

Review

**THE POTENTIAL ROLE OF O-GlcNAc MODIFICATION  
IN CANCER EPIGENETICS**EWA FORMA, PAWEŁ JÓŹWIAK, MAGDALENA BRYŚ  
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**Abstract:** There is no doubt that cancer is not only a genetic disease but that it can also occur due to epigenetic abnormalities. Diet and environmental factors can alter the scope of epigenetic regulation. The results of recent studies suggest that O-GlcNAcylation, which involves the addition of N-acetylglucosamine on the serine or threonine residues of proteins, may play a key role in the regulation of the epigenome in response to the metabolic status of the cell. Two enzymes are responsible for cyclic O-GlcNAcylation: O-GlcNAc transferase (OGT), which catalyzes the addition of the GlcNAc moiety to target proteins; and O-GlcNAcase (OGA), which removes the sugar moiety from proteins. Aberrant expression of O-GlcNAc cycling enzymes, especially OGT, has been found in all studied human cancers. OGT can link the cellular metabolic state and the epigenetic status of cancer cells by interacting with and modifying many epigenetic factors, such as HCF-1, TET, mSin3A, HDAC, and BAP1. A growing body of evidence from animal model systems also suggests an important role for

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Abbreviations used: 5-hmC – 5-hydroxymethylcytosine; BAP1 – BRCA1-associated protein 1; BMI-1 – B lymphoma Mo-MLV insertion region 1 homolog; BMP – bone morphogenic protein; EMT – epithelial-mesenchymal transition; FGF – fibroblast growth factor; GFAT – glutamine:fructose-6-phosphate amidotransferase; H3K4me3 – trimethylation of histone H3 at lysine 4; HBP – hexosamine biosynthetic pathway; HCF-1 – host cell factor 1; HPC – human polycomb; HPH – human polyhomeotic; MLL – myeloid/lymphoid or mixed-lineage leukemia; OGA –  $\beta$ -N-acetylglucosaminidase; OGT – O-linked N-acetylglucosamine transferase; PcG – polycomb group protein; pRb – the product of the retinoblastoma gene; PRC – polycomb repression complex; RING 1A – really interesting new gene 1A; TET – ten eleven translocation; THAP – Thanatos-associated protein; TPA – 12-O-tetradecanoylphorbol 13-acetate; ubH2B – ubiquitylated histone H2B

OGT in polycomb-dependent repression of genes activity. Moreover, O-GlcNAcylation may be a part of the histone code: O-GlcNAc residues are found on all core histones.

**Keywords:** O-GlcNAcylation, Cancer, O-GlcNAc transferase, Histone modifications, Host cell factor 1, Ten-eleven translocation, Polycomb

## INTRODUCTION

Cancer was initially perceived as a genetic disease, but there is now no doubt that it can also arise from epigenetic abnormalities. Epigenetics is defined as hereditary changes in gene expression that occur without a change in the underlying DNA sequence [1]. The main processes that are responsible for epigenetic regulation include DNA methylation, modifications of core histones, nucleosome positioning, and post-transcriptional gene regulation by noncoding RNAs (micro-RNAs) [1, 2]. It is known that in human cancers, aberrant epigenetic regulation of gene expression contributes to different stages of neoplastic development, including initiation, promotion, invasion, metastasis, and resistance to chemotherapy [3, 4]. Diet and environmental factors may alter the scope of epigenetic regulation [5–8]. Different complexes responsible for chromatin modifications, in response to extracellular conditions, can modulate DNA accessibility to the transcriptional and DNA repair machineries. Alterations in these processes could cause aberrant expression of oncogenes, tumor-suppressor genes, and DNA repair genes, resulting in genomic instability and oncogenic transformation, and leading to development of cancer [6].

The results of some epidemiological studies indicate that obesity or hyperglycemia may increase the risk of cancer, including colon, esophageal, liver, pancreas, kidney, endometrial, and breast cancer [9, 10]. Moreover, high blood glucose seems to be an important factor in cancer progression. Glucose and glutamine are the two most abundant extracellular nutrients, and cancer cells are dependent on the availability of these compounds. Cancer cells exhibit an aberrant glucose metabolism characterized by aerobic glycolysis, a phenomenon known as the Warburg effect [11, 12]. Accelerated glycolysis is one of the main characteristics of cancer cells. It allows them to compensate for the inefficient extraction of energy from glucose in order to continue their intensive growth and proliferation.

A family of facilitated glucose transporter proteins named GLUT mediates glucose transport across the plasma membrane. Increased expression of GLUT family members, especially GLUT1 and 3, has been found in many human cancers [13–16]. Roughly 2–5% of the glucose entering a cell fluxes through the hexosamine biosynthesis pathway (HBP) [17]. Increased cancer cell glucose uptake may cause increased HBP flux. Moreover, glutamine is the donor substrate in the conversion of fructose-6-phosphate to glucosamine-6-phosphate by GFAT (glutamine:fructose-6 phosphate amidotransferase) in the HBP. Thus,

there is possibility that excess glutamine uptake in cancer cells also cause the increased flux through the HBP, contributing to an increased level of the HBP end product uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) [18]. This compound is a substrate in the O-linked N-acetyl-glucosaminylation (O-GlcNAcylation) process.

O-GlcNAcylation is a reversible post-translational modification involving the addition of an N-acetylglucosamine moiety to the serine/threonine residues of cytosolic or nuclear proteins [19, 20]. The majority of proteins are modified by O-GlcNAc in response to changes in environmental conditions, such as changes in the availability of glucose and other nutrients or growth factors [20]. Two enzymes are responsible for cyclic O-GlcNAcylation: O-GlcNAc transferase (OGT), which catalyzes the addition of the GlcNAc moiety from UDP-GlcNAc to target proteins, and O-GlcNAcase, (OGA) which catalyzes the hydrolytic removal of the sugar moiety from proteins [21].

Recent studies have revealed the crosstalk between HBP-OGT and the other nutrient-sensitive pathway regulated by AMP-activated protein kinase (AMPK) [22, 23]. AMPK phosphorylates OGT, which regulates the substrate selectivity of this enzyme. On the other hand, OGT O-GlcNAcylates the  $\alpha$  and  $\gamma$  subunits of AMPK, changing its activity. OGT and AMPK seem to cooperate in the integration of information about nutrient availability and metabolic status with the regulation of cellular processes.

Dynamic and reversible O-GlcNAcylation is emerging as an important regulator of diverse cellular processes, such as signal transduction, metabolism, transcription, translation, proteasomal degradation, and protein trafficking [24–27]. Increased glucose uptake and metabolism in nonmalignant cells promote oncogenesis, and O-GlcNAcylation is involved in this process [28, 29]. O-GlcNAcylation and enzymes associated with this modification may also play a key role in the regulation of the epigenome in response to cell metabolic status.

### **ALTERATIONS OF O-GlcNACYLATION IN CANCERS**

Recently, many research groups have shown that elevated O-GlcNAcylation (hyper-O-GlcNAcylation) occurs in many human malignancies, including solid tumors such as lung, prostate, breast, colorectal, liver, and pancreatic cancers, as well as in non-solid cancers such as chronic lymphocytic leukemia [30–38]. Changes in O-GlcNAcylation are associated with changes in OGT and/or OGA levels [30, 36, 39–42]. Aberrant expression of O-GlcNAc-cycling enzymes may be even considered as a biomarker of some human cancers. Low expression of OGA in hepatocellular carcinoma tissues is suggested to be a prognostic marker for tumor recurrence [36]. High expression of OGT and low expression of OGA seem to be characteristic of poorly differentiated breast tumors [39]. It has been shown that lymph node metastasis status in breast cancer patients is significantly associated with decreased OGA mRNA expression [39]. Estimation of the

urinary level of OGT and OGA may be useful as a prognostic tool for bladder cancer [41].

A growing number of studies indicate that O-GlcNAcylation constitutes an important regulator of cancer growth and progression [37, 43]. Numerous transcription factors involved in the control of cell proliferation and cell death are modified by O-GlcNAc, e.g., p53, c-Myc, NFκB, FoxM1, and b-catenin [25, 43]. O-GlcNAcylation of transcription factors may influence their activity, localization, or stability. All these transcription factors are regulated by phosphorylation. There is an interplay between the two modifications. O-GlcNAcylation and phosphorylation both occur on serine and threonine residues. Thus, O-GlcNAc residues and phosphates can compete for occupancy at the same site [44]. This may cause changes in transcription activity that lead to major alterations in gene expression resulting in reduced apoptosis and increased proliferation. Hyper-O-GlcNAcylation is anti-apoptotic in human pancreatic ductal adenocarcinoma cells and contributes to NFκB oncogene activation [37]. Increased O-GlcNAc levels due to downregulation of OGA, an enzyme involved in the removal of O-GlcNAc residues, increases AKT1 activation and the proliferation of thyroid anaplastic cancer cells [45]. The reduction of O-GlcNAc through RNA interference of OGT in breast cancer leads to inhibition of tumor growth. Downregulation of OGT is associated with a decreased level of FoxM1 and increased expression of the cell-cycle inhibitor p27 [32].

O-GlcNAcylation is also involved in invasion and metastasis [30, 31, 46]. Elevation in the O-GlcNAc levels has been implicated in epithelial-mesenchymal transition (EMT) through the regulation of the transcription repressor SNAIL1 [47]. The loss of E-cadherin is regarded as a key step in initiation of EMT [48]. O-GlcNAcylation of SNAIL1 at Ser112 stabilizes it by inhibiting its O-phosphorylation mediated degradation. Enhanced SNAIL1 O-GlcNAc modification under hyperglycemic conditions caused decreased expression of E-cadherin [45]. Moreover, O-GlcNAcylation of E-cadherin in its cytoplasmic domain blocks its cell surface transport, inhibiting intercellular adhesion [49]. Recent studies of Jin et al. have shown that O-GlcNAcylation could enhance ovarian cancer cell migration and cause a decrease in E-cadherin levels [50]. Moreover, Huang et al. showed that the O-GlcNAcylation of cofilin, the actin-binding protein, is required for its proper localization in invadopodia and that this process is implicated in promoting breast cancer cell invasion [46].

### **HISTONE CODE AND O-GlcNAc**

Histones are basic proteins in eukaryotic cell nuclei that take part in packaging the DNA into structural units called nucleosomes. Histones contain a globular domain and a flexible N-terminus known as the histone tail, which protrudes from the nucleosome. Post-translational modifications to histone tails influence the structural status of chromatin and the resulting transcriptional status of genes within a particular locus. Histone modifications include methylation, acetylation,

phosphorylation, ubiquitination, SUMOylation, biotinylation, and ADP-rybosilation [51–53]. Different histone modifications are correlated with transcriptional activation or repression. It has been proposed that these modifications constitute a code that can be recognized by regulatory complexes that determine the transcriptional state of a gene. Histone modifications playing key role in transcriptional regulation are involved in important biological and disease processes, including carcinogenesis [51–53].

Recent studies have shown that O-GlcNAcylation probably is a part of the histone code [54–57]. Using different techniques, such as immunoblotting, selective enzymatic labeling, chemoenzymatic detection, and mass spectrometry, Sakabe et al. revealed O-GlcNAc residues on histones H2A, H2B, and H4 at Thr101, Ser36, and Ser47, respectively [54]. The results of mutagenesis studies suggest that histone H3 can be O-GlcNAcylated at Ser10 [55]. Threonine32 and Ser112 have been identified by mass spectrometry as a O-GlcNAcylation sites on histones H3 and H2B, respectively [56, 57].

## HISTONE H2B MODIFICATIONS

After entering the cell, glucose becomes phosphorylated by hexokinase and is used mainly in glycolysis or glycogen synthesis processes. However, a fraction of the glucose enters the hexosamine biosynthetic pathway and is converted to UDP-GlcNAc, which is a substrate for O-GlcNAc transferase [26]. The relationship between the concentration of extracellular glucose and the intracellular level of O-GlcNAcylation suggest that this modification might be a metabolic sensor. Reversible modification of proteins by O-GlcNAc in response to serum glucose levels may regulate diverse cellular processes. Growing evidence links O-GlcNAcylation, glucose metabolism, and epigenetic regulation.

It is known that extracellular signals and glucose accessibility modulate histone modifications. Using human glioma cells as a model, Gao et al. showed that the level of monoubiquitylation of histone H2B at K120 depends on glucose accessibility in the culture media [58]. Nutrient deprivation causes removal of the ubiquitin moiety from ubH2B. Urasaki et al. showed that loss of ubH2B occurred specifically in cancer cells from a wide array of breast, colon, and lung tumor specimens [59]. By contrast, ubH2B levels remained high in stromal tissues or non-cancerous cells. They suggested that glucose deficiency and the loss of ubH2B are novel properties of cancer cells in vivo and might represent important regulatory mechanisms of tumorigenesis [59]. Moreover, it has been shown that human histone H2B (K120) ubiquitin ligase hRNF20/Bre1 is a putative tumor suppressor acting through selective regulation of a distinct subset of genes, and that depletion of this protein increases cell migration [60]. Chernikova et al. demonstrated that Bre1 deficiency promotes genomic instability, which may be an early step in carcinogenesis [61].

Recently, Fujiki et al. reported that histone H2B is O-GlcNAcylated at serine S112 by OGT in vitro and in living cells [56]. Histone O-GlcNAcylation is

changed in response to extracellular glucose. H2B Ser112 O-GlcNAcylation promotes K120 monoubiquitination, and the GlcNAc moiety can serve as an anchor for a histone H2B ubiquitin ligase. Since H2B Ser112 GlcNAc is frequently located near transcribed genes, those authors suggest that glycosylation of H2B is important for transcriptional activation (Fig. 1).

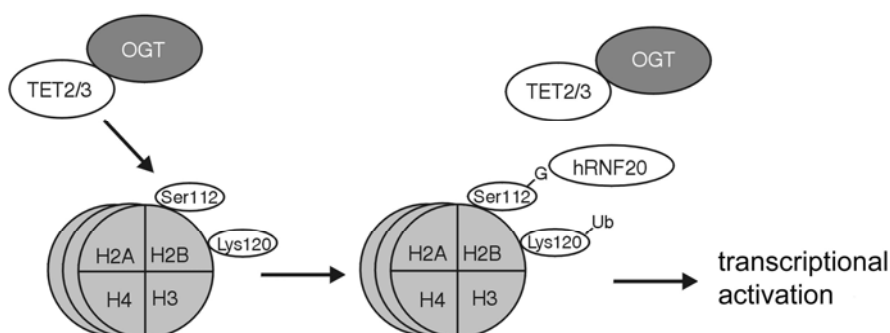


Fig. 1. The role of OGT in histone H2B modifications. OGT activity is promoted by interaction with TET2/3 (ten eleven translocation enzymes 2 and 3) and 5-hydroxymethylcytosine oxidases. OGT glycosylates H2B at Ser112. The O-GlcNAc residue may be an anchor for hRNF20 ubiquitin ligase, which is responsible for monoubiquitination of H2B at Lys120, resulting in transcriptional activation.

It has been shown that H2B Ser112 O-GlcNAcylation depends on TET2/3 (ten eleven translocation enzymes 2 and 3), which catalyze the conversion of 5-methylcytosine to 5-hydroxymethylcytosine [62]. TET2 and 3 directly interact with OGT [62, 63]. However, OGT does not appear to influence the enzymatic activity of TET. Rather, TET promotes OGT activity and facilitates OGT-dependent histone O-GlcNAcylation [62] (for more details see the section on OGT and TET).

Recent studies have also shown an important role of AMPK in O-GlcNAcylation of H2B. Xu et al. found that AMPK can phosphorylate OGT at Thr444 and that this phosphorylation blocks OGT recruitment to chromatin and suppress histone O-GlcNAcylation [23]. However, under high nutrition conditions, the AMPK is inactivated and OGT can bind and modify H2B. These results revealed a crosstalk between the AMPK and HBP-OGT pathways, which cooperate for the sensing of the nutrient state and the regulation of gene transcription.

### O-GlcNAcylation and Histone H3

Alterations in O-GlcNAc levels cause profound effects on chromatin dynamics during mitosis. OGT overexpression has been found to impair histone modifications, which are chromatin marks during mitosis [64]. One of them is a decrease in H3 Ser10 phosphorylation [55, 57, 64]. Histone H3 phosphorylation was initially linked only to chromosome condensation during mitosis. However,

now it is known that phosphorylation of histone H3 at serine 10 also plays an important role in the transcriptional activation of eukaryotic genes [65, 66]. Phosphorylation of H3 Ser10 appears to be a required histone mark for tumor transformation [67, 68]. It has been shown that increased phosphorylation of histone H3 at Ser10 may contribute to the aberrant gene expression and promote oncogene-mediated transformation [69, 70]. Many tumor promotion agents, such as EGF, TPA, ultraviolet radiation, and transformation by the oncogenes H-ras or v-Src, can elevate the level of phosphorylated histone H3 at Ser10 [71, 72]. Aurora kinase B is a kinase responsible for histone H3 phosphorylation on Serine 10 and 28 [65]. It is a chromosomal passenger protein that plays a role in the progression of mitosis and it is essential for chromosome condensation, kinetochore functions, spindle checkpoint activation, and the completion of cytokinesis [73]. The overexpression of Aurora B has been found in some tumor types, and has been associated with a poor prognosis for cancer patients [73]. Protein phosphatase PP1 antagonizes Aurora B function and is responsible for the removal of phosphate from histone H3 [74]. It has been found that Aurora B and PP1 phosphatase are physically associated with OGT and OGA [75, 76]. Tan et al. demonstrated that the mitotic spindle was incorrectly assembled in cells that display OGT or OGA gain of function [77]. The disruption of spindle architecture was partially due to a reduction in histone H3 phosphorylation by Aurora kinase B. These results suggest that O-GlcNAcylation/phosphorylation interplay on spindle proteins may be involved in the regulation of mitotic chromatin dynamics and chromosome segregation. Thus, aberrant activity of O-GlcNAc cycling enzymes might be responsible for aneuploidy, which is one of main features associated with cancers.

### **OGT AND HCF-1**

It is suggested that OGT can link the cellular metabolic state and the proliferation of cells through its impact on the epigenetic cell cycle regulator, host cell factor 1 (HCF-1) [78, 79]. OGT is a metabolic sensor, and cell proliferation is associated with enhanced nutrient requirements. Before division during proliferation, a cell must increase its biomass and replicate its genome. Thus, the cell needs to synthesize a lot of biomolecules and generate enough energy to meet the demands of proliferation. In rapidly dividing cancer cells, it is associated with increased glucose uptake [80]

Ruan et al. suggested that HCF-1, as an adaptor protein, facilitates the recognition of OGT substrates involved in epigenetic regulation [81]. They described the mechanism whereby glucose availability regulates OGT/HCF1 complex formation and subsequent O-GlcNAcylation and stabilization of PGC-1 $\alpha$ , which promotes hepatic gluconeogenesis. Thus, the findings of Ruan et al. define the OGT/HCF1 complex as a glucose sensor and important regulator of gluconeogenesis [81].

HCF-1 was first discovered as a host cell factor for human herpes simplex virus infection. It is an abundant chromatin-associated protein that regulates multiple steps in cell cycle progression [82]. HCF-1 is overexpressed in cancers and this is one of the markers of a poor prognosis in cancer treatment [83]. HCF-1 is also a key regulatory factor in many important processes, like embryonic stem cell pluripotency, stress responses, and development [82].

HCF-1 undergoes a specific process of proteolytic maturation. It is synthesized as a large precursor protein that is subsequently cleaved into two terminal subunits: N (HCF-1<sub>N</sub>) and C (HCF-1<sub>C</sub>). These two domains in the precursor are separated by a large middle region termed the proteolytic processing domain (PPD), which has six conserved 20- to 26-amino acid sequence repeats – HCF-1<sub>PRO</sub> containing many proline and threonine residues and an invariable glutamate that marks the site of proteolysis. The N-terminal subunit HCF-1<sub>N</sub> promotes progression through G1 whereas the C-terminal subunit HCF-1<sub>C</sub> regulates mitosis and cytokinesis [84–86]. Additionally to previous findings suggesting the interaction of OGT with HCF-1 and its glycosylation [87, 88] recent studies have shown that OGT is required for HCF-1 proteolytic processing [78, 89, 90]. Daou et al. demonstrated that a large proportion of the OGT in cells are in complex with HCF-1 and that this interaction is essential for HCF-1 cleavage [89]. On the other hand, HCF-1 is required for stabilizing OGT in the nucleus. Capotosti et al. revealed that OGT not only glycosylated HCF-1 but also directly cleaved HCF-1<sub>PRO</sub> domain [78]. Finally, Lazarus et al. fully elucidated the cleavage process [90]. They reported that the tetratricopeptide-repeat domain of OGT bound the C-terminal portion of a HCF-1 proteolytic repeat such that the cleavage region lay in the glycosyltransferase active site above uridine diphosphate–GlcNAc. Cleavage occurred between the cysteine and glutamate residues. They found that transition of this glutamate into serine converted a HCF-1 proteolytic repeat into a glycosylation substrate. It means that protein glycosylation and HCF-1 cleavage occur in the same active site [90].

HCF-1 has been detected in multiple complexes. Many experiments firmly established the interaction between HCF-1 and E2F transcription factors. Early studies suggested that HCF-1 regulated mammalian cell proliferation and cytokinesis, at least in part, by either directly or indirectly opposing pRb family member function in cell cycle inhibition [91]. Recent findings indicate that pRb and HCF-1 can bind to E2F1 simultaneously. They form repressive (pRb) to activating (HCF-1) transition complex during the G1 to S phase progression [82]. O-GlcNAcylation may be involved in these interactions. Wells et al. [92] showed that pRb and the related p107 protein are modified by O-GlcNAc in an in vitro transcription/translation system [92]. Furthermore, they showed that pRb was more heavily glycosylated in G1 of the cell-cycle in vivo, when pRb is known to be in an active, hypophosphorylated state. E2F1-associated pRb is modified by O-GlcNAc. These studies suggest that regulation of pRb function(s) may be controlled by dynamic O-GlcNAc modification, as well as phosphorylation (Fig. 2).



HCF-1 has been detected in complexes with histone modifying activity. One well-characterized example is a complex consisting of HCF-1, Ash2, WDR5, and MLL proteins [87]. MLL (myeloid/lymphoid or mixed-lineage leukemia) contains the Set domain, which is characteristic for proteins. It catalyzes dimethylation and trimethylation of H3K4. The promoter regions of active genes are often enriched for these modifications.

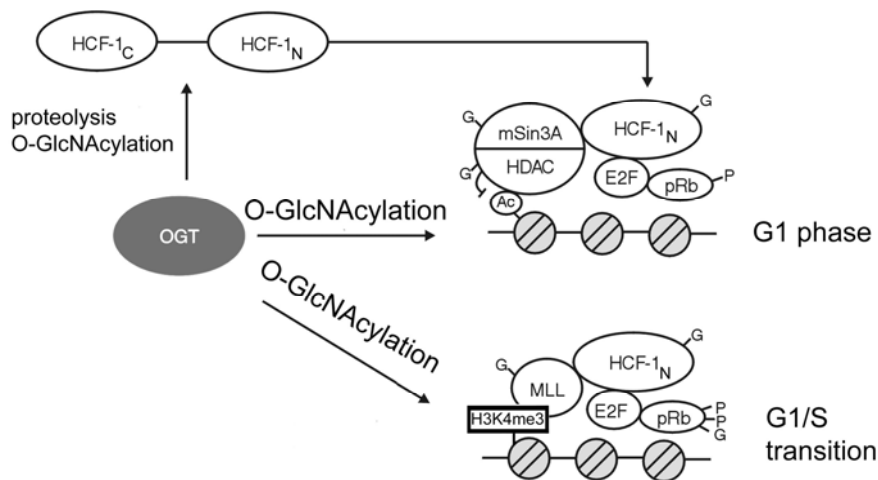


Fig. 2. The impact of OGT on chromatin-associated factors involved in the regulation of cell cycle progression. OGT is responsible for the glycosylation and proteolysis of HCF-1. OGT also interacts with and modifies mSin3A, HDAC, pRb, and MLL.

HCF-1 and OGT associate with ubiquitin carboxy-terminal hydrolase BAP1 (BRCA1-associated protein-1). Somatic BAP1 mutations have been found in cutaneous melanocytic tumors (epithelioid atypical Spitz tumors and melanoma), uveal melanoma, mesothelioma, clear cell renal cell carcinoma, and other tumors [93]. Using hematopoietic-restricted deletion of BAP1, which in adult mice shows similar features to those of human myelodysplastic syndrome (MDS), Dey et al. showed that BAP1 interacted with HCF-1, OGT and the polycomb group proteins ASXL1 and ASXL2 in vivo [94]. OGT and HCF-1 levels were decreased by BAP1 deletion, indicating a critical role for BAP1 in stabilizing these epigenetic regulators [94].

HCF-1 is known to associate with a repressor complex that includes mSin3A/B and HDAC1/2 [87]. mSin3A and HDAC1 are O-GlcNAcylated in HepG2 liver carcinoma cells [95]. Additionally to HDAC, mSin3A can recruit OGT via its TPR domains to specific genes [95]. OGT can thereby contribute along with HDAC to the repression of genes through the addition of O-GlcNAc modifications on transcriptional activators, inhibiting their activity.

HCF-1 interacts with Thanatos-associated proteins (THAP). Twelve THAP proteins have been identified in humans (THAP0 to THAP11). Individual THAP proteins are involved in different physiological processes, including cell

proliferation, regulation of transcription, apoptosis, and the maintenance of embryonic stem cell pluripotency [96–100]. THAP proteins in association with HCF-1 may function in chromatin-dependent processes, and aberrant regulation of these proteins may be involved in many diseases, including cancer. Parker et al. identified THAP11 as a transcriptional regulator differentially expressed in human colon cancer [101]. They provided the characterization of THAP11-dependent gene expression in human colon cancer cells and suggest that the THAP11–HCF-1 complex may be an important transcriptional and cell growth regulator in human colon cancer [101].

Mazars et al. found a physical link between OGT and THAP1 and -3 using HeLa as a cell model [88]. Mutations in the THAP1 DNA-binding domain, an atypical zinc finger (THAP-zf), were recently found to cause DYT6 dystonia, a neurological disease characterized by twisting movements and abnormal postures. The interactions between OGT and THAP1 suggest that OGT and glucose metabolism may play a role in the pathology of dystonia. The interaction of OGT with THAP proteins could conceivably be involved in processes associated with tumorigenesis and cancer progression.

### **OGT AND TET**

A few years ago, it was demonstrated that mammalian DNA contains 5-hydroxymethylcytosine (5hmC), which is generated from 5-methylcytosine (5mC) by three 5mC oxidases, the ten-eleven translocation enzymes TET1, TET2, and TET3 [102–104]. The biological role of 5hmC is still unclear. Growing evidence suggests that 5hmC is an intermediate base in an active or passive DNA demethylation process. TET proteins may also regulate the expression of genes independently of their enzymatic activity by acting in the recruitment of proteins associated with histone modifications.

TET expression varies between cells and tissues. TET1 has its greatest expression during embryogenesis and does not show high expression levels in adult tissues. TET2 is abundantly expressed in hematopoietic cells and TET3 shows ubiquitous expression in adult human tissues [105]. TET1 was discovered as a gene that, as result of a chromosome translocation, was fused in acute myeloid leukemia to the MLL gene coding for the H3K4 histone methyltransferase [106]. However, the role of TET1 in hematopoietic development and leukemogenesis has yet to be fully elucidated. TET2 has been reported as one of the most frequently mutated genes in myeloid malignancies [107, 108]. There is no data concerning a direct correlation between TET3 and cancer.

The levels of 5hmC in cancer are strongly reduced relative to the corresponding normal tissue surrounding the tumor. Jin et al. showed tumor-associated loss of 5hmC for lung, brain, breast, liver, kidney, prostate, intestine, and uterus cancers and melanoma [109]. Reduction of 5hmC in different types of solid tumors was confirmed in many other studies [110–114]. Although decreased 5hmC seems to be a common feature of cancers, there is no straight link with TET expression or

mutation levels and aberrant methylation. Thus, understanding the role of these enzymes in cancer development and progression requires a deeper understanding of all of the mechanisms and factors involved in 5hmC regulation.

Recent studies indicate that TET proteins could interact with OGT. Using protein affinity purification and mass spectrometry analysis, Chen et al. showed that both TET2 and 3 can associate with OGT [62]. TET2 directly interacts with OGT and this interaction is important for the chromatin association of OGT *in vivo*. This specific interaction does not regulate the enzymatic activity of TET2, but it facilitates OGT-dependent H2B histone O-GlcNAcylation at serine 112. At the same time, other researchers also showed a direct interaction of TET2 or 3 with OGT and found that this interaction does not influence 5hmC activity even when TET is O-GlcNAcylated [63, 115]. Deplus et al. found that the TET2 and 3-OGT complex co-localized on chromatin at active promoters enriched in H3K4me3. Reduction of TET or OGT activity resulted in a direct decrease in H3K4me3 levels and concomitant decreased transcription [63]. TET2/3-OGT complex is responsible for HCF1 O-GlcNAcylation, which is a component of the H3K4 methyltransferase SET1/COMPASS complex. These results led Delatte and Fuks to propose a model of TET/OGT action in transcriptional activation through H3K4me3 [107]. According to this model, TET2 and TET3 interact with OGT at GCI promoters and enhance its catalytic activity. OGT glycosylates the H2B on serine 112, which in turn favors H2B ubiquitinylation on lysine 120. Then, OGT glycosylates the SET/COMPASS complex on HCF-1, which stabilizes the complex. SET/COMPASS can bind to H2B K120ub and perform trimethylation on H3K4 [107].

Several studies using mouse embryonic stem (ES) cells indicate that TET proteins are not only transcriptional activators. They can also provide a scaffold for repressive epigenetic complexes and interact for example with polycomb repression complex 2 (PRC2) and mSin3A complex [116–118]. Shi et al. found that OGT controls TET1-mediated 5hmC modification and target gene repression in mouse ES cells [118]. They demonstrated that TET1 interacted with OGT and was O-GlcNAcylated. Depletion of *Ogt* reduced TET1 and 5hmC levels on TET1-targeted genes. Mutation of the putative O-GlcNAcylation site on Tet1 also results in a decreased level of TET1 protein [118].

Although TET1 has been the subject of extensive research in recent years, very little is known about its role in cancer development and progression. Recently, Yang et al. used immunohistochemistry and dot-blot assays to demonstrate that 5hmC is dramatically decreased in gastric cancers compared with matched normal tissues [119]. They also found a strong link between decreased 5hmC and the reduction of TET1 gene expression, but not TET2 or 3. Thus, decreased TET1 expression might be one of the mechanisms underlying 5hmC loss in gastric cancer. It was shown that the tumor suppressive activity of TET1 in gastric cancer can act through downregulation of the EZH2 oncogene and upregulation of the tumor suppressor p53 [120]. Reduced expression of TET1 was also found in prostate and breast cancers. TET1 depletion was associated

with increased invasion and metastasis. TET1 suppresses cancer cell invasion by maintaining the expression of tissue inhibitors of metalloproteinases [121]. Taking into consideration the growing amount of data suggesting an important role for TET1 in carcinogenesis, it would be very interesting to investigate if there is any association between O-GlcNAcylation and reduced expression of TET1 in cancers.

### OGT AND POLYCOMB PROTEINS

The results of studies concerning role of OGT in *Drosophila melanogaster* and *Caenorhabditis elegans* showed that this enzyme may link hexosamine signaling and transcriptional repression caused by polycomb proteins [122–125].

Polycomb group (PcG) proteins are evolutionarily conserved epigenetic regulators of development [126]. These proteins regulate proliferation and differentiation of cells via epigenetic silencing of important growth regulatory genes [127]. PcG proteins function in protein complexes, of which the two best characterized are PRC1 and PRC2. These complexes impact the chromatin condensation for gene repression. PRC2 catalyzes the dimethylation and trimethylation of histone H3 at lysine 27 (H3K27me<sub>2/3</sub>). Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of PRC2. This protein binds other PcG proteins, such as suppressor of zeste 12 (SUZ12), embryonic ectoderm development (EED), and the histone-binding proteins retinoblastoma-associated protein 46 (RbAp46) and RbAp48 [128, 129]. PRC2-mediated repression of gene activity involves histone deacetylation since PRC2 can physically associate with HDAC1 and HDAC2 [128, 129].

PRC1 catalyzes the monoubiquitylation of histone H2A at lysine 119 (K119). RING1 is the catalytic subunit of PRC1 that also contains BMI1, HPH, and HPC subunits. Several studies have suggested that PRC1 is dependent on PRC2 for recruitment to PcG target genes. H3K27me<sub>3</sub> can serve as the binding site of the chromodomain of PRC1 [130].

There is increasing evidence that deregulated expression of PcG proteins contributes to cancer development. Aberrant expression of PcG proteins, in particular BMI1 or EZH2, is associated with several human malignancies, for example myeloid leukemia, non-small cell lung cancer, colorectal cancer, breast and prostate cancers, and head and neck cancers [123]. Overexpression of BMI1 correlates with therapy failure in cancer patients, and BMI1 expression is thought to promote stemness in tumor cells. EZH2 expression promotes neoplastic transformation of immortalized epithelial cells and can increase the invasiveness and metastatic potential of cancer cells [127]. EZH2 activates oncogenic signaling pathways, such as Wnt/ $\beta$ -catenin, Ras, and NF- $\kappa$ B, and inhibits pro-differentiation pathways, such as Notch or BMP, through epigenetic silencing of the negative regulators and positive effectors [131].

Recent studies concerning animal model systems suggest an important role of OGT in PcG repression regulation [122, 123, 125]. It has been established that in

*Drosophila* OGT is encoded by the PcG gene super sex combs (*sxc*). Genome-wide profiling in *Drosophila* revealed that GlcNAc-modified proteins are highly enriched at polycomb response elements [122]. OGT was essential for full repression by PcG, since in the loss *Sxc/Ogt* mutant, genes in PcG binding sites were derepressed. Love et al. carried out ChIP-chip experiments using anti-O-GlcNAc antibodies and whole genome *C. elegans* tiling arrays [124]. They found that over 800 promoters were marked by O-GlcNAc and that a number of transcriptional regulators associated with PcG repression were among those most heavily marked genes. Myers et al. also found a link between OGT and PcG genes in mammals [124]. They showed that PRC2 was necessary to maintain normal levels of OGT and for the correct cellular distribution of O-GlcNAc in mouse embryonic stem cells.

The requirement of *Ogt* activity in *Drosophila* for full repression of PcG-regulated genes suggests that some of proteins within PRC1 and PRC2 complexes could be modified by O-GlcNAc. In fact, Ph protein, which is one of the polycomb proteins in *Drosophila*, has been proven to be O-GlcNAcylated [122]. Glycosylation of PH appears to contribute to the maintenance of polycomb-mediated repression of transcription. Taking into consideration the conservation of OGT during evolution, PcG proteins are predicted to be glycosylated in mammals. Recently, O-GlcNAcylation of EZH2 protein has been demonstrated in human breast cancer cell line MCF7 [133]. OGT-mediated O-GlcNAcylation at Ser75 stabilizes EZH2 and facilitates the trimethylation of histone 3 at K27. The OGT knockdown disrupted PRC2 complex integrity. EZH2 and OGT have been found to co-regulate a set of tumor suppressor genes. These results suggest that the OGT-EZH2 regulatory axis may play a critical role in tumor malignancy [133].

Abnormal OGT expression and O-GlcNAcylation are features of cancer cells but their role in polycomb-dependent gene regulation still remains to be fully elucidated. Since PcG proteins regulate the activities of genes involved in cell differentiation and epithelial mesenchymal transition, which are important for cancer progression, it is necessary to find all of the links between aberrant O-GlcNAcylation in cancer cells and PcG regulation.

## CONCLUSION

Environmental factors and nutrition influence the epigenetic regulation of the genes involved in the initiation and progression of cancer. O-GlcNAcylation, which is a nutrient-responsive modification, may play a pivotal role in linking the metabolic status of cells and chromatin modifications. It is tempting to suggest that the connection between epigenetics and O-GlcNAc might explain the correlations that researchers have found between high fat diets or diabetes and the risk of cancer. However, more work is needed to find out the real mechanistic link between nutrients, O-GlcNAc, and epigenetic regulatory factors in cancer cells.

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