Glycoconj J (2014) 31:185–191
DOI 10.1007/s10719-013-9515-5

Regulation of cancer metabolism by O-GlcNAcylation

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Received: 23 October 2013 / Revised: 25 November 2013 / Accepted: 26 November 2013 / Published online: 10 December 2013

Abstract Cancer cells exhibit increased uptake of glucose and glutamine, and rewire the metabolic flux toward anabolic pathways important for cell growth and proliferation. Understanding how this altered metabolism is regulated has recently emerged as an intense research focus in cancer biology. O-linked β-*N*-acetylglucosamine (O-GlcNAc) is a reversible posttranslational modification of serine and/or threonine residues of nuclear and cytosolic proteins. O-GlcNAcylation has been identified in numerous proteins that are involved in many important cellular functions, including transcription, translation, signal transduction, and stress responses. More recently, increasing evidence indicates that O-GlcNAcylation plays important roles in regulating cancer metabolic reprogramming by modifying key transcription factors, metabolic enzymes and major oncogenic signaling pathways. Thus, O-GlcNAcylation emerges as a novel regulatory mechanism linking altered metabolism to cancer pathogenesis.

Keywords Cancer metabolism · O-GlcNAcylation · Posttranslational modification · Oncogenic signaling

Introduction

One of the hallmarks of cancer is the altered metabolic phenotype [1]. Tumor cells take up glucose at a very high rate compared to normal cells, and convert it principally to lactate relying on glycolysis instead of oxidative phosphorylation, even in the presence of sufficient oxygen [2, 3]. This phenomenon is known as the "Warburg effect" (or aerobic glycolysis). Increasing evidence has shown that this metabolic reprogramming confers both bioenergetic and biosynthetic advantages to proliferating cancer cells by enabling rapid ATP generation, and providing anabolic metabolic precursors important for cell growth and reducing equivalents for maintaining redox homeostasis [4]. It is now accepted that this metabolic rewiring of cancer cells is mainly driven by deregulated oncogenic signaling, including gain-of-function mutations of pro-oncogenes and loss of tumor suppressors [5]. For instance, aberrant activation of PI3K/Akt/mTOR pathway, the most common lesion in human cancers, leads to enhanced uptake of glucose and aerobic glycolysis, contributing to cancer tumorigenesis [5]. Hypoxia-inducible factor 1α (HIF-1α) signaling upregulates glycolytic enzyme expression and increases lactate production in response to hypoxia [6–8]. The c-Myc oncogenic transcription factor signaling induces aerobic glycolysis, glutamine metabolism as well as nucleotide and lipid biosynthesis [7, 9–12]. On the other hand, recent findings indicate that intracellular metabolites can exert feedback control on signaling pathways through posttranslational modifications of key proteins such as metabolism-related transcription factors and metabolic enzymes [13]. These protein modifications exert effects over a fast time scale, allowing cells to respond rapidly and reversibly to a continually changing metabolic requirement. Accumulating data indicate that a particular form of protein glycosylation, O-linked β-*N*-acetyl-glucosamine (O-GlcNAcylation), plays a critical role in sensing cellular metabolism and reciprocally regulating a variety of important biological processes [14]. In this mini-review, we focus our discussion on regulation of cellular metabolism by O-GlcNAcylation particularly in the context of cancer.

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O-GlcNAcylation as a key metabolic sensor

O-GlcNAcylation is defined as a single O-GlcNAc moiety covalently attached to hydroxyl groups of serine and/or threonine residues of cytosolic, nuclear, and mitochondrial proteins. Similar to phosphorylation, O-GlcNAcylation is a reversible, dynamic, and inducible post-translational modification, and presents in all multicellular eukaryotes and tissues [15, 16]. In contrast to phosphorylation that is controlled by over 400 kinases and ~40 phosphatases, O-GlcNAcylation is regulated by only two antagonistic enzymes: O-GlcNAc transferase (OGT, EC 2.4.1.94) and O-linked β-*N*-acetylglucosaminidase (O-GlcNAcase, OGA, EC 3.2.1.52), which are responsible for the addition and removal of O-GlcNAc, respectively [14, 17].

Uridine diphospho-*N*-acetylglucosamine (UDP-GlcNAc), the substrate that drives O-GlcNAcylation, is generated via the hexosamine biosynthetic pathway (HBP) [18]. UDP-GlcNAc constitutes the physical integration of key metabolites and their corresponding metabolic pathways, including glucose associated with glycolysis and carbon supply, glutamine associated with TCA anapleurosis and nitrogen supply, acetyl-CoA associated with fatty acid metabolism and NADPH/reducing equivalent supply, UTP from nucleotide metabolism, energy charge and oxygen availability [15, 19]. Thus, UDP-GlcNAc is ideally constituted to provide the cell with a small-molecule reporter of the status of various pathways simultaneously (Fig. 1). Indeed, recent studies have shown that UDP-GlcNAc and HBP couple growth factor-induced glutamine uptake to glucose metabolism via glycosylation of the IL-3 receptor [20].

Cancer cells increase glucose and glutamine uptake, which can elevate HBP flux, leading to hyper-O-GlcNAcylation. Indeed, increased protein O-GlcNAcylation is observed in most types of cancer including breast, lung, colorectal, liver, bladder, endometrial, prostate, chronic lymphocytic leukemia (CCL) and pancreatic cancer [21–28]. Moreover, reducing hyper-O-GlcNAcylation via inhibition of OGT impaired tumor growth, invasion and metastasis [26, 28–30]. Thus, hyper-O-GlcNAcylation appears to be a general feature of cancer cells, and contributes to cell neoplastic transformation.

Regulation of transcription factors that impact metabolic reprogramming

Recent studies reveal that many transcription factors play important roles in metabolic reprogramming [31–34], and a number of these transcription factors are modified by O-GlcNAc. So, O-GlcNAcylation may contribute to cancer metabolic reprogramming by regulating the activities of transcription factors (Fig. 2).

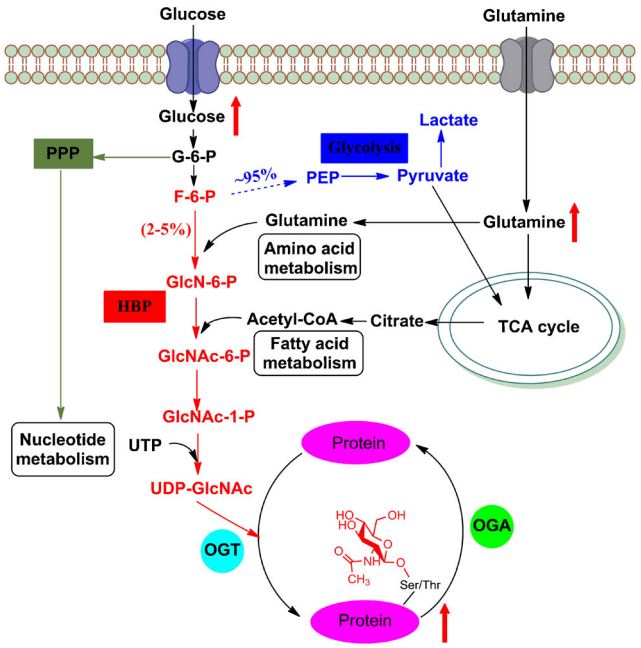

Fig. 1 O-GlcNAcylation as a sensor of metabolic states of cells. Cancer cells exhibit increased uptake of glucose and glutamine, and upregulate metabolic flux through the hexosamine biosynthetic pathway (HBP), leading to hyper-O-GlcNAcylation. A fraction (2~5 %) of glucose entering the cells is channeled into the HBP. The HBP integrates glucose metabolism, amino acid metabolism (glutamine), fatty acid metabolism (acetyl-CoA), and nucleotide metabolism (uridine triphosphate, UTP) to synthesize UDP-GlcNAc, the donor substrate for O-GlcNAcylation of proteins. OGT catalyzes the addition of O-GlcNAc onto hydroxyl groups of serine and/or threonine residues of proteins, whereas OGA catalyzes the hydrolysis of O-GlcNAc. *G-6-P* Glucose-6-phosphate, *F-6-P* Fructose-6-phosphate, *GlcN-6-P* Glucosamine-6-phosphate, *PEP* Phosphoenolpyruvate, *PPP* Pentose phosphate pathway

c-Myc

The oncogenic transcription factor c-Myc is expressed at very low levels in normal cells, but overexpressed in cancer cells [35]. c-Myc not only plays a crucial role in tumorigenesis, but also regulates the metabolism of cancer cells. c-Myc promoted aerobic glycolysis by upregulating transcription of polypyrimidine tract binding protein (PTB), and heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and A2 to favor the generation of pyruvate kinase isoform M2 [9]. c-Myc stimulated glucose uptake and glycolysis by inducing GLUT1 and glycolytic gene expression [7]. c-Myc also transactivated lactate dehydrogenase-A (LDH-A) to increase lactate production [10]. Moreover, c-Myc enhanced glutamine metabolism by transcriptionally upregulating glutamine transporter expression and mitochondrial glutaminase expression [11, 12]. One potential mechanism by which O-GlcNAc regulates the activity of c-Myc is implicated by the identification of the O-GlcNAcylation site of c-Myc at Thr58, a mutational hot spot in lymphomas. Thr58 was shown to undergo phosphorylation by GSK3β, resulting in rapid degradation of c-Myc [36]. Thus, it is postulated that O-GlcNAcylation of c-

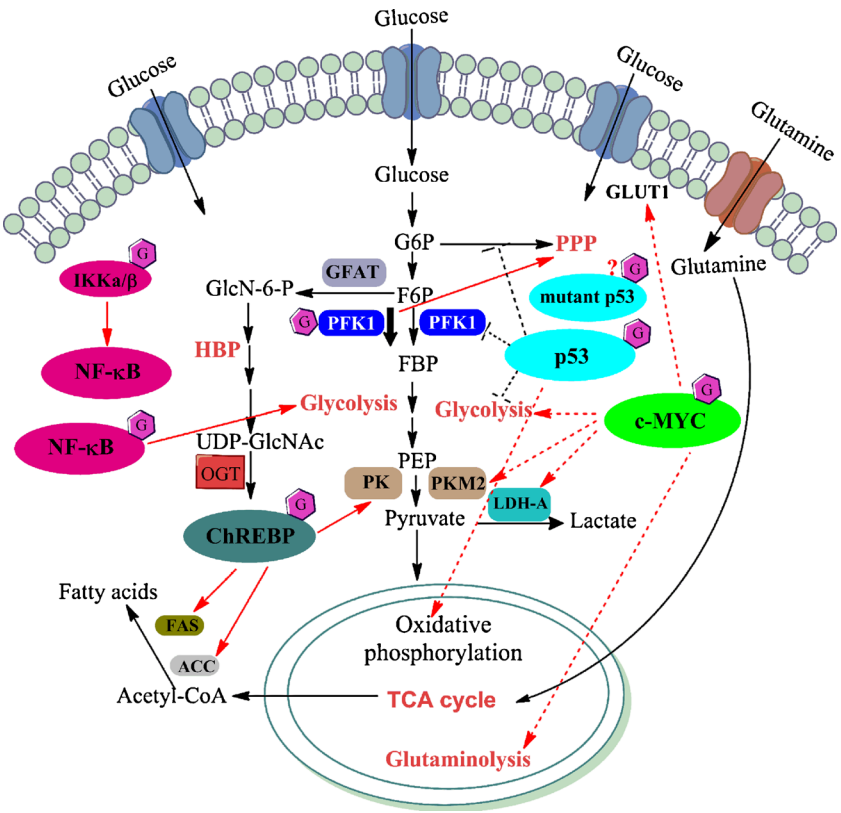

Fig. 2 Regulation of cancer metabolism by O-GlcNAcylation. O-GlcNAcylation regulates activities of key transcription factors (c-Myc, p53, NF-κB and ChREBP) to impact metabolic reprogramming. c-Myc can promote aerobic glycolysis and glutminolysis through upregulating the expression of key proteins. O-GlcNAcylation has shown to stabilize c-Myc. Wild-type p53 has shown to suppress glycolysis and enhance oxidative phosphorylation. Loss of p53 resulted in metabolic shift to aerobic glycolysis. O-GlcNAcylation significantly stabilized p53. However, its influence on metabolic reprogramming remains to be elucidated. Hyper-O-GlcNAcylation of IKKa/b and NF-κB activated (*red arrow*) NF-κB signaling, further promoting the Warburg effect. Hyper-O-GlcNAcylation of ChREBP upregulated aerobic glycolysis and *de novo* lipogenesis. O-GlcNAcylation of a key glycolytic enzyme phosphofructokinase 1 (PFK1) suppressed the enzyme activity (*black bold arrow*), and rerouted glucose flux from glycolysis to the pentose phosphate pathway (PPP). *Dashed arrows* indicate the potential regulation. *Red arrows* and *black lines* indicate promotion and suppression, respectively

Myc on Thr58 antagonizes phosphorylation at the same site, resulting in a more stable and more active c-Myc, promoting glutamine metabolism and tumorigenesis in cancer cells. In agreement with this mechanism, a recent study has revealed that OGT is a central regulator of c-Myc stability in prostate cancer cells, and inhibition of OGT reduced c-Myc protein stability [37]. However, the contribution of c-Myc glycosylation to metabolic reprogramming in cancer still remains to be addressed experimentally.

p53

p53 is a central tumor suppressor who mainly performs tumor suppressive abilities by inducing cell responses such as cell-cycle arrest, apoptosis, and senescence [38, 39]. Increasing evidence indicates that p53 is involved in regulating cellular metabolism [40, 41]. p53 regulated glucose metabolism through the actions of TP53-induced glycolysis and apoptosis regulator (TIGAR) [41], phosphoglyceratemutase (PGM) [42], and through the expression of synthesis of cytochrome c oxidase 2 (SCO2) [43]. p53 induced Guanidinoacetate methyltransferase (GAMT) expression to enhance creatine biosynthesis and fatty acid oxidation (FAO) under the condition of nutrient stress [40]. Lacking of functional p53, by either mutations or loss of wild-type p53, which is a frequent occurrence in cancer [44–46], resulted in a metabolic reprograming that favored aerobic glycolysis [42, 43]. Cancer cells lacking the functional p53 promoted aerobic glycolysis through inducing expression of glucose transporters, glycolytic enzymes, and suppressing oxidative phosphorylation in mitochrondria [42, 43, 47]. In cells, p53 level is intricately controlled by post-translational modifications. Phosphorylation of Thr155 by COP9 signalosome has shown to induce p53 degradation. A recent study by Yang *et al.* showed that O-GlcNAcylation of p53 at Ser149 could effectively inhibit its phosphorylation on Thr155, promoting p53 stabilization by blocking ubiquitin-dependent proteolysis

[31]. This O-GlcNAcylation-induced stabilization of p53 should promote its tumor suppressor activity in the context of wild-type p53, and its pro-oncogenic activity in the context of gain-of-function mutant p53. It is still unclear whether O-GlcNAcylation of p53 plays a role in regulating the metabolic pathways to meet the metabolic demands for tumor growth.

NF-κB

Nuclear factor kappa B (NF-κB) transcription factors are master regulators of immune and inflammation responses, and cell metabolism [48]. They also play a vital role in cancer development and progression [48]. Loss of p53 tumor suppressors can drive NF-κB towards oncogenic and tumor-promoting activity [49]. Studies have shown that NF-κB can promote metabolic switch from oxidative phosphorylation to aerobic glycolysis in cancer cells [50, 51]. Activation of NF-κB can increase the cell surface expression of GLUT3, resulting in enhanced rate of aerobic glycolysis [50]. In the absence of p53, NF-κB family member RelA (p65) repressed mitochondrial gene expression and oxidative phosphorylation, contributing to the switch to glycolysis [51]. Regulation of NF-κB pathway by O-GlcNAcylation has been demonstrated in several recent studies. O-GlcNAc directly modified NF-κB p65 subunit and upstream activating kinases IKKα and IKKβ in pancreatic cancer cells [28]. Inhibition of O-GlcNAcylation resulted in reduction of p65 activating phosphorylation on Ser536 and NF-κB transcriptional activity [28]. O-GlcNAcylation of NF-κB p65 on Thr352 decreased its binding to IκBα and increased the transcriptional activity under hyperglycemic conditions [34]. In the absence of p53, O-GlcNAcylation of IKKβ on Ser733 prevented auto-phosphorylation on the same residue, which was previously shown to inhibit IKKβ activity. Thus, hyper O-GlcNAcylation resulted in sustained IKKβ activity, leading to the constitutive activation of NF-κB pathway, further promoting the Warburg effect [52]. However, further studies are warranted to evaluate whether this O-GlcNAcylation-induced NF-κB pathway-dependent metabolic reprogramming plays a role in tumorigenesis.

ChREBP

Carbohydrate responsive element-binding protein (ChREBP) is a critical transcription factor that mediates glucose-induced gene expression including various glycolytic and lipogenic genes in nonproliferating hepatocytes [53, 54]. Recently, ChREBP has also been found a necessary element for cell proliferation in HCT116 colorectal cancer cells and HepG2 hepatoblastoma cells. Suppression of ChREBP activity led to a metabolic shift from aerobic glycolysis to mitochondrial respiration, and subsequent reduction of cell proliferation and tumor growth [53]. This study demonstrates that ChREBP plays a critical role in directing glucose metabolism into anabolic pathways such as lipid and nucleotide biosynthesis in cancer cells. ChREBP was modified by O-GlcNAc, although the exact sites of modification were not identified [55, 56]. Hyper-O-GlcNAcylation stabilized the ChREBP protein and increased the glucose response of ChREBP, resulting in the increased expression of its target glycolytic (liver pyruvatekinase [L-PK]) and lipogenic genes (acetyl-CoA carboxylase [ACC] and fatty acid synthase [FAS]) [56]. Therefore, hyper-O-GlcNAcylation of ChREBP may represent a molecular mechanism that drives the metabolic programming of glucose into anabolic processes to support cell growth and tumorigenic phenotypes in cancer cells.

Regulation of metabolic enzymes

In addition to impacting metabolic reprogramming by modifying critical transcription factors that have significant roles in modulating cell metabolism, O-GlcNAcylation has recently shown to directly regulate glucose metabolism by affecting the activity of glycolytic enzymes. Studies found that all of the enzymes in the glycolytic pathway were modified by O-GlcNAc [57]. To date, phosphofructokinase 1 (PFK1) is the only metabolic enzyme, whose regulatory mechanism by O-GlcNAc has been elucidated. PFK1 catalyzes a key regulatory step in glycolysis to generate fructose-1,6-bisphosphate from fructose-6-phosphate. O-GlcNAcylation of PFK1 on Ser529, a critical residue involved in the enzyme oligomerization and allosteric regulation, led to the inhibition of the enzymatic activity by blocking the binding of the activator fructose-2,6-bisphosphate (F-2,6-BP) and disrupting oligomerization [29]. The biological outcome resulted from O-GlcNAc-induced inhibition of PFK1 activity is multifaceted. First, it redirected glucose flux from glycolysis down to the oxidative pentose phosphate pathway (PPP), providing cells with pentose sugars for nucleotide and nucleic acid biosynthesis, supporting rapid cell proliferation. Second, enhanced PPP flux generated NADPH, which maintained a pool of reduced glutathione (GSH) and protected cells from oxidative stress conferred by reactive oxygen species (ROS). Blocking O-GlcNAcylation of PFK1 on Ser529 reduced cancer cell proliferation *in vitro* and impaired tumor formation *in vivo* [29]. Therefore, this study reveals that O-GlcNAcylation not only acted as a sensor of altered cell metabolism, but actively involved itself in driving the cancer metabolic reprogramming phenotypes. Considering the large number of metabolic enzymes possessing O-GlcNAcylation, more studies are needed to uncover the link between O-GlcNAcylation, cancer metabolic reprogramming and tumorigenesis.

Regulation of oncogenic signaling pathways by O-GlcNAc

Increasing evidence has shown that major metabolism-sensing signaling pathways (*e.g.* MAPK pathway, mTOR pathway, and AMPK pathway) govern the rewiring of cellular metabolism for cell growth, proliferation and survival. Mitogen activated protein kinase (MAPK) pathway is an important growth-factor-initiated signaling that plays a pivotal role in regulating cell proliferation, differentiation, survival, apoptosis, and metastasis, and is often aberrantly activated in many neoplasms [58, 59]. Due to its tight link to cancer initiation and development, MAPK pathway has become an attractive target for anticancer therapies. Recent studies have demonstrated that O-GlcNAcylation can affect MAPK signaling pathway. People found that addition of glucosamine or high glucose could significantly enhance the phosphorylation of MKK and p38 MAPK, resulting in sustained activation of MAPK pathway [60]. OGT silencing diminished MAPK activation by decreasing the phosphorylation of Erk1/2 [61]. Moreover, several MAPK pathway-associated proteins were directly modified by O-GlcNAc, including Erk2 and Hsp90, a chaperone required for the stability [62, 63]. Thus, these studies point to a potential regulatory role of O-GlcNAcylation in MAPK pathway, although the underlying molecular mechanisms still remain to be elucidated.

Mammalian target of rapamycin (mTOR) pathway is a master regulator of cell growth and metabolism. mTOR responses to a variety of environmental cues, such as growth factors, nutrients, and metabolic stress, and integrate them to impact important biological processes including protein translation, autophagy and metabolism. The PI3K-Akt signaling cascade is the best-studied molecular mechanism of mTOR activation. Aberrant activation of mTOR signaling is a common molecular event detected in the majority of human cancers. Substantial progress has been made in understanding how the mTOR signaling pathway is activated; however, how the mTOR pathway is differentially regulated still awaits further clarification and investigation. Notably, studies have shown that specific proteins in the mTOR pathway are modified by O-GlcNAc [14, 64–66]. These include activating proteins upstream of mTOR (IRS1, PI3K, PDK1, and Akt), proteins serving regulatory roles (GSK3β, FoxO and PP2A), and effector proteins downstream of mTOR (eIF4G and eEF2). This points to an exciting hypothesis that O-GlcNAc might participate in regulating this critical pathway. Indeed, recent studies have generated evidence to support this hypothesis. For example, in MCF-7 breast cancer cells, siRNA-mediated knockdown of OGT expression significantly attenuated serum-induced Akt activation [61]. Consistently, increasing O-GlcNAcylation in MCF-7 cells and 8305C thyroid anaplastic cancer cells resulted in increased Akt phosphorylation and activity [67]. Moreover, increasing O-GlcNAcylation stimulated cellular production of phosphatidylinositol 3,4,5-triphosphate, indicating the activation of PI3K [67].

Adenosine 5′-monophosphate-activated protein kinase (AMPK) plays a critical role in the maintenance of cellular energy and metabolic homeostasis. AMPK is activated by high cellular AMP/ATP ratio, an indicator of declining fuel supply, and subsequently induces catabolic metabolism and inhibits anabolic metabolism [68]. A recent study has demonstrated that in lymphomas loss of AMPK function promoted aerobic glycolysis and channeled metabolic intermediates into anabolic pathways for cellular biosynthesis in a HIF-1α-dependent manner [69]. Possible regulation of AMPK signaling by O-GlcNAcylation has been suggested by several studies. Luo *et al.* discovered that enhancing HBP flux by glucosamine treatment in 3T3L1 adipocytes increased AMPK phosphorylation and activity, leading to stimulation of fatty acid oxidation [70]. Conversely, reducing O-GlcNAcylation by blocking HBP or treatment with hexosaminidase attenuated AMPK activity [70]. AMPK appeared to be modified by O-GlcNAc, even though the exact site of modification remained to be identified. In a reciprocal way, Cheung *et al.* demonstrated that AMPK induced OGT expression and increased O-GlcNAcylation during glucose deprivation in Neuro-2a neuroblastoma cells [71]. These findings indicate a potential regulatory link between AMPK signaling and O-GlcNAcylation.

Conclusion and future perspectives

Cellular metabolism is intricately linked to various critical cellular behaviors including cell proliferation, survival, redox homeostasis, and stress responses. Altered metabolism, triggered by deregulated oncogenic signaling, has been strongly associated with transformed phenotypes of cancer cells. Delineating the molecular mechanisms by which cellular metabolism is regulated in cancer cells provides a solid foundation for understanding the contribution of metabolic reprogramming to the disease, and novel targets for therapeutic intervention. General elevation of O-GlcNAcylation in cancer cells suggests a potential link between deregulation of metabolism and pathophysiology of cancer. O-GlcNAcylation not only serves as a sensor responding to oscillating metabolic states of cells, but also appears to directly participate in regulating metabolism by modifying key transcription factors, metabolic enzymes and major oncogenic signaling pathways. Although increasing evidence indicates O-GlcNAcylation as a new mechanism linking deregulation of metabolism to cancer pathology, such an intriguing hypothesis still remains to be fully addressed experimentally. Future studies are needed to elucidate at molecular levels how protein-specific O-GlcNAcylation contributes to metabolic rewiring and cancer tumorigenesis.

Acknowledgments We thank Dr. Binghui Shen (City of Hope National Cancer Center, USA) for helpful discussions. This work was supported by the National Science Foundation of China (NSFC, grant nos. 31270865 and 31322019), the Thousand-Young-Talents Recruitment Program, the Fundamental Research Funds for the Central Universities, and Cao Guang-biao Research Development Funds.

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