altered gene expression (2, 8). Furthermore, genome-wide localization analyses have revealed enrichment of Tet1 on regulatory regions marked with only H3K4me3 or both H3K4me3 and H3K27me3, suggesting the importance of Tet1 in regulating both pluripotency and differentiation (4, 13, 14).

DNA methylation is generally associated with gene silencing. The ability of Tet1 to hydrolyze 5mC suggests a role of Tet1 in transcriptional activation; however, several studies in mouse ES cells indicate a more complex picture. For example, recent proteomic and genetic studies suggest that chromatin remodeling and histone modification complexes, such as Sin3A and NuRD, may be linked to Tet1 for controlling local 5mC levels and target gene expression (13–15). Immunoprecipitation (IP) and mass spectrometry analysis using 293T cells expressing epitope-tagged Tet1 found it to associate with the chromatin repression Sin3A complex (14). Mouse ES cells knocked down for either Tet1 or Sin3A exhibited similar gene expression profiles, suggesting that Tet1 functions at least in part through the Sin3A repression complex (14), and the polycomb repression
complex 2 (PRC2) appeared to be recruited to its genomic targets in a Tet1-dependent manner in mouse ES cells (13). Indeed, genome-wide ChIP-sequencing results combined with gene expression analyses using cDNA microarray and RNA-seq sequencing revealed an enrichment of mostly derepressed genes, suggesting that Tet1 functions primarily to repress its direct targets (4, 13, 14, 16).

To understand further how Tet1 may recruit chromatin components to its genomic targets for transcriptional silencing, we determined the Tet1-associated protein complex by carrying out large scale IP and mass spectrometry analysis of endogenous Tet1 in mouse ES cells. We found that Tet1 could interact with multiple chromatin repression factors, supporting the notion that Tet1 functions mainly to repress target genes for pluripotency maintenance in mouse ES cells. Despite the wealth of information on Tet1 and other Tet family members, very little is known about how Tet1 is posttranslationally modified. Recent findings indicate that Tet1 could interact with Ogt and this interaction could stabilize Tet1 binding to target promoters (17). However, the precise role of O-GlcNAcylation in regulating Tet1 remains unclear. Through our proteomic study, we also identified O-GlcNAc transferase (Ogt) in the Tet1 complex. We show here that Ogt is important for Tet1-mediated gene repression, where RNAi depletion of Ogt led to decreased Tet1 localization and 5hmC enrichment on Tet1-target genes. Our study provides further evidence that Tet1 is O-GlcNAcylated, and that Tet1 level is regulated by Ogt and O-GlcNAcylation. These findings indicate that Tet1 is a substrate of Ogt, and Ogt-mediated glycosylation of Tet1 in turn regulates its repression function on developmentally important genes. The Ogt-Tet1 link should further our understanding of how posttranslational modifications are integrated into the regulatory networks of ES cell maintenance.

**MATERIALS AND METHODS**

**Cell Lines, Vectors, and siRNA Reagents**—AB2.2 mouse ES cells (passage 18, kindly provided by Darwin Core facility, Baylor college of Medicine, Houston, TX) were maintained on a 0.1% gelatin (Sigma-Aldrich)-coated tissue culture dish in high glucose DMEM (HyClone), supplemented with 15% (v/v) fetal bovine serum, 2 mM GlutaMax-I supplement, 55 μM β-mercaptoethanol, 0.1 mM MEM nonessential amino acid, and 1000 units/ml ESGRO (Millipore) under feeder-free conditions. HEK293T cells were cultured in high glucose (25 mM) containing MEMα (HyClone) supplemented with 10% FBS.

cDNAs encoding murine Tet1 and Ogt were PCR-amplified from AB2.2 cells. Tet1 cDNA was cloned into a pBabe-based retroviral expression vector to be tagged with SFB (5-tag, FLAG tag, and strepavidin-binding peptide). Ogt was cloned into an MSCV-Elf1a-based retroviral expression vector for tagging with both HA and FLAG. A site-directed mutagenesis kit (Stratagene) was used to generate the Tet1 T535A and T535V and Ogt H568A mutations following the manufacturer’s instruction.

The following siRNA oligonucleotides were transfected using Lipofectamine 2000 (Invitrogen): Ctrl KD, 5’-UCCUC-UCCACGCCAGAUAUUAUUA; Tet1 KD1, 5’-CAGACUUAUAAACACAAAACGUAUA; Tet1 KD2, 5’-CCGCGCGCAGGAAA; Ogt KD1, 5’-GCCUCUGUUCACACAAACAAUA; Ogt KD2, 5’-GCCGAUGAGAAGAAUUUGGUAUAA.

**Immunoprecipitation, Western Blotting, Antibodies, and Other Reagents**—Large scale affinity purification, immunoprecipitation, and Western blotting were carried out as described previously (18). The following antibodies were used: anti-Tet1 (09-872, Millipore), anti-Ogt (O6264, Sigma), anti-GlcNAc (MMS-248R, Covance), anti-5-hydroxymethylcytosine (39769, Active Motif), anti-Nanog (A300-397A, Bethyl Laboratories), anti-Oct4 (sc-8628, Santa Cruz Biotechnology), anti-Sox2 (ab15830, Abcam), anti-Ezh2 (39639, Active Motif), anti-Sin3A (ab3479, Abcam), anti-FLAG (F7425, Sigma), anti-GAPDH and anti-β-tubulin (sc-25778 and sc-9104, respectively, Santa Cruz Biotechnology). Cycloheximide, D(-)-glucose, PUGNAc, and alloxan were purchased from Sigma-Aldrich, and GlcNAc was purchased from Vector laboratories.

**Real-time PCR**—Real-time PCR was carried out using an ABI StepOnePlus Real-time PCR System and SYBR Green Master Mix (Applied Biosystems) essentially as previously described (18). Briefly, total RNA was isolated using the RNAeasy Mini Kit (Qiagen) and reverse-transcribed using the iScript Select cDNA Synthesis Kit (Bio-Rad). The primers used for SYBR Green real-time PCR were designed using the Prime Time qPCR Primer Design Software (Integrated DNA Technologies Inc., Coralville, IA) (supplemental Table S1) and tested with the intron-spanning assay.

**Chromatin Immunoprecipitation (ChIP) Assay**—ChIP assays were performed using the fast ChIP protocol (19) with minor modifications. The sonicated chromatin was incubated with antibodies or control IgG in an ultrasonic water bath for 30 min at 4 °C. Immunoprecipitated chromatin fragments were subjected to real-time PCR, and the enrichment of target gene promoter regions was compared with IgG control (see supplemental Table S2 for ChIP primers).

**Succinylated Wheat Germ Agglutinin (sWGA) Affinity Purification**—Whole cell lysate (~50 mg) was first precleared with 30 ml of 50% (v/v) of unconjugated agarose beads (Vector Laboratories) in a total volume of 100 ml of NETN buffer (100 mM NaCl, 20 mM Tris-Cl (pH 8.0), 0.5 mM EDTA, 0.5% (v/v) Nonidet P-40) for 2 h at 4 °C. A total of 30 ml of sWGA-conjugated agarose beads (50% (v/v)) (Vector Laboratories) was added to the supernatant and incubated overnight at 4 °C. The beads were washed three times in lysis buffer and eluted in 30 ml of 2× SDS loading buffer. To minimize indirect association of protein complexes, extract was incubated with sWGA-conjugated agarose beads in the presence of 0.2% SDS.

**RESULTS**

**Endogenous Tet1 Interacts with Repression-associated Chromatin Factors**—To better understand how Tet1 carries out its function in regulating gene expression in ES cells, we performed large scale IP followed by mass spectrometry analysis using mouse ES cells and an antibody against endogenous Tet1 (18). As shown in Fig. 1A, endogenous Tet1 could co-purify with proteins that belong to major chromatin remodeling and repression complexes, including Sin3A, Hdac1/2, Mta3, and Chd4. These results indicate that multiple chromatin repres-
sion factors can interact with Tet1 and potentially participate in Tet1-mediated repression function.

Endogenous Tet1 Complexes with Ogt—Interestingly, Ogt was also one of the top Tet1-interacting candidates (Fig. 1A). Unlike worms (20) and flies (21, 22), only one conserved mammalian O-GlcNAc transferase, Ogt, is known to date, which contains an N-terminal tetratricopeptide domain and a C-terminal catalytic domain (20, 23). Ogt is essential for mouse early development (24). Ogt can also interact with and modify ES cell pluripotency factors such as Oct4, Sox2, and Zfp281 (25–30). To further confirm the interaction between Ogt and Tet1, we carried out co-IP experiments. As shown in Fig. 1B, Tet1 IP could indeed bring down Ogt, in addition to its known interactor Sin3A. Likewise, reciprocal IP with Ogt also pulled down Tet1 and Sin3A. The identification of Ogt in the Tet1 complex suggests cross-talk between Tet1 and Ogt-mediated pathways in maintaining ES cells.

We next examined whether Tet1 could be O-linked GlcNAcylated. Here, we took advantage of the ability of sWGA to specifically bind proteins with the O-GlcNAc moiety. O-GlcNAcylated proteins can bind directly to sWGA, whereas indirectly associated proteins can be washed away in the presence of detergents such as SDS. As expected, we could bring down Ogt using sWGA-conjugated beads because Ogt is itself O-GlcNAcylated (Fig. 1C). Importantly, sWGA also pulled down endogenous Tet1 protein. Furthermore, the sWGA-bound Tet1 proteins could be specifically eluted with free GlcNAc. Alternatively, O-GlcNAcylated proteins can also be detected using anti-O-GlcNAc antibodies (30). When we blotted the precipitates with an anti-O-GlcNAc antibody, Ogt could be readily detected (Fig. 1D), and the same antibody was also able to detect Tet1 proteins that came down with sWGA. These observations indicate that endogenous Tet1 is physically associated with Ogt in ES cells and is modified by Ogt to become O-linked-GlcNAcylated.

Both Tet1 and Ogt Are Required for Maintaining ES Cell Pluripotency—Ogt knockout in mice resulted in embryonic lethality before implantation (24), a clear indication of the essential role of Ogt in early development. To explore the functional significance of Tet1-Ogt interaction, we used siRNA oligonucleotides to deplete Tet1 and Ogt in mouse ES cells. We confirmed the efficacy of Ogt and Tet1 knockdown by RT-qPCR and Western blotting (Fig. 2, A and B). Both siRNA oligonucleotides against Ogt were able to achieve >70% knockdown efficiency of Ogt mRNA and protein expression. Either Tet1 or Ogt knockdown led to reduced alkaline phosphatase...
FIGURE 2. Both Tet1 and Ogt are required for maintaining ES cell pluripotency. A, Tet1 and Ogt knockdown mouse ES cells were examined 2 days after siRNA transfection by Western blotting. Right, densitometry quantification data from three independent experiments. B, real-time qPCR was performed using cells from A to assess the mRNA levels of Tet1 and Ogt. C, mouse ES cells from A were examined by alkaline phosphatase staining 4 days after siRNA transfection. D, real-time qPCR analysis is shown of lineage-specific markers in Tet1 and Ogt knockdown cells from A. E and F, ChIP-qPCR analysis with antibodies against Ezh2 (E) and Sin3A (F) was performed using Tet1 and Ogt knockdown cells. Error bars represent S.D. (n = 3).
staining and increased percentages of differentiated cells (Fig. 2c). When we examined several developmentally important genes, we found that most of the lineage-specific markers we tested, such as ectodermal markers Sox1 and Mash1, endodermal markers Gata6 and Sox17, mesodermal markers Branchyury and Mix1, and trophodermal markers Cdx2 and Eomes, appeared to be derepressed in cells depleted for either Tet1 or Ogt (Fig. 2D). It is interesting to note that the phenotypes exhibited by Ogt knockdown cells appeared more severe, compared with Tet1 knockdown cells. It is likely that Ogt inhibition may have a broader impact on ES cells because Ogt can modify substrates from diverse pathways.

In addition, our proteomic data (Fig. 1A) and results from others indicate that Tet1 functions through communicating with multiple repression-associated chromatin factors (13–15). Indeed, Tet1 knockdown led to reduced genomic targeting of both Ezh2 and Sin3A (Fig. 2, E and F). Similar reduction was also observed in Ogt-depleted cells. These findings underline the importance of both Tet1 and Ogt in repressing developmental genes in ES cells and suggest intersections between the pathways mediated by Tet1 and Ogt.

**Ogt Is Critical for Tet1-mediated Repression of Developmentally Important Genes**—Recent studies indicate that Tet1 is enriched on CpG islands of promoters of genes important for pluripotency and development in ES, and may be responsible for generating 5hmC at these loci (4, 13, 14, 16). To further probe the Tet1-Ogt interaction, we set out to analyze the effect of Ogt depletion on Tet1 and 5hmC enrichment by ChIP and qPCR. As expected, Tet1 knockdown led to reduced Tet1-targeting and 5hmC enrichment on Tet1-target genes (Fig. 3, A and B). Concurrently, the expression of developmentally important genes known to be regulated by Tet1 (e.g. Ssbp2 and Lhx2) also increased (Fig. 3C). When we examined Ogt knockdown cells, we also observed reduced targeting of Tet1 as well as 5hmC enrichment on Tet1-target genes (Fig. 3). Again, this reduction was accompanied by lowered expression of Tet1-controlled genes (Fig. 3D). Taken together with our interaction data, these findings indicate that Ogt modification of Tet1 may regulate Tet1 function.

**O-GlcNAcylation of Tet1 Positively Regulates Its Protein Level**—O-Linked GlcNAcylation of proteins is highly dynamic and impacts protein function. For example, Ogt-mediated GlcNAcylation of Oct4 is important for Oct4 transcriptional activity (30). To probe the functional importance of Tet1 O-GlcNAcylation, we again utilized mouse ES cells depleted for Ogt (Fig. 2). In these cells, Ogt inhibition did not affect the mRNA expression of self-renewal and pluripotency factors such as Nanog, Oct4, or Sox2 (Fig. 2D). Similarly, Ogt knockdown had minimal effect on the mRNA level of Tet1 (Fig. 2, A and B). However, steady-state levels of Tet1 proteins decreased by at least 70% with the two different Ogt siRNAs. The level of inhibition was nearly as effective as Tet1 knockdown itself (Fig. 2A), indicating Ogt-dependent regulation of Tet1 protein stability.

To further assay the effect of Ogt expression on Tet1 levels, we generated 293T cells that co-expressed Tet1 with varying amounts of Ogt to more quantitatively measure Tet1 amount. With increasing concentrations of full-length Ogt, Tet1 protein levels increased as well, indicating dose-dependent regulation of Tet1 level by Ogt (Fig. 4A). In comparison, the Ogt point mutant (Ogt H568A) whose activity was reduced by >95% (31, 32) failed to enhance Tet1 protein levels even when highly overexpressed. We then tested whether this Ogt-dependent increase in Tet1 protein amount was indeed due to O-GlcNAcylation. Here we utilized alloxan, a drug that has been shown to block Ogt (33), and PUGNAc, which inhibits the O-GlcNAc hydrolase OGA (34). We cultured cells in high glucose with or without alloxan and examined the level of Tet1 in these cells. As shown in Fig. 4B, both high glucose in the media (third lane) and PUGNAc treatment (second lane) led to an increase in Tet1 proteins. In comparison, addition of alloxan abolished Tet1 increase that resulted from high glucose in the media (fourth lane). These observations are consistent with the idea that Ogt regulates Tet1 levels through O-GlcNAcylation of Tet1.

Thr-535 was recently identified as a native O-GlcNAcylation site in mouse Tet1 (25). To determine whether Ogt-mediated regulation of Tet1 occurs through O-GlcNAc modification of Thr-535, we generated FLAG-tagged Tet1 mutants with Thr-535 mutated to Ala (T535A) or Val (T535V). O-GlcNAcylated wild-type or mutant Tet1 proteins were subsequently purified using sWGA beads in the presence of 0.2% SDS. As shown in Fig. 4C, whereas Thr-535 mutations did not affect total Tet1 protein levels, reduced amounts of Tet1 Thr-535 mutants were pulled down by sWGA beads compared with wild-type Tet1, indicating Thr-535 as a major in vivo O-GlcNAcylation site and decreased O-GlcNAcylation of Tet1 as a result of Thr-535 mutation. Furthermore, mutating residue Thr-535 abolished the Ogt-dependent stabilization of Tet1 (Fig. 4D). These observations support Ogt-dependent control of Tet1 protein stability, and underscore the importance of O-linked GlcNAc modification and Ogt enzymatic activity in regulating Tet1.

**DISCUSSION**

Tet1 and other Tet family proteins have been under extensive investigation in recent years. In this report, we showed that Tet1 could interact with repression complexes and Ogt and undergo O-linked glycosylation. We also provided evidence that Tet1-mediated repression control depended on Ogt. Through large scale affinity purification of endogenous Tet1 using mouse ES cells, we identified several chromatin remodeling and repression complexes that could associate with Tet1, including the Sin3A and NuRD complexes. This finding provides further support to the model that Tet1 recruits these repression complexes to modulate gene repression. Through direct binding of its CXXC motif to unmethylated CpG, Tet1 can then recruit chromatin factors to generate a repressive chromatin state and inhibit transcriptional activation. Further probing the co-occupancy of Tet1 targets by Tet1 and its associated proteins and the coordinated action of distinct chromatin modifiers will help shed light on the dynamic regulation of chromatin structures.

Our proteomic study also found Ogt in the Tet1 complex. Ogt can add O-GlcNAc moieties to serine/threonine residues of protein substrates. O-Linked GlcNAcylation represents an abundant and essential posttranslational modification event...
FIGURE 3. Ogt inhibition compromises Tet1 function. A and B, ChIP-qPCR analysis for Tet1 targeting (A) and 5hmC enrichment (B) at the promoters of representative Tet1-repressed genes was performed in Tet1-depleted (Tet1 KD) or Ogt-depleted (Ogt KD) ES cells. C and D, the expression levels of Tet1 repressed (C) and activated (D) genes were investigated by RT-qPCR in Tet1 and Ogt KD ES cells. Error bars represent S.D. (n = 3).
By regulating protein activity, localization, and stability, O-GlcNAcylation has proven crucial to diverse biological processes, including nutrient and growth factor sensing, cell cycle progression, and stress response (35–38). Genome-wide O-GlcNAc localization analysis by ChIP-on-chip in Ogt-null worms revealed targeting of O-GlcNAcylation marks to numerous genes involved in longevity, stress, and immunity (39). Drosophila Ogt is encoded by the polycomb group (PcG) gene super sex comb (sxc), and O-GlcNAcylation marks co-localize to PcG protein binding sites on polytene chromosomes (40). In fact, the Drosophila Polycomb protein Ph is a substrate of Ogt and Ogt co-occupies with the polycomb repression complex for gene silencing (22). Furthermore, the N-terminal tetratricopeptide region of Ogt has been shown to interact directly with the transcription co-repressor Sin3A (41). These observations support the notion that Ogt and Ogt-mediated O-GlcNAcylation may be involved in transcriptional repression (22, 40, 41). Indeed, chromatin condensation appeared to

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**FIGURE 4. Ogt regulates Tet1 protein expression.** A, 293T cells transiently co-expressed SFB-tagged Tet1 and FLAG-tagged Ogt or Ogt point mutant Ogt H568A. Tet1 protein levels were then analyzed by Western blotting with the indicated antibodies. Quantification of relative intensity of the Tet1 band (normalized to Smc3) is shown on the right. B, we cultured 293T cells stably expressing FLAG-tagged Tet1 in medium containing high glucose (25 mM) to near confluence (80%) and then replaced with low glucose (5 mM) medium for 24 h. The cells were subsequently maintained in high dose of α-(+)-glucose (25 mM) for 20 h, with or without alloxan (5 mM) before Western blotting analysis. Cells treated with PUGNAc (150 μM) for 20 h were also examined. Right panel, quantification of Tet1 level relative to GAPDH. C, whole-cell lysates from 293T cells stably expressing FLAG-tagged wild-type (WT) or mutant Tet1 (T535A and T535V) were incubated with sWGA-conjugated agarose beads in the presence of 0.2% SDS before Western blotting analysis with anti-FLAG antibodies. Tet1 level was normalized to input, and the numbers under the panels indicate relative amount compared with wild-type Tet1. D, SFB-tagged wild-type or mutant (T535A) Tet1 was co-transfected with or without FLAG-tagged Ogt into 293T cells for 48 h before addition of cycloheximide (20 μg/ml). Cells were harvested at the indicated time points following treatment for Western blot analysis with the indicated antibodies. Relative amount of the Tet1 proteins were quantitated and plotted on the right.
correlate with increased histone O-GlcNAcylation and Ogt amount (42). In mice, homozygous deletion of Ogt led to embryonic lethality at day 5.5 (24), demonstrating its essential role in early development and ES cell derivation. The functional importance of Ogt in ES cell maintenance has become further apparent with a number of recent studies. A screen of O-glycosylated proteins in mouse ES cells revealed a number of in vivo O-glycosylation sites on ES cell transcription factors including Sox2 and Zfp281 (25), and work using mouse and human ES cells suggests Oct4-Ogt interactions and O-GlcNAcylation of Oct4 (26–29). In particular, O-GlcNAcylation of Oct4 appeared to regulate its transcriptional activity, the disruption of which led to altered expression of Oct4-target genes (30).

In this study, we found that Tet1 could interact with Ogt and be modified by O-glycosylation. This is supported by the genome-wide proteomic study using lectin weak affinity chromatography combined with mass spectrometry that identified Tet1 as a candidate for O-GlcNAcylation (25), and it is consistent with recent findings that identified Tet1 as an interacting protein of Ogt (17). We also showed that Ogt depletion led to ES cell differentiation accompanied by derepression of multiple lineage marker genes and reduced Tet1 targeting and 5hmC enrichment on Tet1-target genes. These results are in agreement with previous ChIP analyses showing overlapping Ogt and Tet1 binding sites (17). Furthermore, mutating the putative O-GlcNAcylation site on Tet1 led to decreased Tet1 O-GlcNAcylation. These results provide functional links between Ogt and Tet1 and suggest that Ogt-mediated glycosylation of Tet1 may regulate Tet1 levels and in turn modulate Tet1 function on its target genes.

Recent studies indicate that human TET2 and TET3 could interact with OGT and promote OGT-mediated GlcNAcylation; and TET2, TET3, and OGT show genome-wide co-localization, especially around transcription start sites (43). Whereas Tet3 is not expressed in mouse ES cells (2), Tet2 has been shown to play an important role in mouse ES cells (44). Our study cannot rule out the possibility that Tet2 can also regulate the stability of Tet1 protein through modulating the activity of Ogt.

O-GlcNAcylation may compete for the same serine and threonine residues with other enzymatic modifications including phosphorylation. Previous studies have shown that O-GlcNAcylation contributes to PGC-1α, p53, Myc, and ER-α stabilization (45–49). In the case of Myc, O-GlcNAcylation and phosphorylation of residue Thr-58 can both affect its stability (48), highlighting the interplay between Ogt and kinases in controlling protein function. Another well studied example is RNA polymerase II. O-GlcNAcylation of two serine residues in its C-terminal domain proved antagonistic for the transcriptional activation activity that resulted from phosphorylation of the same residues (50, 51). Alternatively, O-GlcNac addition may alter the interaction between Ogt substrates and other proteins. A recent study showed that O-GlcNAcylation of PGC-1α facilitated its binding to the deubiquitinase BAP1 and thereby enhanced PGC-1α stability (49). Although Tet1 has been the subject of extensive research in recent years, very little is known about its posttranslational modifications. Further studies to unravel whether this Ogt-dependent enhancement of Tet1 level is a result of competing modifications of the same residues or altered Tet1 interaction with the proteolytic pathway should prove particularly informative. Moreover, additional studies are needed to tease out the individual pathways that may be regulated by both Tet1 and Ogt and provide insight into Ogt-dependent and -independent activities of Tet1.

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