O-GlcNAcylation is a key regulator of multiple cellular metabolic pathways

Hongshuo Zhang, Zhen Li, Yufei Wang and Ying Kong

Core Laboratory of Glycobiology and Glycoengineering, College of Basic Medical Sciences, Dalian Medical University, Dalian, Liaoning, China

ABSTRACT

O-GlcNAcylation modifies proteins in serine or threonine residues in the nucleus, cytoplasm, and mitochondria. It regulates a variety of cellular biological processes and abnormal O-GlcNAcylation is associated with diabetes, cancer, cardiovascular disease, and neurodegenerative diseases. Recent evidence has suggested that O-GlcNAcylation acts as a nutrient sensor and signal integrator to regulate metabolic signaling, and that dysregulation of its metabolism may be an important indicator of pathogenesis in disease. Here, we review the literature focusing on O-GlcNAcylation regulation in major metabolic processes, such as glucose metabolism, mitochondrial oxidation, lipid metabolism, and amino acid metabolism. We discuss its role in physiological processes, such as cellular nutrient sensing and homeostasis maintenance. O-GlcNAcylation acts as a key regulator in multiple metabolic processes and pathways. Our review will provide a better understanding of how O-GlcNAcylation coordinates metabolism and integrates molecular networks.

INTRODUCTION

Increasing evidence has suggested that metabolic reprogramming is a key factor in carcinogenesis and tumorigenesis, processes in which many oncogenes and tumor suppressors are metabolic regulators (Jóźwiak et al., 2014). Metabolic reprogramming also occurs in the endometrium during pregnancy (Zuo et al., 2015). In the early 1980s, a new glycosylation modification, O-linked-GlcNAc, was discovered in serine or threonine residues in proteins in the nucleus, cytoplasm, and mitochondria (Yang & Qian, 2017). This process was distinct from traditional O- and N-linked glycosylation which occurred in the endoplasmic reticulum, Golgi, and secretory pathways (Hart, 2019). This discovery generated interest in O-GlcNAc modifications. Research from the past 40 years has indicated that two enzymes regulate this process: O-GlcNAc transferase (OGT) for O-GlcNAc addition, and O-GlcNAcase (OGA) for O-GlcNAc removal (Vocadlo, 2012). O-GlcNAc modifies more than 4,000 cellular proteins and regulates key cellular processes by O-GlcNAcylation proteins, including those involved in signal transduction, mitochondrial activity, cytoskeleton function, and protein degradation (Bacigalupa, Bhadiadra & Reginato, 2018).

How to cite this article

Zhang H, Li Z, Wang Y, Kong Y. 2021. O-GlcNAcylation is a key regulator of multiple cellular metabolic pathways. PeerJ 9:e11443 DOI 10.7717/peerj.11443
O-GlcNAc may also play a role in nutrient sensing and external stress (Hanover, Krause & Love, 2010). UDP-GlcNAc, which precedes O-GlcNAcylation, links glucose, fatty acid, nucleic acid, and nitrogen metabolic pathways. Therefore, the role of O-GlcNAcylation as a nutrient sensor appears crucial for physiological and metabolic processes, such as cell growth, survival, nutritional status sensing, and multiple signaling pathway integration (Slawson, Copeland & Hart, 2010). Similarly, it may have a crucial role in metabolism-related diseases, including diabetes, cancer, cardiovascular disease, and neurodegenerative disease. Thus, O-GlcNAcylation levels may reflect, to some extent, cellular systemic metabolic status. While there is no shortage of information on metabolic regulation by O-GlcNAcylation, information regarding how it regulates coordinating metabolic processes are scarce. We reviewed the effects of O-GlcNAcylation on glucose metabolism, mitochondrial oxidation, lipid metabolism, and amino acid metabolism, and discuss its role in nutrient sensing, homeostatic maintenance, and major signaling pathways in physiological states. Our results yield a comprehensive starting point and general overview for this exciting field.

SURVEY METHODOLOGY

We conducted a literature search using the PubMed search engine with keywords and their combinations: O-GlcNAc; nutrient sensing; glucose uptake; GLUT1; GLUT2; GLUT3; GLUT4; GLUT5; glycolysis; mitochondria; oxidative phosphorylation; TCA; pentose phosphate pathway; glycogen synthesis; glycogenolysis; gluconeogenesis; lipid metabolism; triglyceride; cholesterol; phospholipids; amino acid metabolism; glutamine metabolism; alanine metabolism; AKT; NF-kB; HIF-1α; ERK1/2; β-catenin; c-Myc; Hippo/YAP; SOX2; OCT4; signaling pathway; homeostasis. This range provided objective subject coverage. We filtered articles published within 5- or 10-years based on the number of articles retrieved from various word combinations, as well as some early key articles. Our aim was to describe the regulation of metabolism by O-GlcNAcylation, not the regulation of O-GlcNAcylation by metabolisms, so we excluded articles of low relevance by reading the abstracts. Additionally, some word combinations did not produce results, for example: “O-GlcNAc, GLUT2”; “O-GlcNAc, GLUT5”; “O-GlcNAc, phospholipids”. It is worth noting that this is a comprehensive but not an exhaustive review of the literature.

O-GLCNAC AND NUTRIENT-SENSING

Metabolic homeostasis in mammals depends on interactions between nutrient-sensing components and signaling pathway regulation (Sharma, Saluja & Banerjee, 2018). Nutrient-sensing pathways are interrelated and combine nutrition information in different ways. For example, amino acids regulate the target rapamycin (TOR, mTOR) pathway and AMP/ATP levels affect the AMP-activated protein kinase (AMPK) signal pathway (Johnson, Rabinovitch & Kaeberlein, 2013; Ruan et al., 2013). The hexosamine biosynthetic pathway (HBP) is another branch of glycolysis (Love & Hanover, 2005; Chiaradonna, Ricciardiello & Palorini, 2018). In 1991, Marshall et al. observed that approximately 2–5% of glucose consumed by adipocytes entered the HBP (Marshall, Bacote & Traxinger, 1991). However, current studies present different views on flux into the HBP (Gibb et al., 2017; Zhang et al., 2021, PeerJ, DOI 10.7717/peerj.11443)
Evidence has suggested that HBP activation is also involved in ATP, uridine, glutamine, and acetyl-CoA function (Zachara & Hart, 2004; Hanover, Chen & Bond, 2018), and that the end-product of HBP, UDP-GlcNAc, integrates intracellular nutrient flux information, including glucose, nitrogen, nucleotide, and fatty acid metabolism (Vosseller et al., 2002; Hart et al., 2011; Swamy et al., 2016; Ma et al., 2017; Ohashi et al., 2017) (Fig. 1).

Glutamine fructose-6 phosphate amidotransferase (GFAT) is a rate-limiting enzyme in the HBP which converts fructose 6-phosphate, which is produced by glycolysis and glutamine (from the amino acid synthesis pathway), into glucosamine 6-phosphate (Kornfeld et al., 1964). OGT activity is highly sensitive to UDP-GlcNAc levels (Bacigalapa,
Bhadiadra & Reginato, 2018). A difference or change in the nutrient environment alters UDP-GlcNAc levels and the O-GlcNAcylation of intracellular proteins, thereby triggering changes in physiology or cell fate. Previous evidence has suggested that hyperglycemia causes insulin resistance via the HBP (Teo, Wollaston-Hayden & Wells, 2010) resulting in increased UDP-GlcNAc levels in cells (Traxinger & Marshall, 1991). O-GlcNAc levels increased significantly in the liver, heart, erythrocytes, and leukocytes of diabetic mammals, including humans (Ruan et al., 2012, 2013). Studies have shown that AMPK phosphorylates chromatin-associated fumarate hydratase (FH) at Ser75 under glucose deprivation and that FH is involved in promoter activation, thereby promoting growth arrest gene expression (Fricovsky et al., 2012). However, the O-GlcNAcylation of FH at Ser75 inhibits downstream events mediated by FH, especially in cancer cells with high OGT activity (Fricovsky et al., 2012). UDP-GlcNAc and protein O-GlcNAcylation cellular levels fluctuate in relation to glucose, free fatty acid, uridine, and glutamine availability, which suggests that O-GlcNAc senses nutrients (Johnson, Rabinovitch & Kaeberlein, 2013; Hanover, Chen & Bond, 2018) (Fig. 1).

**O-GlcNAcylation regulates glucose metabolism**

**O-GlcNAcylation regulates glucose uptake**

The first step in glucose metabolism involves transporting glucose through the membrane into cells. This process is undertaken by members of the glucose transporter (GLUT) family of proteins. GLUT1 was one of the first family members to be described and is the most widely expressed and studied transporter (Augustin, 2010). Studies have shown that elevated O-GlcNAcylation indirectly increases GLUT1 expression and glucose uptake via hypoxia-induced factor (HIF)-1α in cancer cells (Ferrer et al., 2014). Our previous work showed that elevated GLUT1 and O-GlcNAcylation levels of the endometrium during embryo implantation were involved in the regulation of endometrial receptivity (Han et al., 2019; Zhang et al., 2020). A mutual regulation between the GLUT1 and O-GlcNAcylation may result in: (1) elevated GLUT1 may enhance O-GlcNAcylation by coordinating glucose flow into HBP (2) elevated O-GlcNAcylation may increase GLUT1 expression through a similar molecular mechanism, which may be HIF-1α. Two recent studies provided evidence for this regulatory effect (Wang et al., 2020; Hu et al., 2021). Infection by hepatitis B promoted glucose uptake by upregulating GLUT1 expression in hepatocytes, providing substrates for the HBP synthesis of UDP-GlcNAc, leading to increased O-GlcNAcylation (Hu et al., 2021). Acyl-CoA ligase 4 (ACSL4) enhanced O-GlcNAcylation via GLUT1 to promote hepatocellular carcinoma growth and survival, and in turn, O-GlcNAcylation increased ACSL4 expression and promoted cancer growth, potentially involving downstream GLUT1 regulation by ACSL4 (Wang et al., 2020). These findings suggested that mutual regulation between the GLUT1 and O-GlcNAcylation may be necessary to maintain high metabolic cell demands.

Insulin-sensitive GLUT4 is also modified by O-GlcNAc (Buse et al., 2002). GLUT4 is stored in GLUT4 storage vesicles (GSVs) in the absence of insulin signaling. During insulin stimulation, signaling responses are generated by receptors binding to transport GSVs to cell membranes, fusing vesicle and cell membranes, resulting in GLUT4 transport to the
cell membrane (Zorzano et al., 1996), and vesicle proteins are also modified by O-GlcNAc (Buse et al., 2002). Evidence has indicated that elevated O-GlcNAcylation inhibits GLUT4 translocation and 2-deoxy-d-glucose (2-DG) uptake during insulin stimulation (Park, Ryu & Lee, 2005). However, increased O-GlcNAcylation of insulin receptor substrate-1 (IRS-1) and Akt2 reduces their phosphorylation in the insulin signaling pathway, leading to impaired glucose utilization and insulin resistance in adipocytes. Whether this is related to the O-GlcNAcylation of GLUT4 and its vesicles is unclear at present, but it remains a strong hypothesis. Therefore, by regulating GLUTs, O-GlcNAcylation affects cellular glucose intake and plays a crucial role in regulating cell fate. However, whether O-GlcNAcylation directly or indirectly regulates other GLUT family members and affects glucose uptake requires additional study.

**O-GlcNAcylation regulates glycolysis**

Glucose is phosphorylated by hexokinase (HK) at cell entry to generate glucose-6-phosphate, which remains in the cell, but whose metabolic fate is determined by cellular physiological requirements (Bacigalupa, Bhadiadra & Reginato, 2018). Some studies have shown that increased OGT enhances HK activity, whereas others have shown that HK is merely modified by O-GlcNAc, and positively regulates its expression (Baldini et al., 2016a). When the cell requires more energy, cellular glucose undergoes glycolysis, during which most key enzymes are also modified by O-GlcNAc (Bacigalupa, Bhadiadra & Reginato, 2018). Phosphofructokinase-1 (PFK1) is the first rate-limiting enzyme of glycolysis and catalyzes the conversion of fructose 6-phosphate to fructose 1, 6-bisphosphate. The O-GlcNAcylation of PFK1 at Ser529 in cancer cells was shown to inhibit its kinase activity (Yi et al., 2012), which may lead to elevated upstream glycolytic intermediates, increasing flux through the pentose phosphate pathway (PPP) and the biosynthetic precursors of cell growth. O-GlcNAc modification levels are further improved by increasing the HBP flux. The last committed step of glycolysis is catalyzed by pyruvate kinase M2 (PKM2), which is modified by O-GlcNAc (Chaiyawat et al., 2015). Recent cancer studies have shown that O-GlcNAcylation destabilizes the active tetramer PKM2 and reduces pyruvate kinase activity, but O-GlcNAcylation increases glucose uptake and consumption and promotes lactate production, and lipid and DNA synthesis (Wang et al., 2017). This potentially shifts the glycolytic flux to anabolic pathways to promote Warburg effects (Wang et al., 2017). Similarly, Singh et al. (2020) reported that PKM2 O-GlcNAcylation inhibited its activity and promoted aerobic glycolysis in cancer cells. Furthermore, these authors found that OGA was highly expressed in several cancers and that PKM2 was a target of OGA-associated acetyltransferase activity. OGA was also found to enhance PKM2 acetylation and facilitate O-GlcNAcylation of PKM2 by OGT (Singh et al., 2020). This study posits the idea that OGA exhibits acetyltransferase activity, which is supported by earlier studies (Toleman et al., 2004), and is now more widely accepted that while there are structural similarities to histone acetyltransferases, OGA is not catalytically active as an acetyltransferase. Indeed, recent structural studies found that human OGA lacks the residues necessary for binding to acetyl-CoA and described this region as a “catalytically incompetent pseudo-AT domain” as reported by Rao et al. (2013).
These studies help us understand the OGA’s pathogenic role, but not OGT’s. Similarly, the cooperation of the two antagonistic enzymes, OGT and OGA, may shed new mechanistic insights on glycolytic metabolism regulation. Previous studies indicated that OGT mediated metabolic reprogramming in cancer cells (Yi et al., 2012; Ferrer et al., 2014), yet OGA may also play a key role in this process. Furthermore, coordinated OGT and OGA actions may provide explanations for the cellular maintenance of the O-GlcNAc cycle in response to metabolic demands.

In their study, Zhang et al. (2017) observed that cells from patients with pre-B acute lymphocytic leukemia (pre-B-ALL) exhibited increased O-GlcNAcylation levels, elevated glycolysis, and O-GlcNAcylation regulation via the PI3K/AKT/c-Myc pathway, which affected cellular glycolysis levels. Furthermore, OGT knockdown in breast cancer cells reduced glycolysis and PPP pathway metabolites (Ferrer et al., 2014). These observations suggested that O-GlcNAcylation induced changes in glycolysis processes and was likely involved in reprogramming the cellular metabolic network (Figs. 2, 3).
identified that fructose bisphosphate aldolase, triosephosphate isomerase, and glyceraldehyde-3-phosphate dehydrogenase were modified by O-GlcNAc for other glycolysis pathway enzymes (Cieniewski-Bernard et al., 2004; Bacigalupa, Bhadiadra & Reginato, 2018). This data indicated that O-GlcNAc modification was directly or indirectly related to glycolytic processes. However, whether O-GlcNAcylation of some enzymes affects their structure, activity, and function remains unclear.

**O-GlcNAcylation regulates PPP**

As a branch of glucose metabolism, the PPP redirects glucose-6-phosphate to produce pentose and nicotinamide adenine dinucleotide phosphate (NADPH). Thus, one of the consequences in the pathway is to provide ribose for nucleic acid biosynthesis and to support lipid synthesis. Few studies have focused on PPP regulation by O-GlcNAcylation. Yehezkel et al. reported that glucose-6-phosphate dehydrogenase (G6PD) expression was increased and PPP enhanced in OGA-silenced colon cancer cells (Yehezkel et al., 2012). Similarly, OGT knockdown in breast cancer cells reduced PPP metabolites (Ferrer et al., 2014). These data suggested that PPP levels were consistent with total O-GlcNAcylation. However, this evidence was generated in cancer cells, therefore, the question remains whether different cell types exhibit different regulatory mechanisms. It was also shown that G6PD was modified by O-GlcNAc under hypoxic conditions and that O-GlcNAcylation activated G6PD activity and increased PPP flow, thereby providing a precursor for
nucleotide and lipid biosynthesis (Rao et al., 2015). These data suggested that O-GlcNAcylation directly regulated PPP through G6PD, which is a key PPP enzyme (Rao et al., 2015) (Fig. 2).

**O-GlcNAcylation regulates glycogen synthesis and glycogenolysis**

Glycogen is the stored form of glucose. Glycogen is stored in the liver and skeletal muscle. Hepatic glycogen is the main source of blood glucose, and muscle glycogen produces ATP to supply energy by rapid anaerobic glycolysis. Glycogen synthase (GS), which controls the final step in glycogen synthesis, was reportedly modified by O-GlcNAc (Parker et al., 2003). Recent studies indicated that total O-GlcNAcylation was increased in hepatoma cells under glucose deprivation which elevated O-GlcNAcylation of GS and decreased its enzyme activity (Taylor et al., 2008), potentially leading to blood glucose retention (Parker et al., 2003). Taylor et al. (2008) suggested that O-GlcNAcylation elevation was caused by increased OGT and decreased OGA levels. A study by Kang et al. (2009) observed that glycogen degradation via glucose deprivation was the source of UDP-GlcNAc for increased O-GlcNAcylation in cancer cells (Kang et al., 2009). The inhibition of glycogen phosphorylase (GP, the rate-limiting enzyme of glycogenolysis) prevented the increase of O-GlcNAcylation, and GFAT increased under glucose deprivation, suggesting that glycogen was the source of the increased O-GlcNAcylation.

GSK-3β inhibited GS activity, and GSK-3β phosphorylation was self-inactivating, thus promoting glycogen synthesis. GSK-3β was modified by O-GlcNAc (Wang, Pandey & Hart, 2007), however, the role of O-GlcNAcylation towards GSK-3β remains unknown. Due to competition between phosphorylation and O-GlcNAcylation, we believe that O-GlcNAcylation inhibits GSK-3β phosphorylation, thereby inhibiting glycogen synthesis, indirectly contributing to glycogenolysis. However, it is unclear whether GP is modified by O-GlcNAc in terms of the direct regulation of glycogenolysis.

**O-GlcNAcylation regulates gluconeogenesis**

Abnormal gluconeogenesis leads to hyperglycemia, which is a feature of the diabetic liver. During the insulin response, FOXO1 phosphorylation by AKT promotes its exit from the nucleus, thereby inhibiting gluconeogenic gene transcription by FOXO1. Studies have reported that FOXO1 was modified by O-GlcNAc (Ruan et al., 2013). Other gluconeogenic transcription factors and cofactors are similarly modified by O-GlcNAc, including cAMP response element-binding protein (CREB), regulated transcription coactivator 2 (CRTC2), peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), and host cell factor C1 (HCF-1) (Fig. 4). OGA overexpression in the liver of high-fat diet fed and db/db mice decreased O-GlcNAcylation levels of CRTC2 and hindered the effects of glucose on gluconeogenesis (Dentin et al., 2008). Studies have also found that PGC-1α promoted FOXO modification and OGT activation (Housley et al., 2009). Ruan et al. (2013) reported that OGT formed a nutrition-sensing complex with HCF-1 and that glycosylation modified PGC-1α via OGT (Ruan et al., 2012). Overall, O-GlcNAcylation promoted gluconeogenesis, thus, O-GlcNAcylation suppression towards key
gluconeogenic transcription factors and co-factors could be a therapeutic strategy for type 2 diabetes.

**O-GLCNACYLATION REGULATES MITOCHONDRIAL OXIDATION**

O-GlcNAcylation regulates oxidative phosphorylation

Mitochondria are essential organelles for nutrient status signaling and energy metabolism regulation. The regulatory role of O-GlcNAcylation in mitochondria has gained considerable traction in recent years. In 2003, Hanover et al. (2003) identified an OGT splice variant, mOGT, which targeted the mitochondrial inner membrane, suggesting that O-GlcNAcylation was present in mitochondria. This increased research interest in O-GlcNAcylation functions in mitochondria suggest that O-GlcNAc protein modifications are highly prevalent in this organelle (Arvanitis et al., 2005; Hu et al., 2009; Zhang et al. (2021), PeerJ, DOI 10.7717/peerj.11443).
A 2005 study reported that the antibody, mAbH, recognized epitope H containing O-GlcNAc residues, which were highly-expressed in mitochondria-rich cells, suggesting the presence of O-GlcNAcylation in the components of respiratory chain and energy transduction in mitochondria (Arvanitis et al., 2005). Subsequently, Clark et al. (2008) identified two mitochondrial ATP synthase α and β subunits, and two complex I components (Nduf-1 and Nduf-2) modified by O-GlcNAc via a chemoenzyme strategy for the selective labeling of O-GlcNAc-glycosylated proteins. Hu et al. (2009) reported that NDUFA9 of complex I, subunits core 1 and 2 of complex III, and mitochondrial DNA-coding subunit I of complex IV (Cox I) in the respiratory chain, were all O-GlcNAcylated in cardiomyocytes. High glucose (30 mM) levels increased O-glycosylation of mitochondrial proteins, impaired the activity of complexes I, III, and IV, and decreased mitochondrial calcium and cellular ATP levels. However, these effects were restored by OGA overexpression (Hu et al., 2009). These observations suggested that increased O-GlcNAcylation of mitochondrial proteins in cardiomyocytes led to impaired mitochondrial functions, by impairing oxidative phosphorylation. Interestingly, Ma et al. (2015) investigated rats that were acutely treated with the OGA inhibitor thiamet-G, using comparative proteomic and O-GlcNAcomic analyses, and identified over 88 O-glycosylated proteins of cardiac mitochondrial function, nearly half of which were localized to the oxidative phosphorylation system. They observed that acute O-GlcNAcylation elevation increased cardiac mitochondrial respiratory capacity, increased oxygen consumption, and regulated oxidative phosphorylation at multiple sites of the respiratory chain, thereby promoting ATP production rates and enhanced Ca\(^{2+}\) uptake. Mitochondrial dysfunction due to elevated O-GlcNAcylation caused by high glucose (Hu et al., 2009) may be the etiology of cardiomyopathy, which is one of the complications of diabetes. Since acute TMG treatment did not alter blood glucose levels, the acute increase in O-GlcNAcylation may have exerted a protective effect on cardiac mitochondria.

Another study reported that O-GlcNAcylation of ATP synthase subunit-α (ATP5A) was reduced in the brains of patients with Alzheimer’s disease (AD), AD mouse models, and amyloid β (Aβ)-treated cells (Cha et al., 2015). Aβ reduced the O-GlcNAcylation of ATP5A by inhibiting the direct interaction of mOGT with ATP5A, thereby decreasing ATPase activity and ATP production (Cha et al., 2015). Recently, Sacoman et al. (2017) proteomically identified 84 candidate mitochondrial glycoproteins, including two mitochondrial DNA-encoded proteins, cytochrome c oxidase subunit 2, and NADH-ubiquinone oxidoreductase chain 4. In addition, by reducing endogenous mOGT levels, membrane potential was lost, which led to mitochondrial content loss. Unexpectedly, OGT knockdown throughout the cell exerted no effects on mitochondrial contents and interconnectivity, suggesting that mOGT contributed to mitochondrial morphology (Sacoman et al., 2017). Furthermore, endogenous mOGT reduction was associated with increased mitochondrial respiration per mitochondrion, suggesting that a compensatory mechanism overcomes reduced mitochondrial contents. Dynamin-related protein 1
(DRP1), which drives mitochondrial fusion and fission, was identified at O-GlcNAc modification sites, and interestingly, elevated O-GlcNAcylation triggered DRP1 translocation from the cytoplasm to the mitochondria (Gawlowski et al., 2012). Moreover, Sacoman et al. (2017) also reported that mitochondrial fragmentation induced by mOGT down-regulation was dependent on DRP1. Banerjee, Ma & Hart (2015) observed OGA (mitochondrial OGA, mOGA) activity in mitochondria, leading to the possibility that mOGT/mOGA was involved in the O-GlcNAcylation regulation of DRP1, and whether mOGT affects DRP1 localization by O-GlcNAcylation. In addition, the protein nucleoplasmic transfer to generate mitochondrial O-GlcNAcylation has been recognized (Trapannone et al., 2016), but there are subunits encoded by mitochondrial DNA that never leave the mitochondria, suggesting that the O-GlcNAcylation of some mitochondrial proteins requires mOGT (Sacoman et al., 2017). Furthermore, UDP-GlcNAc were transported into the mitochondrial matrix via a mitochondrial inner membrane transporter (Banerjee, Ma & Hart, 2015). This suggests that an intra-mitochondrial O-GlcNAc cycle exists and that it has a vital role in mitochondrial metabolism and function. This cycle may also be influenced by intracytoplasmic O-GlcNAc (Fig. 5). The above data demonstrated that the O-GlcNAcylation was involved in mitochondrial metabolism regulation (Lozano et al., 2014), and supported the association of O-GlcNAcylation in mitochondrial oxidative phosphorylation.

**O-GlcNAcylation regulates the tricarboxylic acid cycle (TCA)**

The TCA cycle is a common pathway for sugar, fat, and amino acid catabolism. These major nutrients can be transformed into each other through this cycle under certain conditions. However, there is not much research on O-GlcNAcylation in TCA cycle

![Figure 5 O-GlcNAc cycling in mitochondria.](https://DOI:10.7717/peerj.11443/fig-5)
regulation. In 2008, Clark et al. reported that both the α and β subunits of the pyruvate dehydrogenase complex were O-GlcNAc modified. This was followed by Cao et al. (2013) who observed that malate dehydrogenase was a substrate for O-GlcNAcylation. In 2014, a proteomic analysis of mitochondrial protein changes, observed that protein abundance in mitochondrial respiratory chain and TCA cycle processes were decreased, regardless of OGT or OGA overexpression (Tan et al., 2014), suggesting O-GlcNAcylation effects on the TCA cycle. Interestingly, Tan et al. (2014) reported that adenovirus-mediated OGT/OGA overexpression in SY5Y neuroblastoma cells showed similar changes in the mitochondrial pathway. Subsequently, Ferrer et al. (2014) used liquid chromatography-mass spectrometry to measure TCA cycle intermediates in triple negative breast cancer cells (MDA-MB-231) and observed that O-GlcNAcylation inhibition by short-hairpin OGT increased TCA cycle metabolites. In 2016, Ma et al. (2016) using comparative proteomics techniques, observed that the vast majority of enzymes in the TCA cycle were O-GlcNAcylated and that some had multiple modification sites. It will be interesting to note if the O-GlcNAcylation of these enzymes translates to regulatory roles in the TCA cycle. A recent study reported that phosphoglycerate kinase 1 (PGK1), the first enzyme to produce ATP during glycolysis, was modified by O-GlcNAc at Thr255 (Nie et al., 2020). This modification promoted PGK1 activity, increased lactate production, induced PGK1 translocation to the mitochondria, and reduced mitochondrial oxidative phosphorylation by inhibiting the pyruvate dehydrogenase complex. However, blocking PGK1 Thr255 O-GlcNAcylation appeared to inhibit glycolysis, enhancing the TCA cycle (Fig. 2) and suppressing cancer cell proliferation (Nie et al., 2020). This study provided important insights into coordinated metabolic signals between glycolysis and the TCA cycle in O-GlcNAcylation (Figs. 2, 3). Associations between the O-GlcNAc cycle, mitochondrial biogenesis, and target proteins are being increasingly reported (Nugent & Bale, 2015; Ohashi et al., 2017; Tan et al., 2017). This evidence has suggested that O-GlcNAcylation plays important roles in the mitochondria. Indeed, O-GlcNAcylation regulation could be mechanistically exploited to respond to the reprogramming of mitochondrial metabolism.

**O-GlcNAcylation regulates lipid metabolism**

Lipids are widely distributed in cells and are important structural components of membranes and biofilms. They also serve as second messengers of intracellular signal transmission, and important energy sources during nutrient deficiency or cell growth (van Meer, Voelker & Feigenson, 2008; Holhuis & Menon, 2014; Efeyan, Comb & Sabatini, 2015). Lipid metabolism dysregulation is associated with the progression of a variety of metabolic diseases (Cohen, Horton & Hobbs, 2011; Schwartz et al., 2013). In 2005, Hanover et al. (2005) reported that a Caenorhabditis elegans OGT homozygous deletion mutant exhibited striking metabolic changes, exemplified by increased glycogen storage and decreased triglyceride levels. However, it was unclear whether triglyceride reduction was due to decreased synthesis or increased triglyceride mobilization. Fülöp et al. (2008) studied the effects of age on tissue O-GlcNAc levels and observed increased O-GlcNAcylation with age in all tissues examined, while serum triglyceride levels
increased. This could indicate a link between O-GlcNAcylation and triglycerides. In 2011 Guinez et al. observed that O-GlcNAcylation of carbohydrate response element binding protein (ChREBP) increased self-stabilization, and transcriptional activity on the lipogenic genes, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase 1 (SCD1). OGT overexpression increased ChREBP levels in the mouse liver, enhancing lipogenic gene expression, and the excessive deposition of hepatic triglycerides. OGA overexpression reduced ChREBP levels and decreased adipogenic protein levels of ACC and FAS, and ChREBP deglycosylation reduced lipid droplet accumulation in hepatocytes (Guinez et al., 2011). Similarly, O-GlcNAcylation of ChREBP induced lipid accumulation in mesangial cells (Park et al., 2014a). This evidence indicated that O-GlcNAcylation may induce increased triglyceride synthesis via increased ChREBP levels. In 2018, Sodi et al. reported that OGT downregulation in breast cancer cells decreased unsaturated fatty acid biosynthesis, pantothenate/coenzyme A biosynthesis, and fatty acid biosynthesis (Sodi et al., 2018). OGT inhibition degraded sterol regulatory element-binding protein 1 (SREBP-1) via phosphorylation of the AMPK pathway, and reduced expression of its transcriptional targets, ATP-citrate lyase (ACLY) and FAS, thereby decreasing lipid synthesis and causing cancer cell death (Sodi et al., 2018). Thus, this study identified a key role for the O-GlcNAcylation regulation of lipid metabolism in tumor cells.

Further studies revealed that liver X receptors (LXRs) maintained the expression of SREBP-1c, ChREBP-α, and the newly identified short isoform, ChREBP-β (Bindesbøll et al., 2015). LXRs were O-GlcNAcylated (Anthonisen et al., 2010). Bindesbøll et al. (2015) reported that LXRs and OGT interacted and were localized to the nucleus; they also discovered that LXRs deletion reduced nuclear O-GlcNAc signal transduction, including the O-GlcNAcylation of ChREBP-α (Bindesbøll et al., 2015) (Fig. 4). As the key to de novo fatty acid synthesis, FAS is also modified by O-GlcNAc; increased O-GlcNAcylation prevented FAS degradation and elevated its expression (Baldini et al., 2016b). Interestingly, Groves et al. (2017) observed that OGA interacted with FAS and decreased self-activity, whereas FAS overexpression increased stress-induced O-GlcNAcylation in U2OS cells (Groves et al., 2017). This suggests that there is a feedback mechanism for the FAS regulation of O-GlcNAcylation via OGA inhibition.

In addition to regulating glucose nutrient substrates, lipid synthesis is also controlled by insulin. SREBP-1c is one such transcription factor. Recent studies indicated that O-GlcNAcylation was a negative regulatory mechanism of insulin signaling transduction (Yang et al., 2008b; Whelan et al., 2010; Zhang, Yin & Yang, 2014). Increased OGT levels in mouse livers led to insulin resistance and dyslipidemia (Yang et al., 2008b). In addition, hepatic lipogenic pathways in animals and humans with diabetes were hyperactivated (Ruan et al., 2013) and associated with the abnormality of O-GlcNAcylation of the key regulatory factor (ACC, FAS, and SCD1) of lipogenesis. Furthermore, in adipose cells OGT caused the accumulation of N-arachidonyl ethanolamine, an endogenous appetite-inducing cannabinoid, and also activated de novo lipid desaturation, causing hyperorexia and promoting obesity (Li et al., 2018). In the mechanism, this regulation is related to the O-GlcNAcylation of PPARγ.
O-GLCNACYLATION REGULATES AMINO ACID METABOLISM

O-GlcNAcylation regulates glutamine metabolism

The amino acid glutamine is widely-distributed in mammalian physiology (Bergström et al., 1974; Wishart et al., 2013) and acts as a nitrogen donor for cellular growth. Glutamine is also used as an energy substrate for cell metabolism in the TCA cycle of the mitochondria. It participates in amino acid and lipid synthesis (DeBerardinis et al., 2007; Dang, Le & Gao, 2009), and is a key precursor for UDP-GlcNAc synthesis (Altman, Stine & Dang, 2016). The glutamine-driven HBP leads to the O-GlcNAc modification of intracellular target proteins (Swamy et al., 2016; Abramowitz et al., 2019; Akella, Ciraku & Reginato, 2019). Petrus et al. (2020) reported that high glutamine levels decreased glycolysis and intra-nuclear O-GlcNAcylation in human adipocytes. This observation exemplified the diversity and complexity of O-GlcNAcylation regulation by glutamine. However, there is little direct evidence on O-GlcNAcylation regulation towards glutamine metabolism. Studies have indicated some enzymes are modified by O-GlcNAc during glutamine metabolism, including glutamine synthetase and glutamine dehydrogenase (Clark et al., 2008). However, it is unclear if these modifications directly affect enzyme activity. Another recent study indicated that c-Myc regulated glutamine absorption and metabolism (Wise et al., 2008), and that c-Myc itself was modified and regulated by O-GlcNAc, suggesting an indirect regulatory role for O-GlcNAcylation in glutamine metabolism. Glutamine metabolism was also regulated by HIF-1α and SIRT1, both of which were modified by O-GlcNAc (Stegen et al., 2016; Ren et al., 2017; Bacigalupa, Bhadiadra & Reginato, 2018), potentially indicating another indirect regulatory link to glutamine metabolism.

Itkonen et al. (2016) recently reported that alanine levels decreased in prostate cancer cells treated with OGT inhibitors, suggesting that O-GlcNAcylation was involved in alanine metabolism. Few studies have reported O-GlcNAcylation regulation in amino acid metabolism, and future studies should be conducted to further our understanding of the O-GlcNAcylation regulatory mechanism on protein, glucose, and lipid metabolism.

O-GlcNAcylation regulates signaling pathways

Intracellular signal transduction coordinates physiological activities in response to different stimuli. Signal integration not only requires multiple signal inputs from different molecules, but also the triggering of multiple signal outputs via spatiotemporal regulation (Ong, Han & Yang, 2018). Given the ongoing interest in O-GlcNAcylation, it is theorized O-GlcNAcylation regulates various cellular processes, and functions as a key signal junction, or integrator (Hart, Housley & Slawson, 2007; Bond & Hanover, 2015). A typical example involves insulin signaling. After we eat, insulin binds to its receptor, phosphorylating downstream PI3K, and activating AKT. Then, AKT phosphorylates and inactivates GSK3 (Fang et al., 2000), promoting glycogen synthesis. AKT inhibits gluconeogenesis by phosphorylating FOXO, which promotes gluconeogenic gene transcription (Manning & Cantley, 2007) from the nucleus. In adipocytes, elevated
O-GlcNAcylation promoted insulin signaling attenuation through key signaling O-GlcNAcylation proteins, including IRS-1 (Whelan et al., 2010), PI3K (Yang et al., 2008b), and AKT (Wang et al., 2012) (Fig. 4). O-GlcNAcylation at Thr305/312 reduced AKT activity by suppressing Thr308 phosphorylation (Wang et al., 2012). Interestingly, Onodera, Nam & Bissell (2014) reported that the downregulation of O-GlcNAcylation levels in three-dimensional cultured breast cancer cells inhibited AKT signaling. This observation suggested that AKT signaling regulation by O-GlcNAcylation may depend on cell fate. PI3K/AKT signaling was shown to regulate glucose uptake and utilization (Vander Heiden, Cantley & Thompson, 2009) and stimulated lipogenic gene expression (Chang et al., 2005). Another major downstream molecule of AKT is mTOR, which is a serine/threonine kinase. mTOR has been shown to be influenced by OGT stability and thus regulates O-GlcNAc modification (Park et al., 2014b). This may represent a feedback regulatory mechanism that responds to high O-GlcNAcylation levels.

HIF responds to oxygen changes in physiological and disease environments. HIF-1α is transcribed to activate GLUT1, 3, and glycolytic enzyme expression (Semenza, 2010). Under a normoxic state, hydroxylated HIF-1α is recognized by the E3 ligase von Hippel-Lindau (VHL) and then ubiquitinated and degraded in the proteasome. Under hypoxic conditions, the hydroxylation of HIF-1α with oxygen and α-ketoglutarate as substrates is inhibited, and its expression stabilized. A recent study by Ferrer et al. (2014) showed that reduced O-GlcNAcylation promoted HIF-1α hydroxylation and its interaction with VHL, causing HIF-1α degradation and a reduction in GLUT1 expression, thereby affecting metabolic reprogramming. The transcription factor, c-Myc was shown to participate in cell glycolysis and anabolism regulation (Soga, 2013). The O-GlcNAcylation of c-Myc stabilizes itself and thus affects cell metabolism (Chou, Hart & Dang, 1995; Chou & Hart, 2001). Similarly, the transcription factor, NF-κB promoted tumor cell switching from oxidative phosphorylation to glycolytic metabolism (Wang & Jin, 2010), with O-GlcNAcylation regulating its activation (Ramakrishnan et al., 2013). The O-GlcNAcylation of NF-κB p65 by high glucose was shown to inhibit the interaction of NF-κB with 1κB, leading to the nuclear translocation of NF-κB and the activation of its target genes (Yang et al., 2008a). The O-GlcNAcylation promoted NF-κB activation in intestinal epithelial cells (Sun et al., 2020), and similarly, the activation of NF-κB by O-GlcNAcylation has also been confirmed in the placenta of hyperglycemia rats (Dela Justina et al., 2017). Elevated O-GlcNAcylation in the tumor microenvironment also promoted cancer progression via the downregulation of p38 MAPK activity and upregulation of the ERK1/2 signaling pathway (Moriwaki & Asahi, 2017). O-GlcNAcylation also played a role in myocardial hypertrophy by activating ERK1/2 (Ding et al., 2013). β-catenin was also O-GlcNAcylated, with elevated O-GlcNAcylation increasing β-catenin stability, nuclear accumulation, and transcriptional activity (Zhou et al., 2016; Harosh-Davidovich & Khalaila, 2018; Gao et al., 2019). β-catenin overexpression upregulated O-GlcNAcylation levels (Gao et al., 2019) to reveal a reciprocal regulatory mechanism between both molecules. Several examples of this phenomenon have been cited in the literature, e.g., O-GlcNAcylation influenced tumorigenesis by regulating the Hippo/YAP pathway (Peng et al., 2017), and cellular development and
differentiation by regulating SOX2 and OCT4 activity (Jang et al., 2012). This evidence strongly supports the role of O-GlcNAcylation in regulating cellular metabolic processes, through a pervasive role in signaling pathways (Fig. 6).

**Maintaining O-GlcNAc homeostasis**

O-GlcNAcylation is important for the spatiotemporal regulation of cell metabolism, and cellular O-GlcNAcylation homeostasis is vital for the maintenance of cell functions and the continuation of normal physiological processes. Thus, imbalanced homeostasis is directly implicated in the pathogenesis of many human diseases. However, the conditions under which imbalanced O-GlcNAcylation arise, and the mechanism for the maintenance of O-GlcNAcylation homeostasis are still unknown.

A buffer system has been proposed to maintain this intracellular O-GlcNAcylation balance through the mutual expression and activity regulation between OGT and OGA (Yang & Qian, 2017). Increased OGT activity leads to a concomitant increase in OGA activity and vice versa, thus buffering cells from drastic O-GlcNAcylation transformation (Burén et al., 2016). Furthermore, it was suggested that OGA functioned as a coactivator to upregulate OGT gene transcription by binding to the transcription factor, C/EBP-β (Qian et al., 2018) and the absence or inhibition of OGT reduced OGA expression (Kazemi et al., 2010). OGA’s pharmacological inhibition increased OGA gene transcription (Zhang et al., 2014). Park et al. (2017) reported that under high O-GlcNAcylation conditions, OGT introns were retained and OGT expression was suppressed, whereas, under low O-GlcNAcylation conditions, OGT introns were removed and OGT expression increased. A conservative OGT intron clipping muffler (ISS) was necessary for the retention of OGT introns, whereas the absence of an ISS disrupted OGT intron retention, and responses to metabolic conditions, thereby altering O-GlcNAcylation levels in cells (Park et al., 2017).

**Figure 6** O-GlcNAcylation is associated with multiple signaling pathways. O-GlcNAcylation directly or indirectly regulates different signaling pathways affecting a variety of cellular processes.

DOI: 10.7717/peerj.11443/fig-6
et al., 2017). This suggested a self-compensating mechanism to maintain the vital balance, however, autoregulation may be limited to tolerating fluctuations in moderate or acute stimuli (Yang & Qian, 2017) (Fig. 7). Typically the exposure to prolonged, severe, and chronic stress causes the O-GlcNAc homeostatic balance to be disrupted, generating physiological and metabolic cellular changes, and potentially impacting survival or resulting in specific cellular damage.

CONCLUSIONS
Remarkable advances in research have been made over the past 40 years in our understanding of the various biological functions underpinning O-GlcNAcylation. It is our current understanding that O-GlcNAcylation regulates some aspects of cellular metabolism. Our understanding of the cellular metabolic regulation in normal and disease states is increased by identifying O-GlcNAcylation mechanisms in functional proteins. There is considerable evidence to suggest that O GlcNAcylation is implicated in metabolic reprogramming, however, several molecular regulatory mechanisms require elucidation. Common O-GlcNAc signals in pathological states may become viable therapeutic targets. In this review, we summarized the roles of O-GlcNAcylation in glucose, lipid, biological oxidation, and amino acid metabolism, and discussed its role in physiological processes, such as cellular nutrient sensing, and homeostasis maintenance. O-GlcNAcylation typically acts as a regulator in multiple metabolic processes and pathways, signifying an influencing molecular interconnectivity between these processes. This notion provides us with an integrated understanding and foundation of potential communication exchanges between these metabolic pathways, all mediated by
O-GlcNAcylation. O-GlcNAcylation response to stress may not be limited to key proteins, but more to the regulation of signaling and metabolism. Additional studies are needed to determine how O-GlcNAcylation coordinates and integrates related cellular and molecular networks.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
The authors received no funding for this work.

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Hongshuo Zhang conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Zhen Li performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Yufei Wang performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Ying Kong conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:
This work is a literature review without data.

REFERENCES

Abramowitz LK, Harly C, Das A, Bhandoola A, Hanover JA. 2019. Blocked O-GlcNAc cycling disrupts mouse hematopoietic stem cell maintenance and early T cell development. Scientific Reports 9(1):12569 DOI 10.1038/s41598-019-48991-8.

Akella NM, Ciraku L, Reginato MJ. 2019. Fueling the fire: emerging role of the hexosamine biosynthetic pathway in cancer. BMC Biology 17(1):52 DOI 10.1186/s12915-019-0671-3.

Altman BJ, Stine ZE, Dang CV. 2016. From Krebs to clinic: glutamine metabolism to cancer therapy. Nature Reviews Cancer 16(10):619–634 DOI 10.1038/nrc.2016.71.

Anthonisen EH, Berven L, Holm S, Nygård M, Nebb HI, Gronning-Wang LM. 2010. Nuclear receptor liver X receptor is O-GlcNAc-modified in response to glucose. The Journal of Biological Chemistry 285(3):1607–1615 DOI 10.1074/jbc.M109.082685.

Arvanitis DL, Arvanitis LD, Panourias IG, Kitsoulis P, Kanavaros P. 2005. Mitochondria-rich normal, metaplastic, and neoplastic cells show overexpression of the epitope H recognized by the monoclonal antibody H. Pathology, Research and Practice 201(4):319–324 DOI 10.1016/j.prp.2005.01.007.

Augustin R. 2010. The protein family of glucose transport facilitators: it’s not only about glucose after all. IUBMB Life 62(5):315–333 DOI 10.1002/iub.315.
Bacigalupa ZA, Bhadiadra CH, Reginato MJ. 2018. O-GlcNAcylation: key regulator of glycolytic pathways. Journal of Bioenergetics and Biomembranes 50(3):189–198 DOI 10.1007/s10863-018-9742-3.

Baldini SF, Steenackers A, Olivier-Van Stichelen S, Mir AM, Mortuaire M, Lefebvre T, Guinez C. 2016a. Glucokinase expression is regulated by glucose through O-GlcNAc glycosylation. Biochemical and Biophysical Research Communications 478(2):942–948 DOI 10.1016/j.bbrc.2016.08.056.

Baldini SF, Wavelet C, Hainault I, Guinez C, Lefebvre T. 2016b. The nutrient-dependent O-GlcNAc modification controls the expression of liver fatty acid synthase. Journal of Molecular Biology 428(16):3295–3304 DOI 10.1016/j.jmb.2016.04.035.

Banerjee PS, Ma J, Hart GW. 2015. Diabetes-associated dysregulation of O-GlcNAcylation in rat cardiac mitochondria. Proceedings of the National Academy of Sciences of the United States of America 112(19):6050–6055 DOI 10.1073/pnas.1424017112.

Bergström J, Fürst P, Norée LO, Vinnars E. 1974. Intracellular free amino acid concentration in human muscle tissue. Journal of Applied Physiology 36(6):693–697 DOI 10.1152/jappl.1974.36.6.693.

Bindesbøll C, Fan Q, Nørgaard RC, MacPherson L, Ruan HB, Wu J, Pedersen TÅ, Steffensen KR, Yang X, Matthews J, Mandrup S, Nebb HI, Grønning-Wang LM. 2015. Liver X receptor regulates hepatic nuclear O-GlcNAc signaling and carbohydrate responsive element-binding protein activity. Journal of Lipid Research 56(4):771–785 DOI 10.1194/jlr.M049130.

Bond MR, Hanover JA. 2015. A little sugar goes a long way: the cell biology of O-GlcNAc. The Journal of Cell Biology 208(7):869–880 DOI 10.1083/jcb.201501101.

Burén S, Gomes AL, Teijeiro A, Fawal MA, Yilmaz M, Tummala KS, Perez M, Rodrigue-Justo M, Campos-Olivas R, Megías D, Djouder N. 2016. Regulation of OGT by URI in response to glucose confers c-MYC-dependent survival mechanisms. Cancer Cell 30(2):290–307 DOI 10.1016/j.ccell.2016.06.023.

Buse MG, Robinson KA, Marshall BA, Hresko RC, Mueckler MM. 2002. Enhanced O-GlcNAc protein modification is associated with insulin resistance in GLUT1-overexpressing muscles. American Journal of Physiology-Endocrinology and Metabolism 283(2):E241–E250 DOI 10.1152/ajpendo.00060.2002.

Cao W, Cao J, Huang J, Yao J, Yan G, Xu H, Yang P. 2013. Discovery and confirmation of O-GlcNAcylated proteins in rat liver mitochondria by combination of mass spectrometry and immunological methods. PLOS ONE 8(10):e76399 DOI 10.1371/journal.pone.0076399.

Cha MY, Cho HJ, Kim C, Jung YO, Kang MJ, Murray ME, Hong HS, Choi YJ, Choi H, Kim DK, Choi H, Kim J, Dickson DW, Song HK, Cho JW, Yi EC, Kim J, Jin SM, Mook-Jung I. 2015. Mitochondrial ATP synthase activity is impaired by suppressed O-GlcNAcylation in Alzheimer’s disease. Human Molecular Genetics 24(22):6492–6504 DOI 10.1093/hmg/ddv358.

Chaiyawat P, Chokchaichamnanakit D, Lirdprapamongkol K, Srisosmp S, Svasti J, Champattanachai V. 2015. Alteration of O-GlcNAcylation affects serine phosphorylation and regulates gene expression and activity of pyruvate kinase M2 in colorectal cancer cells. Oncology Reports 34(4):1933–1942 DOI 10.3892/or.2015.4178.

Chang Y, Wang J, Lu X, Thewke DP, Mason RJ. 2005. KGF induces lipogenic genes through a PI3K and JNK/SREBP-1 pathway in H292 cells. Journal of Lipid Research 46(12):2624–2635 DOI 10.1194/jlr.M500154-JLR200.

Chiaradonna F, Ricciardiello F, Palorini R. 2018. The nutrient-sensing hexosamine biosynthetic pathway as the hub of cancer metabolic rewiring. Cells 7(6):53 DOI 10.3390/cells7060053.
Chou TY, Hart GW. 2001. O-linked N-acetylglucosamine and cancer: messages from the glycosylation of c-Myc. *Advances in Experimental Medicine and Biology* **491**:413–418 DOI 10.1007/978-1-4615-1267-7_26.

Chou TY, Hart GW, Dang CV. 1995. c-Myc is glycosylated at threonine 58, a known phosphorylation site and a mutational hot spot in lymphomas. *The Journal of Biological Chemistry* **270**(32):18961–18965 DOI 10.1074/jbc.270.32.18961.

Cieniewski-Bernard C, Bastide B, Lefebvre T, Lemoine J, Mounier Y, Michalski JC. 2004. Identification of O-linked N-acetylglucosamine proteins in rat skeletal muscle using two-dimensional gel electrophoresis and mass spectrometry. *Molecular & Cellular Proteomics: MCP* **3**(6):577–585 DOI 10.1074/mcp.M400024-MCP200.

Clark PM, Dweck JF, Mason DE, Hart CR, Buck SB, Peters EC, Agnew BJ, Hsieh-Wilson LC. 2008. Direct in-gel fluorescence detection and cellular imaging of O-GlcNAc-modified proteins. *Journal of the American Chemical Society* **130**(35):11576–11577 DOI 10.1021/ja8030467.

Cohen JC, Horton JD, Hobbs HH. 2011. Human fatty liver disease: old questions and new insights. *Science* **332**(6037):1519–1523 DOI 10.1126/science.1204265.

Dang CV, Le A, Gao P. 2009. MYC-induced cancer cell energy metabolism and therapeutic opportunities. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* **15**(21):6479–6483 DOI 10.1158/1078-0432.CCR-09-0889.

DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB. 2007. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **104**(49):19345–19350 DOI 10.1073/pnas.0709747104.

Dela Justina V, Gonçalves JS, de Freitas RA, Fonseca AD, Volpato GT, Tostes RC, Carneiro FS, Lima VV, Giachini FR. 2017. Increased O-Linked N-Acetylglucosamine modification of NF-KB and augmented cytokine production in the placenasts from hyperglycemic rats. *Inflammation* **40**(5):1773–1781 DOI 10.1007/s10753-017-0620-7.

Dentin R, Hedrick S, Xie J, Yates J III, Montminy M. 2008. Hepatic glucose sensing via the CREB coactivator CRTC2. *Science* **319**(5868):1402–1405 DOI 10.1126/science.1151363.

Ding F, Yu L, Wang M, Xu S, Xia Q, Fu G. 2013. O-GlcNAcylation involvement in high glucose-induced cardiac hypertrophy via ERK1/2 and cyclin D2. *Amino Acids* **45**(2):339–349 DOI 10.1007/s00726-013-1504-2.

Efeyan A, Comb WC, Sabatini DM. 2015. Nutrient-sensing mechanisms and pathways. *Nature* **517**(7534):302–310 DOI 10.1038/nature14190.

Fang X, Yu SX, Lu Y, Bast RC, Woodgett JR, Mills GB. 2000. Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. *Proceedings of the National Academy of Sciences of the United States of America* **97**(22):11960–11965 DOI 10.1073/pnas.220413597.

Ferrer CM, Lynch TP, Sodi VL, Falcone JN, Schwab LP, Peacock DL, Vocadlo DJ, Seagroves TN, Reginato MJ. 2014. O-GlcNAcylation regulates cancer metabolism and survival stress signaling via regulation of the HIF-1 pathway. *Molecular Cell* **54**(5):820–831 DOI 10.1016/j.molcel.2014.04.026.

Fricovsky ES, Suarez J, Ihm SH, Scott BT, Suarez-Ramirez JA, Banerjee I, Torres-Gonzalez M, Wang H, Ellrott I, Maya-Ramos L, Villarreal F, Dillmann WH. 2012. Excess protein O-GlcNAcylation and the progression of diabetic cardiomyopathy. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **303**(7):R689–R699 DOI 10.1152/ajpregu.00548.2011.

Fülöp N, Feng W, Xing D, He K, Nótt LG, Brochs CA, Marchase RB, Miller AP, Chatham JC. 2008. Aging leads to increased levels of protein O-linked N-acetylglucosamine in heart, aorta,
brain and skeletal muscle in Brown-Norway rats. *Biogerontology* 9(3):139
DOI 10.1007/s10522-007-9123-5.

Gao S, Miao Y, Liu Y, Liu X, Fan X, Lin Y, Qian P, Zhou J, Dai Y, Xia L, Zhu P, Zhu J. 2019. Reciprocal regulation between O-GlcNAcylation and β-Catenin facilitates cell viability and inhibits apoptosis in liver cancer. *DNA and Cell Biology* 38(4):286–296
DOI 10.1089/dna.2018.4447.

Gawlowski T, Suarez J, Scott B, Torres-Gonzalez M, Wang H, Schwappacher R, Han X, Yates JR III, Hoshijima M, Dillmann W. 2012. Modulation of dynamin-related protein 1 (DRP1) function by increased O-linked-β-N-acetylglucosamine modification (O-GlcNAc) in cardiac myocytes. *The Journal of Biological Chemistry* 287(35):30024–30034
DOI 10.1074/jbc.M112.390682.

Gibb AA, Lorkiewicz PK, Zheng YT, Zhang X, Bhatnagar A, Jones SP, Hill BG. 2017. Integration of flux measurements to resolve changes in anabolic and catabolic metabolism in cardiac myocytes. *The Biochemical Journal* 474(16):2785–2801
DOI 10.1042/BCJ20170474.

Gu Y, Ande SR, Mishra S. 2011. Altered O-GlcNAc modification and phosphorylation of mitochondrial proteins in myoblast cells exposed to high glucose. *Archives of Biochemistry and Biophysics* 505(1):98–104
DOI 10.1016/j.abb.2010.09.024.

Guinez C, Filhoulaud G, Rayah-Benhamed F, Marmier S, Dubuquoy C, Dentin R, Moldes M, Burnol AF, Yang X, Lefebvre T, Girard J, Postic C. 2011. O-GlcNAcylation increases ChREBP protein content and transcriptional activity in the liver. *Diabetes* 60(5):1399–1413
DOI 10.2337/db10-0452.

Han X, Li X, Liu H, Zhang H, Li A, Dong M, Xu Y, Yan B, Sui L, Kong Y. 2019. O-GlcNAc modification influences endometrial receptivity by promoting endometrial cell proliferation, migration and invasion. *Oncology Reports* 42(5):2065–2074
DOI 10.3892/or.2019.7317.

Hanover JA, Chen W, Bond MR. 2018. O-GlcNAc in cancer: an oncometabolism-fueled vicious cycle. *Journal of Bioenergetics and Biomembranes* 50(3):155–173
DOI 10.1007/s10863-018-9751-2.

Harosh-Davidovich SB, Khalaila I. 2018. O-GlcNAcylation affects β-catenin and E-cadherin expression, cell motility and tumorigenicity of colorectal cancer. *Experimental Cell Research* 364(1):42–49
DOI 10.1016/j.yexcr.2018.01.024.

Hart GW. 2019. Nutrient regulation of signaling and transcription. *The Journal of Biological Chemistry* 294(7):2211–2231
DOI 10.1074/jbc.AW119.003226.
Hart GW, Housley MP, Slawson C. 2007. Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. *Nature* **446**(7139):1017–1022 DOI 10.1038/nature05815.

Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O. 2011. Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. *Annual Review of Biochemistry* **80**(1):825–858 DOI 10.1146/annurev-biochem-060608-102511.

Holthuis JC, Menon AK. 2014. Lipid landscapes and pipelines in membrane homeostasis. *Nature* **510**(7503):48–57 DOI 10.1038/nature13474.

Housley MP, Udeshi ND, Rodgers JT, Shabanowitz J, Puigserver P, Hunt DF, Hart GW. 2009. A PGC-1alpha-O-GlcNAc transferase complex regulates FoxO transcription factor activity in response to glucose. *The Journal of Biological Chemistry* **284**(8):5148–5157 DOI 10.1074/jbc.M808890200.

Hu J, Gao Q, Yang Y, Xia J, Zhang W, Chen Y, Zhou Z, Chang L, Hu Y, Zhou H, Liang L, Li X, Long Q, Wang K, Huang A, Tang N. 2021. Hexosamine biosynthetic pathway promotes the antiviral activity of SAMHD1 by enhancing O-GlcNAc transferase-mediated protein O-GlcNAcylation. *Theranostics* **11**(2):805–823 DOI 10.7150/thno.50230.

Hu Y, Suarez J, Fricovsky E, Wang H, Scott BT, Trauger SA, Han W, Hu Y, Oyeye MO, Dillmann WH. 2009. Increased enzymatic O-GlcNAcylation of mitochondrial proteins impairs mitochondrial function in cardiac myocytes exposed to high glucose. *The Journal of Biological Chemistry* **284**(1):547–555 DOI 10.1074/jbc.M808518200.

Itkonen HM, Gorad SS, Duveau DY, Martin SE, Barkovskaya A, Bathen TF, Moestue SA, Mills IG. 2016. Inhibition of O-GlcNAc transferase activity reprograms prostate cancer cell metabolism. *Oncotarget* **7**(11):12464–12476 DOI 10.18632/oncotarget.7039.

Jang H, Kim TW, Yoon S, Choi SY, Kang TW, Kim SY, Kwon YW, Cho EJ, Youn HD. 2012. O-GlcNAc regulates pluripotency and reprogramming by directly acting on core components of the pluripotency network. *Cell Stem Cell* **11**(1):62–74 DOI 10.1016/j.stem.2012.03.001.

Johnson SC, Rabinovitch PS, Kaebelstein M. 2013. mTOR is a key modulator of ageing and age-related disease. *Nature* **493**(7432):338–345 DOI 10.1038/nature11861.

Jóźwiak P, Forma E, Bryś M, Krześlak A. 2014. O-GlcNAcylation and metabolic reprogramming in cancer. *Frontiers in Endocrinology* **5**:145 DOI 10.3389/fendo.2014.00145.

Kang JG, Park SY, Ji S, Jang I, Park S, Kim HS, Kim SM, Yook JJ, Park YI, Roth J, Cho JW. 2009. O-GlcNAc protein modification in cancer cells increases in response to glucose deprivation through glycogen degradation. *The Journal of Biological Chemistry* **284**(50):34777–34784 DOI 10.1074/jbc.M109.026351.

Kazemi Z, Chang H, Haserodt S, McKen C, Zachara NE. 2010. O-linked beta-N-acetylglucosamine (O-GlcNAc) regulates stress-induced heat shock protein expression in a GSK-3beta-dependent manner. *The Journal of Biological Chemistry* **285**(50):39096–39107 DOI 10.1074/jbc.M110.024173.

Kornfeld S, Kornfeld R, Neufeld EF, O'Brien PJ. 1964. The feedback control of sugar nucleotide biosynthesis in liver. *Proceedings of the National Academy of Sciences* **52**(2):371–379 DOI 10.1073/pnas.52.2.371.

Li MD, Vera NB, Yang Y, Zhang B, Ni W, Ziso-Qejvanaj E, Ding S, Zhang K, Yin R, Wang S, Zhou X, Fang EX, Xu T, Erion DM, Yang X. 2018. Adipocyte OGT governs diet-induced hyperphagia and obesity. *Nature Communications* **9**(1):5103 DOI 10.1038/s41467-018-07461-x.

Love DC, Hanover JA. 2005. The hexosamine signaling pathway: deciphering the "O-GlcNAc code". *Science's STKE : Signal Transduction Knowledge Environment* **2005**(312):re13 DOI 10.1126/stke.3122005re13.
Lozano L, Lara-Lemus R, Zenteno E, Alvarado-Vásquez N. 2014. The mitochondrial O-linked N-acetylglucosamine transferase (mOGT) in the diabetic patient could be the initial trigger to develop Alzheimer disease. *Experimental Gerontology* 58(Suppl. 1):198–202 DOI 10.1016/j.exger.2014.08.008.

Ma J, Banerjee P, Whelan SA, Liu T, Wei AC, Ramirez-Correa G, McComb ME, Costello CE, O’Rourke B, Murphy A, Hart GW. 2016. Comparative proteomics reveals dysregulated mitochondrial O-GlcNAcylation in diabetic hearts. *Journal of Proteome Research* 15(7):2254–2264 DOI 10.1021/acs.jproteome.6b00250.

Ma J, Liu T, Wei AC, Banerjee P, O’Rourke B, Hart GW. 2015. O-GlcNAcomic profiling identifies widespread O-linked β-N-acetylglucosamine modification (O-GlcNAcylation) in oxidative phosphorylation system regulating cardiac mitochondrial function. *The Journal of Biological Chemistry* 290(49):29141–29153 DOI 10.1074/jbc.M115.691741.

Ma X, Liu H, Li J, Wang Y, Ding YH, Shen H, Yang Y, Sun C, Huang M, Tu Y, Liu Y, Zhao Y, Dong MQ, Xu P, Tang TS, Guo C. 2017. Polη O-GlcNAcylation governs genome integrity during translesion DNA synthesis. *Nature Communications* 8(1):1941 DOI 10.1038/s41467-017-02164-1.

Manning BD, Cantley LC. 2007. AKT/PKB signaling: navigating downstream. *Cell* 129(7):1261–1274 DOI 10.1016/j.cell.2007.06.009.

Marshall S, Bacote V, Traxinger RR. 1991. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. *The Journal of Biological Chemistry* 266(8):4706–4712 DOI 10.1016/S0021-9258(19)67706-9.

Moriwaki K, Asahi M. 2017. Augmented TME O-GlcNAcylation promotes tumor proliferation through the inhibition of p38 MAPK. *Molecular Cancer Research: MCR* 15(9):1287–1298 DOI 10.1158/1541-7786.MCR-16-0499.

Nie H, Ju H, Fan J, Shi X, Cheng Y, Cang X, Zheng Z, Duan X, Yi W. 2020. O-GlcNAcylation of PGK1 coordinates glycolysis and TCA cycle to promote tumor growth. *Nature Communications* 11(1):36 DOI 10.1038/s41467-019-13601-8.

Nugent BM, Bale TL. 2015. The omniscient placenta: metabolic and epigenetic regulation of fetal programming. *Frontiers in Neuroendocrinology* 39:28–37 DOI 10.1016/j.yfrne.2015.09.001.

Ohashi N, Morino K, Ida S, Sekine O, Lemecha M, Kume S, Park SY, Choi CS, Ugi S, Maegawa H. 2017. Pivotal role of O-GlcNAc modification in cold-induced thermogenesis by brown adipose tissue through mitochondrial biogenesis. *Diabetes* 66(9):2351–2362 DOI 10.2337/db16-1427.

Olson AK, Bouchard B, Zhu WZ, Chatham JC, Des Rosiers C. 2020. First characterization of glucose flux through the hexosamine biosynthesis pathway (HBP) in ex vivo mouse heart. *The Journal of Biological Chemistry* 295(7):2018–2033 DOI 10.1074/jbc.RA119.010565.

Ong Q, Han W, Yang X. 2018. O-GlcNAc as an integrator of signaling pathways. *Frontiers in Endocrinology* 9:599 DOI 10.3389/fendo.2018.00599.

Onodera Y, Nam JM, Bissell MJ. 2014. Increased sugar uptake promotes oncogenesis via EPAC/RAP1 and O-GlcNAc pathways. *The Journal of Clinical Investigation* 124(1):367–384 DOI 10.1172/JCI63146.

Park MJ, Kim DI, Lim SK, Choi JH, Han HJ, Yoon KC, Park SH. 2014a. High glucose-induced O-GlcNAcylated carbohydrate response element-binding protein (ChREBP) mediates mesangial cell lipogenesis and fibrosis: the possible role in the development of diabetic nephropathy. *The Journal of Biological Chemistry* 289(19):13519–13530 DOI 10.1074/jbc.M113.530139.
Park S, Pak J, Jang I, Cho JW. 2014b. Inhibition of mTOR affects protein stability of OGT. Biochemical and Biophysical Research Communications 453(2):208–212 DOI 10.1016/j.bbrc.2014.05.047.

Park SK, Zhou X, Pendleton KE, Hunter OV, Kohler JJ, O'Donnell KA, Conrad NK. 2017. A conserved splicing silencer dynamically regulates O-GlcNAc transferase intron retention and O-GlcNAc homeostasis. Cell Reports 20(5):1088–1099 DOI 10.1016/j.celrep.2017.07.017.

Park SY, Ryu J, Lee W. 2005. O-GlcNAc modification on IRS-1 and Akt2 by PUGNAc inhibits their phosphorylation and induces insulin resistance in rat primary adipocytes. Experimental & Molecular Medicine 37(3):220–229 DOI 10.1038/emm.2005.30.

Parker GJ, Lund KC, Taylor RP, McClain DA. 2003. Insulin resistance of glycogen synthase mediated by O-linked N-acetylglucosamine. The Journal of Biological Chemistry 278(12):10022–10027 DOI 10.1074/jbc.M207787200.

Peng C, Zhu Y, Zhang W, Liao Q, Chen Y, Zhao X, Guo Q, Shen P, Zhen B, Qian X, Yang D, Zhang JS, Xiao D, Qin W, Pei H. 2017. Regulation of the hippo-YAP pathway by glucose sensor O-GlcNAc. Molecular Cell 68(3):591–604.e5 DOI 10.1016/j.molcel.2017.10.010.

Petrus P, Lecoutre S, Dollet L, Wiel C, Sulen A, Gao H, Tavira B, Laurenciukiene J, Rooyackers O, Checa A, Douagi I, Wheelock CE, Arner P, Bergo MO, Edgar L, Choudhury RP, Aouadi M, Krook A, Rydén M. 2020. Glutamine links obesity to inflammation in human white adipose tissue. Cell Metabolism 31(2):375–390.e11 DOI 10.1016/j.cmet.2019.11.019.

Qian K, Wang S, Fu M, Zhou J, Singh JP, Li MD, Yang Y, Zhang K, Wu J, Nie Y, Ruan HB, Yang X. 2018. Transcriptional regulation of O-GlcNAc homeostasis is disrupted in pancreatic cancer. The Journal of Biological Chemistry 293(36):13989–14000 DOI 10.1074/jbc.RA118.004709.

Ramakrishnan P, Clark PM, Mason DE, Peters EC, Hsieh-Wilson LC, Baltimore D. 2013. Activation of the transcriptional function of the NF-κB protein c-Rel by O-GlcNAc glycosylation. Science Signaling 6(290):ra75 DOI 10.1126/scisignal.2004097.

Rao FV, Schüttelkopf AW, Dorfmueller HC, Ferenbach AT, Navratilova I, van Aalten DM. 2013. Structure of a bacterial putative acetyltransferase defines the fold of the human O-GlcNAcase C-terminal domain. Open Biology 3(10):130021 DOI 10.1098/rsob.130021.

Rao X, Duan X, Mao W, Li X, Li Z, Li Q, Zheng Z, Xu H, Chen M, Wang PG, Wang Y, Shen B, Yi W. 2015. O-GlcNAcylation of G6PD promotes the pentose phosphate pathway and tumor growth. Nature Communications 6(1):8468 DOI 10.1038/ncomms9468.

Ren N, Ji M, Tokar EJ, Busch EL, Xu X, Lewis D, Li X, Jin A, Zhang Y, Wu W, Huang W, Li L, Fargo DC, Keku TO, Sandler RS, Li X. 2017. Haploinsufficiency of SIRT1 enhances glutamine metabolism and promotes cancer development. Current Biology: CB 27(4):483–494 DOI 10.1016/j.cub.2016.12.047.

Ruan HB, Han X, Li MD, Singh JP, Qian K, Azarhoush S, Zhao L, Bennett AM, Samuel VT, Wu J, Yates JR, Yang X. 2012. O-GlcNAc transferase/host cell factor C1 complex regulates gluconeogenesis by modulating PGC-1α stability. Cell Metabolism 16(2):226–237 DOI 10.1016/j.cmet.2012.07.006.

Ruan HB, Singh JP, Li MD, Wu J, Yang X. 2013. Cracking the O-GlcNAc code in metabolism. Trends in Endocrinology and Metabolism: TEM 24(6):301–309 DOI 10.1016/j.tem.2013.02.002.

Sacoman JL, Dagda RY, Burnham-Marusch A, Dagda RK, Berninsone PM. 2017. Mitochondrial O-GlcNAc transferase (mOGT) regulates mitochondrial structure, function, and survival in HeLa cells. The Journal of Biological Chemistry 292(11):4499–4518 DOI 10.1074/jbc.M116.726752.
Schwartz MW, Seeley RJ, Tschöp MH, Woods SC, Morton GJ, Myers MG, D’Alessio D. 2013. Cooperation between brain and islet in glucose homeostasis and diabetes. Nature 503(7474):59–66 DOI 10.1038/nature12709.

Semenza GL. 2010. HIF-1: upstream and downstream of cancer metabolism. Current Opinion in Genetics & Development 20(1):51–56 DOI 10.1016/j.gde.2009.10.009.

Sharma NS, Saluja AK, Banerjee S. 2018. “Nutrient-sensing” and self-renewal: O-GlcNAc in a new role. Journal of Bioenergetics and Biomembranes 50(3):205–211 DOI 10.1007/s10863-017-9735-7.

Singh JP, Qian K, Lee J-S, Zhou J, Jiang M, Ruan H-B, Li M-D, Zhang K, Ding Z, Lee P, Singh K, Wu J, Herzog RI, Kaech S, Wendel H-G, Yates JR III, Han W, Sherwin RS, Nie Y, Yang X. 2020. O-GlcNAcase targets pyruvate kinase M2 to regulate tumor growth. Oncogene 39(3):560–573 DOI 10.1038/s41388-019-0975-3.

Slawson C, Copeland RJ, Hart GW. 2010. O-GlcNAc signaling: a metabolic link between diabetes and cancer. Trends in Biochemical Sciences 35(10):547–555 DOI 10.1016/j.tibs.2010.04.005.

Sodi VL, Bacigalupa ZA, Ferrer CM, Lee JV, Gocal WA, Mukhopadhyay D, Wellen KE, Ivan M, Reginato MJ. 2018. Nutrient sensor O-GlcNAc transferase controls cancer lipid metabolism via SREBP-1 regulation. Oncogene 37(7):924–934 DOI 10.1038/onc.2017.395.

Soga T. 2013. Cancer metabolism: key players in metabolic reprogramming. Cancer Science 104(3):275–281 DOI 10.1111/cas.12085.

Stegen S, van Gastel N, Eelen G, Ghesquière B, D’Anna F, Thienpont B, Goveia J, Torrekens S, Van Looveren R, Luyten FP, Maxwell PH, Wielockx B, Lambrechts D, Fendt SM, Carmeliet P, Carmeliet G. 2016. HIF-1α promotes glutamine-mediated redox homeostasis and glycogen-dependent bioenergetics to support postimplantation bone cell survival. Cell Metabolism 23(2):265–279 DOI 10.1016/j.cmet.2016.01.002.

Sun QH, Wang YS, Liu G, Zhou HL, Jian YP, Liu MD, Zhang D, Ding Q, Zhao RX, Chen JF, Li YN, Liang J, Li YL, Quan CS, Xu ZX. 2020. Enhanced O-linked GlcNAcylation in Crohn’s disease promotes intestinal inflammation. EBioMedicine 53(10080):102693 DOI 10.1016/j.ebiom.2020.102693.

Swamy M, Pathak S, Grzes KM, Damerow S, Sinclair LV, van Aalten DM, Cantrell DA. 2016. Glucose and glutamine fuel protein O-GlcNAcylation to control T cell self-renewal and malignancy. Nature Immunology 17(6):712–720 DOI 10.1038/ni.3439.

Tan EP, Villar MT, E L, Lu J, Selfridge JE, Artigues A, Swerdlow RH, Slawson C. 2014. Altering O-linked β-N-acetylglucosamine cycling disrupts mitochondrial function. The Journal of Biological Chemistry 290(36):14940–14962 DOI 10.1074/jbc.M113.499965.

Taylor RP, Parker GJ, Hazel MW, Soesanto Y, Fuller W, Yazzie MJ, McClain DA. 2008. Glucose deprivation stimulates O-GlcNAc modification of proteins through up-regulation of O-linked N-acetylglucosaminyltransferase. The Journal of Biological Chemistry 283(10):6050–6057 DOI 10.1074/jbc.M707328200.

Teo CF, Wollaston-Hayden EE, Wells L. 2010. Hexosamine flux, the O-GlcNAc modification, and the development of insulin resistance in adipocytes. Molecular and Cellular Endocrinology 318(1–2):44–53 DOI 10.1016/j.mce.2009.09.022.
Toleman C, Paterson AJ, Whisenhunt TR, Kudlow JE. 2004. Characterization of the histone acetyltransferase (HAT) domain of a bifunctional protein with activable O-GlcNAcase and HAT activities. *The Journal of Biological Chemistry* **279**(51):53665–53673 DOI 10.1074/jbc.M410406200.

Trapannone R, Mariappa D, Ferenbach AT, van Aalten DM. 2016. Nucleocytoplasmic human O-GlcNAc transferase is sufficient for O-GlcNAcylation of mitochondrial proteins. *The Biochemical Journal* **473**(12):1693–1702 DOI 10.1042/BCJ20160092.

Traxinger RR, Marshall S. 1991. Coordinated regulation of glutamine: fructose-6-phosphate amidotransferase activity by insulin, glucose, and glutamine. Role of hexosamine biosynthesis in enzyme regulation. *The Journal of Biological Chemistry* **266**(16):10148–10154 DOI 10.1016/S0021-9258(18)99202-1.

van Meer G, Voelker DR, Feigenson GW. 2008. Membrane lipids: where they are and how they behave. *Nature Reviews Molecular Cell Biology* **9**(2):112–124 DOI 10.1038/nrm2330.

Vander Heiden MG, Cantley LC, Thompson CB. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* **324**(5930):1029–1033 DOI 10.1126/science.1160809.

Vocadlo DJ. 2012. O-GlcNAc processing enzymes: catalytic mechanisms, substrate specificity, and enzyme regulation. *Current Opinion in Chemical Biology* **16**(5–6):488–497 DOI 10.1016/j.cbpa.2012.10.021.

Vosseller K, Wells L, Lane MD, Hart GW. 2002. Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. *Proceedings of the National Academy of Sciences of the United States of America* **99**(8):5313–5318 DOI 10.1073/pnas.072072399.

Wang J, Wang Z, Yuan J, Wang J, Shen X. 2020. The positive feedback between ACSL4 expression and O-GlcNAcylation contributes to the growth and survival of hepatocellular carcinoma. *Sedentary Life and Nutrition* **12**(9):7786–7800 DOI 10.18632/aging.103092.

Wang S, Huang X, Sun D, Xin X, Pan Q, Peng S, Liang Z, Luo C, Yang Y, Jiang H, Huang M, Chai W, Ding J, Geng M. 2012. Extensive crosstalk between O-GlcNAcylation and phosphorylation regulates Akt signaling. *PLOS ONE* **7**(5):e37427 DOI 10.1371/journal.pone.0037427.

Wang X, Jin H. 2010. The epigenetic basis of the Warburg effect. *Epigenetics* **5**(7):566–568 DOI 10.4161/epi.5.7.12662.

Wang Y, Liu J, Jin X, Zhang D, Li D, Hao F, Feng Y, Gu S, Meng F, Tian M, Zheng Y, Xin L, Zhang X, Han X, Aravind L, Wei M. 2017. O-GlcNAcylation destabilizes the active tetrameric PKM2 to promote the Warburg effect. *Proceedings of the National Academy of Sciences of the United States of America* **114**(52):13732–13737 DOI 10.1073/pnas.1704145115.

Wang Z, Pandey A, Hart GW. 2007. Dynamic interplay between O-linked N-acetylglucosaminylation and glycogen synthase kinase-3-dependent phosphorylation. *Molecular & Cellular Proteomics* **6**(8):1365–1379 DOI 10.1074/mcp.M600453-MCP200.

Whelan SA, Dias WB, Thiruneelakantapillai L, Lane MD, Hart GW. 2010. Regulation of insulin receptor substrate 1 (IRS-1)/AKT kinase-mediated insulin signaling by O-Linked beta-N-acetylglucosamine in 3T3-L1 adipocytes. *The Journal of Biological Chemistry* **285**(8):5204–5211 DOI 10.1074/jbc.M109.077818.

Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, Thompson CB. 2008. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proceedings of the
Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu J, Djoumbou Y, Mandal R, Aziat F, Dong F, Bouatra S, Sinelnikov I, Arndt D, Xia J, Liu P, Yallou F, Bjorndahl T, Perez-Pineiro R, Eisner R, Allen F, Neveu V, Greiner R, Scalbert A. 2013. HMDB 3.0—the human metabolome database in 2013. *Nucleic Acids Research* **41**(D1):D801–D807. DOI 10.1093/nar/gks1065.

Yang WH, Park SY, Nam HW, Kim DH, Kang JG, Kang ES, Kim YS, Lee HC, Kim KS, Cho JW. 2008a. NFkappaB activation is associated with its O-GlcNAcylation state under hyperglycemic conditions. *Proceedings of the National Academy of Sciences* **105**(45):17345–17350. DOI 10.1073/pnas.0806198105.

Yang X, Ongusaha PP, Miles PD, Havstad JC, Zhang F, So WV, Kudlow JE, Michell RH, Olefsky JM, Field SJ, Evans RM. 2008b. Phosphoinositide signalling links O-GlcNAc transferase to insulin resistance. *Nature* **451**(7181):964–969. DOI 10.1038/nature06668.

Yang X, Qian K. 2017. Protein O-GlcNAcylation: emerging mechanisms and functions. *Nature Reviews Molecular Cell Biology* **18**(7):452–465. DOI 10.1038/nrm.2017.22.

Yehezkel G, Cohen L, Kliger A, Manor E, Khalaila I. 2012. O-linked β-N-acetylglucosaminylation (O-GlcNAcylation) in primary and metastatic colorectal cancer clones and effect of N-acetyl-β-D-glucosaminidase silencing on cell phenotype and transcriptome. *The Journal of Biological Chemistry* **287**(34):28755–28769. DOI 10.1074/jbc.M112.345546.

Zachara NE, Hart GW. 2004. O-GlcNAc a sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress. *Biochimica et Biophysica Acta* **1673**(1–2):13–28. DOI 10.1016/j.bbagen.2004.03.016.

Zhang B, Zhou P, Li X, Shi Q, Li D, Ju X. 2017. Bitterness in sugar: O-GlcNAcylation aggravates pre-B acute lymphocytic leukemia through glycolysis via the PI3K/Akt/c-Myc pathway. *American Journal of Cancer Research* **7**(6):1337–1349.

Zhang H, Qi J, Wang Y, Sun J, Li Z, Sui L, Fan J, Liu C, Shang Y, Kong L, Kong Y. 2020. Progesterone regulates glucose metabolism through glucose transporter 1 to promote endometrial receptivity. *Frontiers in Physiology* **11**:543148. DOI 10.3389/fphys.2020.543148.

Zhang K, Yin R, Yang X. 2014. O-GlcNAc: a bittersweet switch in liver. *Frontiers in Endocrinology* **5**(6):221. DOI 10.3389/fendo.2014.00221.

Zhang Z, Tan EP, VandenHull NJ, Peterson KR, Slawson C. 2014. O-GlcNAcase expression is sensitive to changes in O-GlcNAc homeostasis. *Frontiers in Endocrinology* **5**(5):206. DOI 10.3389/fendo.2014.00206.

Zhou F, Huo J, Liu Y, Liu H, Liu G, Chen Y, Chen B. 2016. Elevated glucose levels impair the WNT/β-catenin pathway via the activation of the hexosamine biosynthesis pathway in endometrial cancer. *The Journal of Steroid Biochemistry and Molecular Biology* **159**(Suppl 2):19–25. DOI 10.1016/j.jsbmb.2016.02.015.

Zorzano A, Muñoz P, Camps M, Mora C, Testar X, Palacín M. 1996. Insulin-induced redistribution of GLUT4 glucose carriers in the muscle fiber. In search of GLUT4 trafficking pathways. *Diabetes* **45**(1):S70–S81. DOI 10.2337/diab.45.1.S70.

Zuo RJ, Gu XW, Qi QR, Wang TS, Zhao XY, Liu JL, Yang ZM. 2015. Warburg-like glycolysis and lactate shuttle in mouse decidua during early pregnancy. *The Journal of Biological Chemistry* **290**(35):21280–21291. DOI 10.1074/jbc.M115.656629.