Cancer Metabolism and Elevated O-GlcNAc in Oncogenic Signaling

Zhiyuan Ma and Keith Vosseller

From the Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102

O-Linked β-N-acetylglucosamine (O-GlcNAc) is a covalent addition of a GlcNAc sugar moiety to hydroxyl groups of serine and/or threonine residues of cytosolic and nuclear proteins. An analogous to phosphorylation, O-GlcNAcylation plays crucial regulatory roles in cellular signaling. Recent work indicates that increased O-GlcNAcylation is a general feature of cancer and contributes to transformed phenotypes. In this minireview, we discuss how hyper-O-GlcNAcylation may be linked to various hallmarks of cancer, including cancer cell proliferation, survival, invasion, and metastasis; energy metabolism; and epigenetics. We also discuss potential therapeutic modulation of O-GlcNAc levels in cancer treatment.

Introduction to O-GlcNAc in Cancer

O-Linked β-N-acetylglucosamine (O-GlcNAc), which was discovered in the early 1980s (1), is the covalent addition of a GlcNAc sugar moiety to hydroxyl groups of serine and/or threonine residues of cytosolic and nuclear proteins. The O-GlcNAc transferase (OGT) transfers the GlcNAc moiety from the high-energy donor UDP-GlcNAc to substrate proteins, whereas O-GlcNAcase (OGA) hydrolyzes O-GlcNAc from proteins (2–6). UDP-GlcNAc is an end product of the hexosamine biosynthetic pathway (HBP). Increased glucose flux through the HBP elevates UDP-GlcNAc and drives increased cellular O-GlcNAcylation. Energy metabolism in cancer involves a shift away from mitochondrial oxidative phosphorylation to less efficient glycolysis (Warburg effect), necessitating greatly increased glucose uptake and increased HBP flux. This cancer-specific metabolism is associated with elevated O-GlcNAcylation (hyper-O-GlcNAcylation) in all human malignancies examined, including breast (7, 8), prostate (9), lung (10), colorectal (10, 11), liver (12), and nonsolid cancers such as chronic lymphocytic leukemia (13). Additionally, deregulation of the enzyme that adds O-GlcNAc to proteins (OGT) appears to be involved in cancer cell hyper-O-GlcNAcylation, as levels of OGT are elevated in all cancers examined (7, 9, 10, 14, 15). Knockdown of OGT reduces cancer cell hyper-O-GlcNAcylation and inhibits transformed phenotypes, indicating that elevated O-GlcNAc contributes to cancer progression. Given the diverse regulatory roles of O-GlcNAc and the complex molecular changes in cancer, potential links between cancer cell hyper-O-GlcNAcylation and underlying oncogenicity are just beginning to be understood. In this minireview, known and potential mechanisms through which O-GlcNAcylation supports cancer phenotypes will be discussed in the context of various cellular hallmarks of cancer (16, 17). We also discuss the clinical potential of therapeutically targeting hyper-O-GlcNAcylation in cancer.

O-GlcNAc and Cancer Cell Metabolic Reprogramming

One of the remarkable features of cancer cells is aerobic glycolysis, a phenomenon also known as the Warburg effect (18–20), in which cancer cells rely preferentially on glycolysis instead of oxidative phosphorylation as the main energy source even in the presence of high oxygen tension. The switch to glycolysis in cancer cells is driven in part by tumor cell hypoxia, oncogenes (e.g. ras and myc), and mutant tumor suppressors (e.g. TP53) (21), leading to up-regulation of glycolytic enzymes and glucose transporters, increasing glucose import into tumor cells (22). The abundance of glucose in cancer cytoplasm not only contributes to increased glycolysis but also increases flux into metabolic branch pathways of glycolysis, including the pentose phosphate pathway (PPP) and HBP (Fig. 1). Thus, increased cancer cell glucose uptake likely drives increased HBP flux. Indeed, certain oncogenes such as kras up-regulate levels of not only glucose transporters and glycolytic enzymes but also GFAT1 (glutamine-fructose-6-phosphate amidotransferase 1), the rate-limiting enzyme in the HBP (23). As O-GlcNAc levels rise in response to elevated UDP-GlcNAc, increased cancer cell HBP flux may be expected to drive hyper-O-GlcNAcylation. In support of this, loss of the p53 tumor suppressor in mouse embryonic fibroblasts increases the rate of aerobic glycolysis, Glut3 expression (24), and HBP flux and leads to elevation of O-GlcNAc (25). Cancer cells are also addicted to glutamine (26). Cancer cells consume glutamine at high rates in vivo and, compared with non-transformed cells, require high concentrations of glutamine to survive and proliferate (27). Oncogenes can up-regulate glutamine uptake. For example, c-Myc transcriptionally up-regulates glutamine transporter expression (26, 28, 29). Tumor hypoxia and hypoxia-inducible factor-1α (HIF-1α) transcriptionally induce the expression of GFAT (30), whereas down-regulation of oncogenic Kras or c-Myc in pancreatic cancer decreases the expression of GFAT (23). Glutamine is the donor substrate in the conversion of fructose 6-phosphate to glucosamine 6-phosphate by GFAT in the HBP. Thus, excess glutamine uptake in cancer cells could contribute to increased flux through the HBP, ultimately contributing to increased levels of the HBP end product UDP-GlcNAc. Indeed, in vivo glutamine administration...
tion itself has been shown to increase HBP flux (31, 32). Such metabolic reprogramming in cancer cells meets energetic demands for rapid cell proliferation and supplies increased intermediates for cancer cell biosynthesis. Consequently, cancer cell metabolic changes, including increased glucose uptake due to the Warburg effect and increased glutamine uptake, likely cooperate to drive increased HBP flux. In support of this, we found that the end products of the HBP (including UDP-GlcNAc) are elevated in pancreatic cancer cells (15). Thus, it appears that increased HBP flux and elevated UDP-GlcNAc are general features of cancer cells that contribute to hyper-O-GlcNAcylation.

Hyper-O-GlcNAcylation itself may regulate cancer metabolism in a feedback manner. Due to the responsiveness of O-GlcNAcylation to glucose flux, O-GlcNAc has been termed a “nutritional sensor” and may provide feedback signals that modulate metabolism in response to changing cellular nutrient status. Several studies now link hyper-O-GlcNAcylation to cancer-associated metabolic reprogramming. O-GlcNAc has been reported to modify a variety of glycolytic enzymes (33–35). In particular, a role for O-GlcNAc modification has been described at Ser-529 of PFK1 (phosphofructokinase 1), which catalyzes the rate-limiting step of glycolysis to generate fructose 1,6-bisphosphate from fructose 6-phosphate (36). Ser-529 is vital for allosteric activation of PFK1 by fructose 1,6-bisphosphate (37). O-GlcNAcylation of PFK1 at Ser-529 inhibits its kinase activity, possibly resulting from the O-GlcNAc moiety blocking the binding of fructose 1,6-bisphosphate to PFK1 and disrupting activating oligomerization of PFK1 (36), leading to increased levels of glycolytic intermediates. This impacts cancer cells in several ways. First, flux through the PPP is increased, leading to increased nucleotide production for cancer cell proliferation. Second, increased PPP flux increases NADPH production, which cancer cells use to cope with oxidative stress. Third, flux through the HBP is increased, which could further elevate O-GlcNAc by increasing UDP-GlcNAc levels. Of note, O-GlcNAcylation of PFK1 is increased only in transformed cells, but not in highly proliferating non-transformed T-cells or epithelial cells (36).

**FIGURE 1. O-GlcNAc and cancer cell metabolism.** Increased levels of glucose are imported into cancer cells by glucose transporters (e.g. Glut1). Glucose is phosphorylated by hexokinase to generate glucose 6-phosphate (G6P), which can be either shunted into the PPP, leading to production of nucleotides and NADPH, or converted into fructose 6-phosphate (F6P). Although most fructose 6-phosphate fluxes through glycolysis, a portion is directed into the HBP, leading to production of UDP-GlcNAc. OGT utilizes UDP-GlcNAc in addition to O-GlcNAc on hydroxyl groups of serine and/or threonine residues of cytosolic and nuclear proteins. O-GlcNAc is removed by OGA. Cancer cell metabolic reprogramming is facilitated by hyper-O-GlcNAcylation. For example, cancer cell-specific O-GlcNAcylation of PFK1 at Ser-529 inhibits PFK1 kinase activity, leading to several consequences. 1) More glycolytic intermediates shunt into branch pathways such as the PPP, leading to increased nucleotide production for cancer cell proliferation. 2) Increased PPP flux increases NADPH production, which cancer cells use to cope with oxidative stress. 3) Increased flux through the HBP contributes further to elevated O-GlcNAc. In addition, HIF-1α and ChREBP are stabilized by elevated O-GlcNAc in cancer. HIF-1α and ChREBP are implicated in aerobic glycolysis, de novo lipogenesis, and nucleotide biosynthesis. Proteins shown in red are modified by O-GlcNAc. HK, hexokinase; FBP, fructose 1,6-bisphosphate; PEP, phosphoenolpyruvate; PFK, phosphofructokinase; PK, pyruvate kinase.
In addition to direct regulation of glycolytic enzymes, O-GlcNAcylation also regulates transcription factors to modulate metabolic reprogramming. The transcription factor HIF-1α transcriptionally controls the majority of enzymes involved in glycolysis, contributing to the Warburg effect in cancer cells (21). It has been reported that elevated O-GlcNAcylation in cancer cells stabilizes HIF-1α, thereby promoting the Warburg effect. Conversely, reduction of hyper-O-GlcNAcylation reverses cancer cell aerobic glycolysis to oxidative phosphorylation (38). Moreover, carbohydrate-responsive element-binding protein (ChREBP) contributes to metabolic reprogramming in tumors through inhibition of mitochondrial respiration and up-regulation of aerobic glycolysis, de novo lipogenesis, and nucleotide biosynthesis (39). ChREBP O-GlcNAcylation stabilizes the protein, leading to increased transcription of ChREBP glycolytic and lipogenic target genes L-type pyruvate kinase, acetyl-CoA carboxylase, and fatty acid synthase (40, 41). O-GlcNAcylation of the transcription factor Sp1 activates a cholesterolgenic program through de novo lipogenesis, and nuclear transcription of ChREBP glycolytic and lipogenic target genes (42). Although the O-GlcNAc sites on ChREBP have not been mapped, cancer cell hyper-O-GlcNAcylation may stabilize/activate ChREBP and Sp1, driving cancer cell-increased aerobic glycolysis and lipogenesis, representing a possible mechanism through which hyper-O-GlcNAcylation contributes to transformed phenotypes.

Another potential link between hyper-O-GlcNAcylation and cancer metabolism is the transcription factor c-Myc. Oncogenic c-Myc induces expression of glycolytic enzymes and lactate dehydrogenase to promote glycolytic flux and also up-regulates glutaminase expression, which is important in cancer cell conversion of glutamine to glutamate in mitochondria for anaplerotic resupply of tricarboxylic acid intermediates used in biosynthesis (21). c-Myc phosphorylation at Thr-58 (at least in part by glycogen synthase kinase 3β) (43) causes rapid degradation of c-Myc and reduces its target gene expression (44). Oncogenic mutations at Thr-58 (a mutational hot spot in many lymphomas) stabilize c-Myc (45). c-Myc is also O-GlcNAcylated at Thr-58 (46). Thus, increased c-Myc Thr-58 O-GlcNAcylation could compete with phosphorylation and potentially stabilize c-Myc. Indeed, reducing O-GlcNAc has been shown to cause the degradation of c-Myc protein in prostate cancer cells (47). These results suggest that hyper-O-GlcNAcylation may contribute to oncogenicity and cancer metabolic reprogramming through stabilizing oncogenic c-Myc.

**O-GlcNAc and Cancer Cell Proliferation**

Sustaining proliferative signaling and evading growth suppressors are two hallmarks of cancer cells (Fig. 2) (16). Oncogenic changes often promote cell cycle progression and bypass cell cycle checkpoints. FoxM1 acts as a key positive regulator of cell cycle progression by up-regulating transcription of genes involved in the G1/S and G2/M transition, and its overexpression is linked to oncogenesis (48). O-GlcNAcylation is implicated in the stability of FoxM1.

Reducing breast cancer cell hyper-O-GlcNAcylation decreases levels of FoxM1 and its target genes and increases the cyclin-dependent kinase cell cycle inhibitor p27Kip1. Consistent with this, reducing hyper-O-GlcNAcylation decreases cell cycle progression in breast and prostate cancer (7, 9). Cyclin D1 is a positive regulator of the G1/S transition. Recent work with different cancer types has shown that reducing cancer cell hyper-O-GlcNAcylation either genetically or pharmacologically attenuates the expression of cyclin D1 (15, 49, 50). Thus, cancer cell hyper-O-GlcNAcylation appears to contribute in part to excessive growth through up-regulation of key proteins that drive cell cycle progression such as cyclin D1 and down-regulation of cell cycle inhibitory proteins such as p27Kip1.

**O-GlcNAc and Cancer Cell Survival**

Cancer cells evolve to resist cell death in part through up-regulation of anti-apoptotic mechanisms that allow cancer cells to elude barriers to unrestricted cell growth (16). Conditional knock-out of OGT in mice results in loss of O-GlcNAc and T-cell apoptosis (51), suggesting that hyper-O-GlcNAcylation in cancer may play an anti-apoptotic role. Indeed, we found that reducing hyper-O-GlcNAcylation in pancreatic cancer BxPC-3 cells protects against suspension-induced apoptosis (15). Conversely, increasing O-GlcNAc in pancreatic cancer BxPC-3 cells protects against suspension-induced apoptosis (15). Up-regulated/constitutively activated NF-κB contributes to transformation in many cancers, in part through anti-apoptotic influences (52). We have shown that the NF-κB p65 subunit and the upstream IκB kinase (IKK) α and IKKβ are hyper-O-GlcNAcylated in pancreatic cancer cell lines. Reducing hyper-O-GlcNAcylation decreases cancer cell p65, activating phosphorylation (Ser-536), nuclear translocation, NF-κB

**FIGURE 2. O-GlcNAc and hallmarks of cancer.** The known links between O-GlcNAc and cancer hallmarks are depicted. Proteins shown in red are modified by O-GlcNAc.

**MINIREVIEW: O-GlcNAc in Cancer**
transcriptional activity, and target gene expression. Conversely, mimicking cancer cell hyper-O-GlcNAcylation through pharmacological elevation of O-GlcNAcylation increases IKKα and p65 O-GlcNAcylation, accompanied by activation of NF-κB signaling (15). Therefore, cancer cell hyper-O-GlcNAcylation appears to be anti-apoptotic, possibly in part through activation of NF-κB signaling. In addition to activation of NF-κB signaling, it has been reported that elevation of O-GlcNAcylation protects breast cancer MCF-7 cells from 4-OH-tamoxifen-induced apoptosis through down-regulation of estrogen receptor α (53).

Cancer cells are also subject to a number of physiologic stresses that may trigger cell death in non-transformed cells, such as nutrient stress, proteotoxic stress, oxidative stress, endoplasmic reticulum (ER) stress, and hypoxia (17). Mechanisms developed by cancer cells to cope with these stresses are essential to cancer cell survival. Emerging evidence indicates that hyper-O-GlcNAcylation may contribute to pro-oncogenic cancer cell survival by combating such stresses.

Reactive oxygen species (ROS) can be either generated endogenously by normal aerobic metabolism or derived exogenously from the extracellular milieu (54). Excessive ROS production exceeding capacity of cellular antioxidant defenses leads to oxidative stress, which can lead to severe damage of nuclear or mitochondrial DNA, intracellular lipids, and proteins. The relationship between oxidative stress and cancer is complex. Cancer cells experience increased oxidative stress due to many reasons, including rapid growth, impaired mitochondrial function, and a reactive stroma (55). Oxidative stress may contribute to oncogenesis (56). Conversely, excessive oxidative stress in cancer cells must be combated to avoid cell death (54).

One potential connection between cancer cell protection against oxidative stress and hyper-O-GlcNAcylation is PFK1, the rate-limiting enzyme in glycolysis (Fig. 3A). Reduced glutathione is critical in combating oxidative stress. The cofactor NADPH is required to maintain pools of reduced glutathione. O-GlcNAcylation of PFK1 inhibits its activity in cancer cells (36), decreasing rates of flux through glycolysis and thus increasing shunt of fructose 6-phosphate into the PPP. A consequence of increased PPP flux is increased generation of NADPH. Thus, hyper-O-GlcNAcylation in cancer cells would likely increase PPP flux and NADPH production, contributing to maintaining a pool of reduced glutathione to combat ROS-induced cell death (36).

Cellular protein homeostasis, or proteostasis, refers to a cell’s capacity to maintain proper protein folding, trafficking, and avoidance of toxic protein aggregation (57). Proteostasis is maintained, under stress conditions, by a number of cellular response mechanisms, including the heat shock response (HSR). The HSR in mammals is mediated by six groups of closely related proteins: HSP100, HSP90, HSP70, HSP60, HSP40, and small HSPs, which are transcriptionally regulated by HSF1 (heat shock transcription factor 1) (57, 58). The HSR is frequently activated/up-regulated in cancer cells and helps counter excessive proteotoxic stress due to various cancer-associated states, including increased gene copy number linked to aneuploidy and increased protein translation, misfolding, and/or trafficking (17). Several lines of evidence suggest that hyper-O-GlcNAcylation may potentially support cancer cell growth through increasing the levels and/or activity of HSPs (Fig. 3B). For example, cell stress-induced elevation of O-GlcNAc is protective against cell damage at least in part through induction of increased HSP levels, including HSP70 and HSP90 (59). Lowering O-GlcNAcylation in mouse embryonic fibroblasts decreases the expression of HSP70 and HSP40 (59, 60). Moreover, increasing HBP pathway flux by glutamine treatment to elevate O-GlcNAc enhances expression of HSF1 and HSP70 in a septic mouse model and in isolated rat cardiomyocytes (32, 61). HSP70 and HSP90 are modified by O-GlcNAc (62, 63). Additionally, HSP70 displays lectin-like binding activity specifically toward O-GlcNAc (64), and HSP90 inhibition destabilizes OGT and reduces O-GlcNAcylation (65). It has also been reported that increased HBP flux by gain-of-function mutation of GFAT1 or exogenous addition of GlcNAc controls protein quality control through increased autophagy, ER-associated degradation, and proteasome activ-
ity, thereby exerting cytoprotective effects in *Caenorhabditis elegans* disease models and extending lifespan (Fig. 3B) (66). Furthermore, we and others have recently shown that O-GlcNAc on proteins such as tau, TAK1-binding protein, α-synuclein, and PKF1 inhibits oligomerization or aggregation in the context of disease states (36, 67, 68). Taken together, these results suggest that hyper-O-GlcNAcylation in cancer cells may reduce proteotoxic stress through several mechanisms, including transcriptional induction of HSPs, stabilization of HSPs through direct O-GlcNAc modification, recruitment of HSPs to O-GlcNAc-modified targets through the lectin-like activity seen in the case of HSP70, and protein quality control.

Disruption of the equilibrium between protein synthesis and ER protein folding capacity can cause ER stress, as often seen in cancer cells (69). Elevated O-GlcNAc may inhibit ER stress as a prosurvival mechanism (Fig. 3). In response to ER stresses, the unfolded protein response is activated, which has been shown to activate the HBP through Xbp1 (X-box-binding protein 1)-dependent transcriptional induction of key enzymes involved in the HBP (e.g. GFAT1) (70). Induction of HBP by stimulation of Xbp1 in the heart protects cardiomyocytes from ischemia/reperfusion injury (70). Furthermore, genetically or pharmacologically increasing O-GlcNAcylation protects against cardiomyocyte cell death in response to ER stress (71). This protection is associated with decreased expression of proteins involved in the ER stress maladaptive response, including the folding enzyme chaperones Grp74 and Grp90 and CHOP (CCAAT/enhancer-binding protein homologous protein) (71). Indeed, it has been shown that reduction of hyper-O-GlcNAcylation in cancer induces metabolic stress, thereby activating the CHOP/Bim pathway (38). Therefore, hyper-O-GlcNAcylation could contribute to cancer cell survival by mitigating ER stress through the inhibition of CHOP.

**O-GlcNAc and Cancer Angiogenesis**

Induction of angiogenesis during tumorigenesis is another hallmark of cancer (16). Not only is angiogenesis essential for delivery of oxygen and nutrients to the interior of a tumor, but it also contributes to allowing cancer cells to invade surrounding tissue and disseminate to distant organs (72). A variety of pro-angiogenic factors have been linked to proliferation and differentiation of endothelial cells, e.g. VEGFs (73). Recent evidence indicates that tumor angiogenesis may be supported in part by hyper-O-GlcNAcylation. Reducing hyper-O-GlcNAcylation in the prostate cancer cell line PC-3ML by knocking down OGT inhibits VEGF expression and *in vitro* angiogenesis (9). To sustain angiogenesis, the tumor stroma requires continuous remodeling, a process in which matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9 are vital in the proteolytic degradation of the extracellular matrix (74). MMP-9 has also been shown to be important in liberating matrix-bound VEGF-A in mouse models (75). Reducing hyper-O-GlcNAcylation in prostate cancer PC-3ML and liver cancer HepG2 cells suppresses the expression of MMP-2 and MMP-9 (9, 12). Conversely, elevation of O-GlcNAcylation using the OGA inhibitor Thiamet-G or knockdown of OGA enhances the activity of MMP-2 and MMP-9 in chondrocytes and increases the expression of MMP-1, MMP-2, and MMP-3 (12, 76). Therefore, hyper-O-GlcNAcylation in tumors may contribute to angiogenesis through up-regulation of VEGF and MMPs.

**O-GlcNAc and Cancer Cell Invasion and Metastasis**

Tumor metastasis (not the primary tumor) accounts for >90% of cancer mortality (77). Emerging evidence suggests that hyper-O-GlcNAcylation in cancers may be involved in tumor invasion and metastasis. Whereas increasing hyper-O-GlcNAcylation enhances the migration/invasion of breast and liver cancer cells, lowering hyper-O-GlcNAcylation by knockdown of OGT inhibits tumor invasion and metastasis *in vivo* and *in vitro* in breast and prostate cancer cells (7–9, 78). The mechanisms by which hyper-O-GlcNAcylation may regulate tumor invasion and metastasis are only beginning to be understood. One mechanism that contributes to metastasis is epithelial-to-mesenchymal transition (EMT) (79, 80). EMT involves loss of epithelial markers such as E-cadherin and gain of mesenchymal markers such as vimentin and N-cadherin (79, 81). We and others have found that reducing hyper-O-GlcNAcylation in cancers increases expression of the epithelial marker E-cadherin and decreases expression of the mesenchymal marker vimentin, whereas elevating O-GlcNAc decreases expression of the epithelial marker E-cadherin in breast and liver cancer (8, 15, 78). The loss of E-cadherin is regarded as a key step in initiation of EMT (79), and transcription factors, including Snail, E47, and Zeb, can directly bind to the promoter of E-cadherin, thereby repressing its expression (82). There are several potential links between hyper-O-GlcNAcylation, repression of E-cadherin expression, and enhancement of invasion and metastasis. O-GlcNAcylation of E-cadherin in its cytoplasmic domain during ER stress blocks its cell surface transport, thereby inhibiting intercellular adhesion (83) and thus potentially promoting migration/metastasis. Also, direct O-GlcNAcylation of Snail on Ser-112 stabilizes it, increasing repression of E-cadherin expression and increasing cancer cell invasion and metastasis (78). Finally, the stability of Snail can be indirectly enhanced by NF-κB activity (84), suggesting that cancer cell-associated increased NF-κB activity due to hyper-O-GlcNAcylation (15) may contribute to Snail stability and thus metastasis. In addition, O-GlcNAcylation of cofillin at Ser-108 has been shown to promote cell invasion through its localization to invadopodia at the leading edge of breast cancer cells (85).

**O-GlcNAc and Cancer Cell Epigenetics**

Dynamic O-GlcNAcylation in response to changing nutrient status has been linked to epigenetic changes through a variety of mechanisms. The role of O-GlcNAc in epigenetics is reviewed in detail in Ref. 98, whereas a few specific examples potentially relevant to cancer will be noted here.

It appears that O-GlcNAc is part of the histone code, regulating gene transcription in an epigenetic manner (86). O-GlcNAc modification of histone H2B facilitates its monoubiquitination, associated with active gene transcription (87). Thus, cancer cell hyper-O-GlcNAcylation directly on histones may lead to epigenetic procarcin gene regulation. O-GlcNAc
may also contribute to epigenetic control through regulation of chromatin-modifying enzymes and complexes. The TET (ten-eleven translocation) family proteins are 5-methylcytosine oxidases required during DNA demethylation at a step in 5-hydroxymethylcytosine formation, a modification that is strongly reduced in cancer tissues (88, 89). TET1 binds OGT and is O-GlcNAcylated, leading to nuclear export of TET1 and inhibition of 5-hydroxymethylcytosine formation (90). Cancer cell hyper-O-GlcNAcylation and elevated OGT levels potentially contribute to TET inhibition and thus the reduced 5-hydroxymethylcytosine observed in tumors. However, mechanisms that link reduced 5-hydroxymethylcytosine to oncogenicity are not understood. Independent of their enzymatic activity, TET proteins may also recruit histone-modifying proteins to chromatin. The TET2 and TET3 proteins bind OGT and are O-GlcNAcylated, which facilitates O-GlcNAcylation of histone H2B (91) and H3K4me3 histone modification associated with activated gene transcription (92). Although no specific links between hyper-O-GlcNAcylation and cancer cell epigenetic contribution to transformation have been established, it seems likely that such connections will be operating in cancer cells.

Clinical Implications and Therapeutic Targeting

Key aspects of treating human cancer will involve reliable biomarkers for early diagnosis, prognosis, and detection of recurrence. O-GlcNAc levels and expression of enzymes regulating O-GlcNAcylation, including OGT, OGA, and GFAT, may serve as biomarkers for early detection and prognosis. Higher levels of hyper-O-GlcNAcylation are associated with poor overall survival in prostate cancer patients (14). Additionally, although OGT mRNA was undetectable in the urine of 143 healthy individuals, 51.7% of 176 urine samples obtained from bladder cancer patients contained OGT transcripts (93). Furthermore, the mRNA expression of OGT is correlated with the differentiation of bladder tumors, with the poorly differentiated (grade III), most aggressive form manifesting the highest OGT mRNA levels (93). Although OGA mRNA is found in urine from bladder cancer patients and healthy individuals at a comparable rate, the mRNA levels of OGA are inversely correlated with the differentiation state of bladder tumors (93). Furthermore, low levels of OGA correlate with tumor recurrence in hepatocellular carcinoma patients with liver transplants (12). Moreover, O-GlcNAc levels in blood and spleen are positively correlated with the burden of chronic lymphocytic leukemia (94). In addition, high expression of GFAT2 correlates with poor prognosis in breast cancer patients (95).

A key consideration in potential anticancer therapeutic approaches is cancer cell selectivity versus more general toxicity. There are indications that OGT as a potential target may offer some degree of cancer cell selectivity. Genetically reducing hyper-O-GlcNAcylation by knocking down OGT inhibits breast, prostate, and pancreatic cancer cell survival but does not affect the cell proliferation of counterpart immortalized but non-transformed cells (7, 9, 15), suggesting that a therapeutic window may exist within which the cancer cell-specific effects of targeting OGT may be achieved.

The cancer cell-specific susceptibility to targeting OGT is reminiscent of “oncogene addiction,” in which cancer cells are uniquely sensitive to loss of an acquired oncogenic molecular process. Several OGT inhibitors have been described. An OGT inhibitor identified in a screen of a small molecule library (96) reduces breast cancer cell hyper-O-GlcNAcylation and blocks anchorage-independent growth (7). However, the potency of the inhibitor is relatively low, necessitating use of high concentrations in vitro. 5-Thioglycosamine (5SGlcNAc) and its per-O-acetylated analog Ac-5SGlcNAc have been developed as alternative OGT inhibitors (97). Ac-5SGlcNAc can be converted into UDP-5SGlcNAc via the GlcNAc salvage pathway, thereby competing with UDP-GlcNAc and inhibiting O-GlcNAcylation (97). We have shown that Ac-5SGlcNAc is able to reduce pancreatic cancer cell hyper-O-GlcNAcylation and inhibit cell growth in vitro (15). With the development of more bioavailable and efficacious OGT inhibitors, it is expected that suppression of hyper-O-GlcNAcylation by targeting OGT may serve as a novel therapeutic intervention for a variety of cancers.

REFERENCES

MINIREVIEW: O-GlcNAc in Cancer


Tomic, J., McCaw, L., Li, Y., Hough, M. R., Ben-David, Y., Moffat, J., and