Cancer Metabolism and Elevated O-GlcNAc in Oncogenic Signaling*

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O-Linked β-N-acetylglucosamine (O-GlcNAc) is a carbohydate post-translational modification on hydroxyl groups of serine and/or threonine residues of cytosolic and nuclear proteins. 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 (11, 12), 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).

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 oncosgenes 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-GlcNAcylation (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 administra-

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1 To whom correspondence should be addressed. E-mail: keith.vosseller@drexelmed.edu.
2 The abbreviations used are: OGT, O-GlcNAc transferase; OGA, O-GlcNAcase; HBP, hexosamine biosynthetic pathway; PPP, pentose phosphate pathway; HIF-1α, hypoxia-inducible factor-1α; ChREBP, carbohydrate-responsive element-binding protein; IKK, IκB kinase; ER, endoplasmic reticulum; ROS, reactive oxygen species; HSR, heat shock response; HSP, heat shock protein; MMP, metalloproteinase; EMT, epithelial-to-mesenchymal transition; S5GlcNAc, S-thioglucosamine.
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 6-phosphate 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, 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-GlcNAcylation 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 transcriptionally increasing the expression of the SREBP1 (sterol response element-sp1 activates a cholesterolgenic program through fatty acid synthase (40, 41)).

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 transcriptionally increasing the expression of the SREBP1 (sterol response element-binding protein 1) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (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 glycolgen 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 knockout 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 the pancreatic ductal adenocarcinoma cell lines MiaPaCa-2 and PANC-1 decreases the expression of the anti-apoptotic protein Bcl-xL and induces pro-apoptotic cleavage of caspase-9 and caspase-3. These results indicate that reducing pancreatic cancer cell hyper-O-GlcNAcylation triggers the intrinsic apoptotic pathway (15). Conversely, increasing O-GlcNAc in pancreatic cancer BxPC-3 cells protects against suspension-induced apoptosis (15). Up-regulated/constitutively activated NF-kB contributes to transformation in many cancers, in part through anti-apoptotic influences (52). We have shown that the NF-kB 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-kB...
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-

![Diagram](image-url)
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 PFK1 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 Thimet-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 cofilin 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 proancer 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 relative low, necessitating use of high concentrations in vitro. 5-Thioglucoasamine (5S-GlcNAc) and its per-O-acetylated analog Ac-5S-GlcNAc have been developed as alternative OGT inhibitors (97). Ac-5S-GlcNAc can be converted into UDP-5S-GlcNAc via the GlcNAc salvage pathway, thereby competing with UDP-GlcNAc and inhibiting O-GlcNAcylation (97). We have shown that Ac-5S-GlcNAc 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

1. Torres, C. R., and Hart, G. W. (1984) Topography and polypeptide distribution of terminal N-acetylglucosamnine residues on the surfaces of intact lymphocytes. Evidence for O-linked GlcNAc. J. Biol. Chem. 259, 3308–3317
2. Hart, G. W. (1997) Dynamic O-linked glycosylation of nuclear and cytoskeletal proteins. Annu. Rev. Biochem. 66, 315–335
3. Hart, G. W., Housley, M. P., and Slawson, C. (2007) Cycling of O-linked β-N-acetylglucosamine on nucleocepoyl proteins. Nature 446, 1017–1022
4. Wells, L., Vosseller, K., and Hart, G. W. (2001) Glycosylation of nucleocytoplasmic proteins: signal transduction and O-GlcNAc. Science 291, 2376–2378
5. Zachara, N. E., and Hart, G. W. (2004) O-GlcNAc, a sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress. Biochim. Biophys. Acta 1673, 13–28
6. Haltiwanger, R. S., Blomberg, M. A., and Hart, G. W. (1992) Glycosylation of nuclear and cytoplasmic proteins. Purification and characterization of a uridine diphospho-N-acetylglucosamine:polypeptide β-N-acetylglucosaminytransferase. J. Biol. Chem. 267, 9005–9013
7. Caldwell, S. A., Jackson, S. R., Shahriari, K. S., Lynch, T. P., Sethi, G., Walker, S., Vosseller, K., and Reginato, M. J. (2010) Nutrient sensor O-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1. Oncogene 29, 2831–2842
8. Gu, Y., Mi, W., Ge, Y., Liu, H., Fan, Q., Han, C., Yang, J., Han, F., Lu, X., and Yu, W. (2010) O-GlcNAcylacylation plays an essential role in breast cancer metastasis. Cancer Res. 70, 6344–6351
9. Lynch, T. P., Ferrer, C. M., Jackson, S. R., Shahriari, K. S., Vosseller, K., and Reginato, M. J. (2012) Critical role of O-linked β-N-acetylglucosamine transferase in prostate cancer invasion, angiogenesis, and metastasis. J. Biol. Chem. 287, 11070–11081
10. Mi, W., Gu, Y., Han, C., Liu, H., Fan, Q., Zhang, X., Cong, Q., and Yu, W. (2011) O-GlcNAcylacylation is a novel regulator of lung and colon cancer malignancy. Biochim. Biophys. Acta 1812, 514–519
11. Yehezkel, G., Cohen, L., Kliger, A., Manor, E., and Khalaila, I. (2012) O-linked β-N-acetylglucosaminyltransferase (O-GlcNAcylacylation) in primary and metastatic colorectal cancer clones and effect of N-acetyl-β-D-glucosaminidase silencing on cell phenotype and transcriptome. J. Biol. Chem. 287, 28755–28769
12. Zhu, Q., Zhou, L., Yang, Z., Lai, M., Xie, H., Wu, L., Xing, C., Zhang, F., and Zheng, S. (2012) O-GlcNAcylacylation plays a role in tumor recurrence of hepatocellular carcinoma following liver transplantation. Med. Oncol. 29,
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78, 452–458

34. Ohn, T., Kedersha, N., Hickman, T., Tisdale, S., and Anderson, P. (2008) A functional RNAi screen links O-GlcNAc modification of ribosomal proteins to stress granule and processing body assembly. Nat. Cell Biol. 10, 1224–1231

35. Teo, C. F., Ingle, S., Wolfert, M. A., Elsayed, G. A., Nöt, L. G., Chatham, J. C., Wells, L., and Boons, G. J. (2010) Glycopeptide–specific monoclonal antibodies suggest new roles for O-GlcNAc. Nat. Chem. Biol. 6, 338–343

36. Yi, W., Clark, P. M., Mason, D. E., Keenan, M. C., Hill, C., Goddard, W. A., 3rd, Peters, E. C., Driggers, E. M., and Hsieh-Wilson, L. C. (2012) Phosphofructokinase 1 glycosylation regulates cell growth and metabolism. Science 337, 975–980

37. Sola-Penna, M., Da Silva, D., Coelho, W. S., Marinho-Carvalho, M. M., and Zancan, P. (2010) Regulation of mammalian muscle type 6-phosphofructo-1-kinase and its implication for the control of the metabolism. ILIUM Life 62, 791–796

38. Ferrer, C. M., Lynch, T. P., Sodi, V. L., Falcone, J. N., Schwab, L. P., Peacock, D. L., Vocadlo, D. J., Seagroves, T. N., and Regnato, M. J. (2014) O-GlcNAcylation regulates cancer metabolism and survival stress signaling via regulation of the HIF-1 pathway. Mol. Cell 54, 820–831

39. Tong, X., Zhao, F., Mancuso, A., Gruber, J. I., and Thompson, C. B. (2009) The glucose-responsive transcription factor ChREBP contributes to glucose-dependent anaerobic synthesis and cell proliferation. Proc. Natl. Acad. Sci. U.S.A. 106, 21660–21665

40. Sakiyama, H., Fujiwara, N., Noguchi, T., Eguchi, H., Yoshihara, D., Uyeda, K., and Suzuki, K. (2010) The role of O-linked GlcNAc modification on the glycosylation response of ChREBP. Biochem. Biophys. Res. Commun. 402, 784–789

41. Guiney, C., Filholoul, G., Rayah-Benhamed, F., Marmier, S., Dubuquoy, C., Dentin, R., Moldes, M., Burnol, A. F., Yang, L., Lefebvre, T., Girard, J., and Postic, C. (2011) O-GlcNAcylation increases ChREBP protein content and transcriptional activity in the liver. Diabetes 60, 1399–1413

42. Penque, B. A., Hoffgott, A. M., Herring, B. P., and Elmendorf, J. S. (2013) Hexosamine biosynthesis impairs insulin action via a cholesterologenic response. Mol. Endocrinol. 27, 536–547

43. Gregory, M. A., Qi, Y., and Hann, S. R. (2003) Phosphorylation by glycosynogenesis kinase-3 controls c-Myc proteolysis and subnuclear localization. J. Biol. Chem. 281, 3431–3436

44. Vervoorts, J., Lüschér-Firzlaff, J., and Lüscher, B. (2006) The ins and outs of MYC regulation by posttranslational mechanisms. J. Biol. Chem. 281, 34725–34729

45. Pouyssegur, J., Salmon, A. C., and Preiss, M. (2013) Sphingosine kinase: a key player in glucose homeostasis. J. Biol. Chem. 288, 21797–21800

46. Manzari, B., Kudlow, J. E., Fardin, P., Merello, E., Ottaviano, C., Puppo, M., Singleton, K. D., and Wischmeyer, P. E. (2008) Glutamine induces heat shock protein expression via O-glycosylation and phosphorylation of HSF-1 and Sp1. J. Physiol. 583.5, 789–806

47. Itkonen, H. M., Minner, S., Guldvik, I. J., Sandmann, M. J., Tsourlakis, M. C., Berge, V., Svindland, A., Schlomm, T., and Mills, I. G. (2013) O-GlcNAc transferase regulates metabolic pathways to regulate the stability of c-MYC in human prostate cancer. Cancer Res. 73, 5277–5287

48. Myers, S. S., and Lam, E. W. (2007) The emerging roles of forkhead box (Fox) proteins in cancer. Nat. Rev. Cancer 7, 847–859

49. Kwee, K. A., Baker, J. I., and Pelham, R. H. (2012) Modulators of sensitivity and resistance to inhibition of PI3K identified in a pharmacogenomic screen of the NCI-60 human tumor cell line collection. PLoS ONE 7, e46518

50. Olivier-Van Stichelen, S., Drouget, L., Dehennault, V., El Yazidi-Belkoura, I., Guiney, C., Mir, A. M., Michalski, J. C., Vercoutter-Edouart, A. S., and Postic, C. (2011) O-GlcNAcylation increases ChREBP protein content and transcriptional activity in the liver. Diabetes 60, 1399–1413

51. O’Donnell, N., Zachara, N. E., Hart, G. W., and Marth, J. D. (2004) O-GlcNAc modification of ribosomal proteins is significantly associated with poor prognosis of patients. Prostate Cancer Prostatic Dis. 7, 18–22

52. Kawauchi, K., Araki, K., Tobiume, K., and Tanaka, N. (2009) Loss of p53 through regulation of anabolic glucose metabolism. J. Biol. Chem. 284, 15121–15130

53. Shi, Y., Tomic, J., Wen, F., Shaha, S., Bahlo, A., Harrison, R., Dennis, J. W., Williams, R., Gross, B. J., Walker, S., Zuccolo, J., Deans, J. P., Hart, G. W., and Spaner, D. E. (2010) Aberrant O-GlcNAcylation characterizes chronic lymphocytic leukemia. Leukemia 24, 1588–1598

54. Kamigaito, T., Okane, T., Kawakubo, M., Shimojo, H., Nishizawa, O., and Nakayama, J. (2014) Overexpression of O-GlcNAc by prostate cancer cells is significantly associated with poor prognosis of patients. Prostate Cancer Prostatic Dis. 17, 18–22

55. Hanahan, D., and Weinberg, R. A. (2011) Hallmarks of cancer: the next generation. Cell 144, 646–674

56. Luo, J., Solimini, N. L., and Elledge, S. J. (2009) Principles of cancer therapy: oncogene and non-oncogene addiction. Cell 136, 823–837

57. Warburg, O. (1956) On the origin of cancer cells. J. Gen. Physiol. 38, 519–530

58. Warburg, O. (1956) On the origin of cancer cells. Science 123, 309–314

59. Warburg, O. (1956) On respiratory impairment in cancer cells. Science 124, 269–270

60. DeBerardinis, R. J., Lum, J. I., Hatzivassiliou, G., and Thompson, C. B. (2012) Oncogenic Kras maintains pancreatic tumors in the body. Nature 480, 608–611

61. DeBerardinis, R. J., Lum, J. I., Hatzivassiliou, G., and Thompson, C. B. (2012) Oncogenic Kras maintains pancreatic tumors in the body. Nature 480, 608–611

62. Manzari, B., Kudlow, J. E., Fardin, P., Merello, E., Ottaviano, C., Puppo, M., Singleton, K. D., and Wischmeyer, P. E. (2008) Glutamine induces heat shock protein expression via O-glycosylation and phosphorylation of HSF-1 and Sp1. J. Physiol. 583.5, 789–806

63. Manzari, B., Kudlow, J. E., Fardin, P., Merello, E., Ottaviano, C., Puppo, M., Singleton, K. D., and Wischmeyer, P. E. (2008) Glutamine induces heat shock protein expression via O-glycosylation and phosphorylation of HSF-1 and Sp1. J. Physiol. 583.5, 789–806

64. Manzari, B., Kudlow, J. E., Fardin, P., Merello, E., Ottaviano, C., Puppo, M., Singleton, K. D., and Wischmeyer, P. E. (2008) Glutamine induces heat shock protein expression via O-glycosylation and phosphorylation of HSF-1 and Sp1. J. Physiol. 583.5, 789–806
MINIREVIEW: O-GlcNAc in Cancer

Masson, E., Hampe, C., and Issad, T. (2013) O-GlcNAcylation-inducing treatments inhibit estrogen receptor α expression and confer resistance to 4-OH-tamoxifen in human breast cancer-derived MCF-7 cells. PLoS ONE 8, e69150

Martiindale, J. L., and Holbrook, N. J. (2002) Cellular response to oxidative stress: signaling for suicide and survival. J. Cell. Physiol. 192, 1–15

Benz, C. C., and Yao, C. (2008) Ageing, oxidative stress and cancer: paradigms in parallel. Nat. Rev. Cancer 8, 875–879

Reuter, S., Gupta, S. C., Chaturvedi, M. M., and Aggarwal, B. B. (2010) Oxidative stress, inflammation, and cancer: how are they linked? Free Radic. Biol. Med. 49, 1603–1616

Balch, W. E., Morimoto, R. I., Dillin, A., and Kelly, J. W. (2008) Adapting proteostasis for disease intervention. Science 319, 916–919

Dai, C., Dai, S., and Cao, J. (2012) Proteotoxic stress of cancer: implication of the heat-shock response in oncogenesis. J. Cell. Physiol. 227, 2982–2987

Zachara, N. E., O’Donnell, N., Cheung, W. D., Mercer, J. J., Martir, J. D., and Hart, G. W. (2004) Dynamic O-GlcNAc modification of nucleocytoplasmic proteins in response to stress. A survival response of mammalian cells. J. Biol. Chem. 279, 30133–30142

Kazemi, Z., Chang, H., Haserodt, S., McKen, C., and Zachara, N. E. (2010) O-Linked β-N-acetylglucosamine (O-GlcNAc) regulates stress-induced heat shock protein expression in a GSK-3β-dependent manner. J. Biol. Chem. 285, 39096–39107

Gong, J., and Jing, L. (2011) Glutamate induces heat shock protein 70 expression via O-GlcNAc modification and subsequent increased expression and transcriptional activity of heat shock factor-1. Minerva Anestesiol. 77, 488–495

Walgren, J. L., Vincent, T. S., Schey, K. L., and Buse, M. G. (2003) High glucose and insulin promote O-GlcNAc modification of proteins, including α-tubulin. Am. J. Physiol. Endocrinol. Metab. 284, E424–E434

Frank, L. A., Sutton-McDowall, M. L., Brown, H. M., Russell, D. L., Gilchrist, R. B., and Thompson, J. G. (2014) Spliced X-box binding protein 1 couples H2B facilitates its monoubiquitination. J. Biol. Chem. 289, 1179–1192

Guineu, C., Lemoine, J., Michalski, J. C., and Lefebvre, T. (2004) 70-kDa heat shock protein presents an adjustable lectinic activity towards N-linked O-acetylglucosamine modification in endo-

O-glycosylation of cofilin promotes breast cancer cell invasion. J. Biol. Chem. 288, 36418–36425

Zhu, W., Leber, B., and Andrews, D. W. (2001) Cytoplasmic O-glycosylation prevents cell surface transport of E-cadherin during apoptosis. EMBO J. 20, 5999–6007

Wu, Y., Deng, J., Rychahou, P. G., Qiu, S., Evers, B. M., and Zhou, B. P. (2009) Stabilization of Snail by NF-κB is required for inflammation-induced cell migration and invasion. Cancer Cell 15, 416–428

Huang, X., Pan, Q., Sun, D., Chen, W., Shen, A., Huang, M., Ding, J., and Geng, M. (2013) O-GlcNAcylation of collagen promotes breast cancer cell invasion. J. Biol. Chem. 288, 36418–36425

Sakabe, K., Wang, Z., and Hart, G. W. (2010) β-N-Acetylgalcosamine (O-GlcNAc) is part of the histone code. Proc. Natl. Acad. Sci. USA. 107, 19915–19920

Fujiki, R., Hashiba, W., Sekine, H., Yokoyama, A., Chikanishi, T., Ito, S., Imai, Y., Kim, J., He, H. H., Igarashi, K., Kanno, J., Ohtake, F., Kitagawa, H., Roeder, R. G., Brown, M., and Kato, S. (2011) GlcNAcylation of histone H2B facilitates its monoubiquitination. Nature 480, 557–560

Ito, S., D’Alessio, A. C., Taranova, O. V., Hong, K., Sowers, L. C., and Zhang, Y. (2010) Role of Tet proteins in 5mC to 5hmC conversion, ESC-cell self-renewal and inner cell mass specification. Nature 466, 1129–1133

Jin, S. G., Jiang, Y., Qiu, R., Rauch, T. A., Wang, Y., Schackert, G., Krek, D., Lu, Q., and Pfeifer, G. P. (2011) S-Hydroxymethylcytosine is strongly depleted in human cancers but its levels do not correlate with IDH1 mutations. Cancer Res. 71, 7360–7365

Zhang, Q., Liu, X., Gao, W., Li, P., Hou, J., Li, J., and Wong, J. (2014) Differential regulation of the ten–eleven translocation (TET) family of dioxygenases by O-linked β-N-acetylglucosamine transferase (OGT). J. Biol. Chem. 289, 5986–5996

Chen, Q., Chen, Y., Bian, C., Fujiki, R., and Yu, X. (2013) TET2 promotes histone O-GlcNAcylation during gene transcription. Nature 493, 561–564

Deplus, R., Delatte, B., Schwinn, M. K., Défrance, M., Méndez, J., Murphy, N., Dawson, M. A., Volkmar, M., Putmans, P., Calonne, E., Shih, A. H., Levine, R. L., Bernard, O., Mercher, T., Solary, E., Urh, M., Daniels, D. L., and Fuchs, F. (2013) TET2 and TET3 regulate GlcNAcylation and H3K4 methylation through OGT and SET1/COMPASS. EMBO J. 32, 645–655

Rozanski, W., Krzeslak, A., Forma, E., Brys, M., Blewniewski, M., Wozniak, P., and Lipinski, M. (2012) Prediction of bladder cancer based on urinary content of MGEAS5 and OGT mRNA level. Clin. Lab. 58, 579–583

Tomic, J., McCaw, L., Li, Y., Hough, M. R., Ben-David, Y., Moffat, J., and...
Spaner, D. E. (2013) Resveratrol has anti-leukemic activity associated with decreased O-GlcNAcylated proteins. *Exp. Hematol.* **41**, 675–686
95. Onodera, Y., Nam, J. M., and Bissell, M. J. (2014) Increased sugar uptake promotes oncogenesis via EPAC/RAPI and O-GlcNAc pathways. *J. Clin. Invest.* **124**, 367–384
96. Gross, B. J., Kraybill, B. C., and Walker, S. (2005) Discovery of O-GlcNAc transferase inhibitors. *J. Am. Chem. Soc.* **127**, 14588–14589
97. Gloster, T. M., Zandberg, W. F., Heinonen, J. E., Shen, D. L., Deng, L., and Vocadlo, D. J. (2011) Hijacking a biosynthetic pathway yields a glycosyltransferase inhibitor within cells. *Nat. Chem. Biol.* **7**, 174–181
98. Lewis, B. A., and Hanover, J. A. (2014) O-GlcNAc and the epigenetic regulation of gene expression. *J. Biol. Chem.* **289**, 34440–34448