



# Cross-Dysregulation of O-GlcNAcylation and PI3K/AKT/mTOR Axis in Human Chronic Diseases

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The hexosamine biosynthetic pathway (HBP) and the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway are considered as nutrient sensors that regulate several essential biological processes. The hexosamine biosynthetic pathway produces uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), the substrate for O-GlcNAc transferase (OGT), the enzyme that O-GlcNAcylation proteins on serine (Ser) and threonine (Thr) residues. O-linked  $\beta$ -N-acetylglucosaminylation (O-GlcNAcylation) and phosphorylation are highly dynamic post-translational modifications occurring at the same or adjacent sites that regulate folding, stability, subcellular localization, partner interaction, or activity of target proteins. Here we review recent evidence of a cross-regulation of PI3K/AKT/mTOR signaling pathway and protein O-GlcNAcylation. Furthermore, we discuss their co-dysregulation in pathological conditions, e.g., cancer, type-2 diabetes (T2D), and cardiovascular, and neurodegenerative diseases.

**Keywords:** O-GlcNAcylation, PI3K/AKT/mTOR, cancer, diabetes, cardiovascular, neurodegenerative diseases

## INTRODUCTION

O-linked  $\beta$ -N-acetylglucosaminylation (O-GlcNAcylation) is a dynamic modification of serine (Ser) and threonine (Thr) amino acids of cytoplasmic, nuclear (1), and mitochondrial (2) proteins with a single residue of N-acetylglucosamine (GlcNAc). This post-translational modification is controlled by two single antagonist enzymes: O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), which transfer and remove the GlcNAc moiety, respectively. The nucleotide sugar donor, uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), is the final product of the hexosamine biosynthetic pathway that is at the nexus of glucose, amino acid, lipid, and nucleotide metabolisms (**Figure 1**). Consequently, O-GlcNAcylation is considered as a cellular nutrient sensor linking nutrient availability to intracellular signaling and biological responses. To date, thousands of O-GlcNAcylation proteins endowing a wide range of functions have been identified and most of them are also phosphoproteins (3). In fact, O-GlcNAcylation and phosphorylation can modulate each other at the same or adjacent sites (4).

Over the last decade, emerging evidence has indicated that a cross-talk exists between O-GlcNAcylation and phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway (5). The PI3K/AKT/mTOR signaling pathway is a key transducer of metabolic and mitogen signals (such as energy, amino acids, insulin or growth factors) that modulate gene expression, protein translation and cellular metabolism, thus regulating cell growth and proliferation (**Figure 1**). Aberrant activation of this signaling pathway as well as altered protein O-GlcNAcylation have both described in several types of cancer, cardiovascular and metabolic diseases, aging, and neurodegeneration in human (6–8). This mini-review summarizes and discusses recent evidence linking cross-regulation and co-dysregulation of O-GlcNAcylation and PI3K/AKT/mTOR signaling pathway in physiological conditions and in human chronic diseases, respectively.

## THE PI3K/AKT/MTOR SIGNALING PATHWAY AND ITS CROSS-REGULATION WITH PROTEIN O-GLCNACYLATION

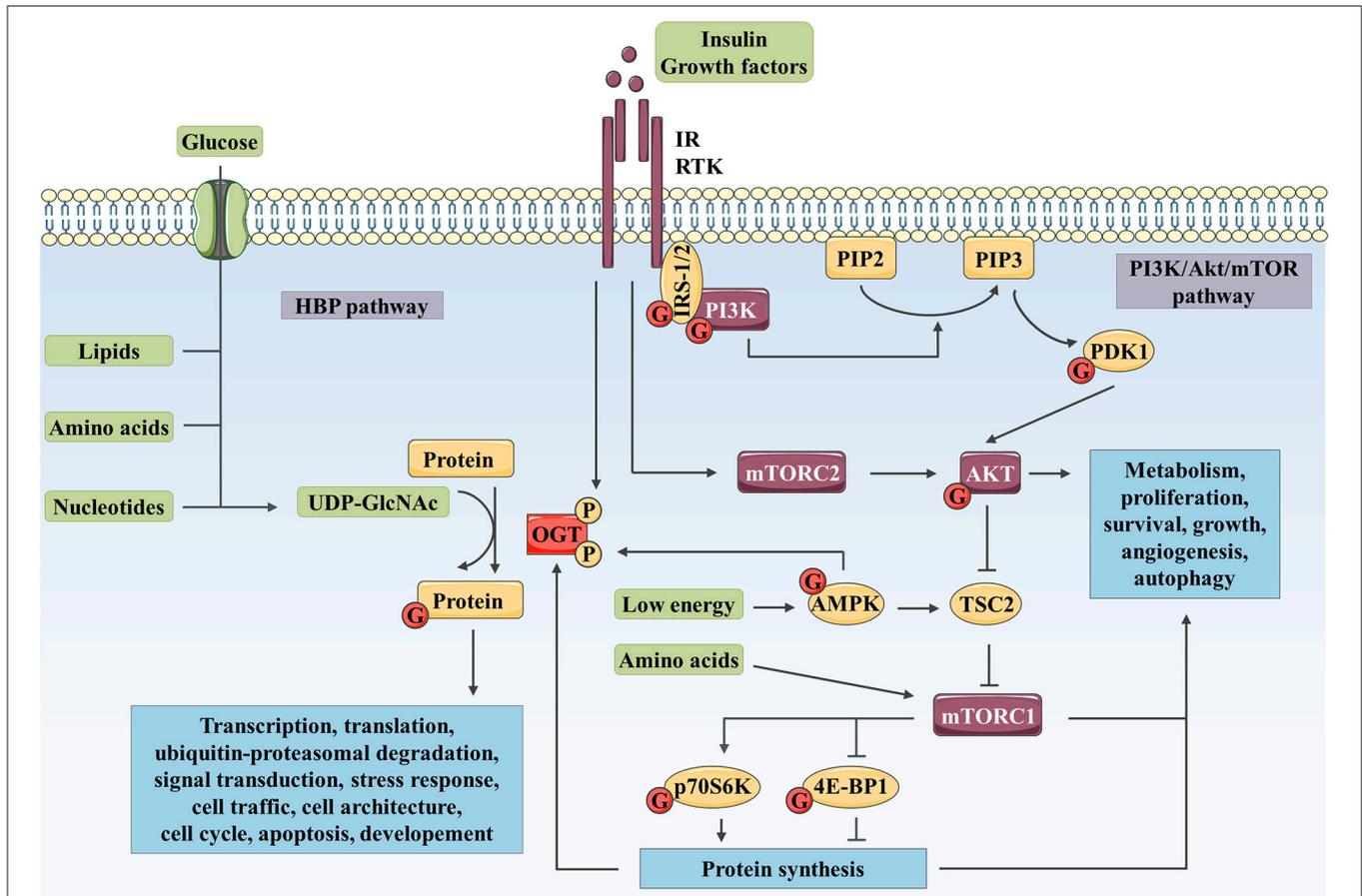
Binding of insulin or growth factors to their plasma membrane tyrosine kinase receptors (RTK) triggers the phosphorylation of PI3K, either directly by the RTK or indirectly *via* phosphorylation of adapter signaling proteins such as insulin receptor substrate-1 or 2 (IRS-1/2; **Figure 1**). Phospho-PI3K catalyzes the formation of membrane phosphatidylinositol-3,4,5-trisphosphate (PIP3) which drives the activation of the phosphoinositide-dependent protein kinase-1 (PDK-1) and the recruitment of AKT. The latter is partially activated through initial phosphorylation at Thr308 by PDK-1 and fully activated after phosphorylation at Ser473 by the mTOR complex 2 (mTORC2) (9). Once activated, AKT phosphorylates several downstream effectors [e.g., mTOR, forkhead box proteins O (FoxO), glycogen synthase kinase 3  $\beta$  (GSK3 $\beta$ ), BCL-2-associated agonist of cell death (BAD) or endothelial nitric oxide synthase (eNOS)] that in return regulate and coordinate a variety of cellular responses including cell proliferation, survival and growth, glucose metabolism, and angiogenesis (9). Tuberous sclerosis complex 2 (TSC2), inhibited by AKT-dependent phosphorylation, is a critical negative regulator of mTOR complex 1 (mTORC1). mTORC1 induces protein synthesis through phosphorylation of eukaryotic translation initiation factor 4E (eIF4E)-binding protein-1 (4E-BP1) and ribosomal

protein S6 kinase (p70S6K) (9). mTORC1 also regulates nucleotide, lipid and glucose metabolisms, angiogenesis and autophagy processes by regulating alpha-activating transcription factor 4 (ATF4), lipin-1, hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) or Unc-51 like autophagy activating kinase 1 (ULK1) (10, 11). In response to amino acid stimulation, mTORC1 is recruited to the lysosomal surface where it is activated by Ras homolog enriched in brain (Rheb) (9). Upon elevated AMP/ATP ratio, AMP-activated protein kinase (AMPK) phosphorylates, and activates TSC2 leading to inhibition of mTORC1 activity (10, 11).

In parallel, O-GlcNAcylation targets proteins involved in transcription, translation, ubiquitin-proteasomal degradation, signal transduction, stress response, cellular trafficking and architecture, cell cycle, apoptosis, and development (12). OGT activity is sensitive to UDP-GlcNAc levels, thus, addition of glucose or glucosamine globally increases levels of O-GlcNAcylation (13).

Many studies have established a complex interplay between PI3K/AKT/mTOR signaling pathway and protein O-GlcNAcylation (**Figure 1**). After insulin stimulation, the C-terminal PIP-binding domain of OGT (PPO) allows its translocation from the nucleus to the plasma membrane in murine 3T3-L1 adipocytes (14) and African green monkey COS-7 fibroblasts (15), and possibly to lipid rafts as observed in the human hepatic cancer cell line HepG2 (16). This translocation possibly facilitates the tyrosine phosphorylation of OGT by the insulin receptor (IR), which increases its enzymatic activity (17). The cellular energy sensor AMPK also regulates OGT. AMPK phosphorylates OGT at Thr444, which induces its nuclear translocation in differentiated C2C12 skeletal muscle myotubes (18) and promotes its dissociation from chromatin in human embryonic kidney 293T cells (19). In HepG2 cells, it has been further shown that OGT phosphorylation by AMPK inhibits histone H2B O-GlcNAcylation and gene transcription (19). In contrast, OGT targets several actors from the PI3K/AKT/mTOR signaling pathway, including IRS-1 (17, 20–24), PI3K (23), PDK1 (17), AKT (21, 25–27), AMPK (18, 19), 4E-BP1 (28), and p70S6K (29). Indeed, these proteins are O-GlcNAc-modified in IR and insulin growth factor-1 receptor (IGF-1R) expressing cell types including adipocytes, myocytes, hepatocytes, pancreatic beta ( $\beta$ ) cells, endothelial cells, kidney and retina cells (30). However, only few studies have investigated the molecular impacts of O-GlcNAcylation on PI3K/AKT/mTOR signaling pathway and the subsequent biological effects under physiological conditions. O-GlcNAc modification of IRS-1 and AKT inhibits their activity either by disruption of their interaction with PI3K and PDK1 kinases, respectively, in 3T3-L1 adipocytes and MCF-7 breast cancer cell lines (17, 26), either by a “Yin-Yang” competition mechanism with activating phosphorylation as described in rat primary adipocytes and INS-1 pancreatic  $\beta$  cell lines (25, 27). O-GlcNAcylation also enhances 4E-BP1 stability *in vitro* in rat retinal TR-MUL Müller cells an *in vivo* in murine retinal cells, potentially by preventing its phosphorylation-dependent ubiquitin-mediated degradation (28). Protein O-GlcNAcylation could hence potentiate cellular nutrient sensing capacity of the PI3K/AKT/mTOR signaling pathway in order to regulate crucial intracellular processes.

**Abbreviations:** 4E-BP1, eukaryotic translation initiation factor 4E-binding protein-1; AD, Alzheimer’s disease; AMPK, adenosine monophosphate-activated protein kinase; BAD, BCL-2-associated agonist of cell death; eNOS, endothelial nitric oxide synthase; FoxO, forkhead box protein O; GlcNAc, N-acetylglucosamine; GLUT, glucose transporter; GSK3 $\beta$ , glycogen synthase kinase 3  $\beta$ ; HBP, hexosamine biosynthetic pathway; HIF-1 $\alpha$ , hypoxia-inducible factor-1 $\alpha$ ; IR, insulin receptor; IRS-1, insulin receptor substrate-1; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; O-GlcNAcylation, O-linked  $\beta$ -N-acetylglucosaminylation; OGA, O-GlcNAcase; OGT, O-GlcNAc transferase; p70S6K, ribosomal protein S6 kinase; PDK-1, phosphoinositide-dependent protein kinase-1; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol-3,4,5-trisphosphate; T2D, type-2 diabetes; Tau, tubulin-associated unit; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine.



**FIGURE 1 |** Complex interplay between O-GlcNAcylation and PI3K/AKT/mTOR signaling pathway controls numerous biological processes. The HBP integrates a fraction of the glucose entering the cell as well as lipid, nucleotide, and amino acid metabolites to produce UDP-GlcNAc. Then, OGT uses UDP-GlcNAc as a nucleotide sugar donor substrate to add a GlcNAc group on serine and threonine residues of target proteins. Like phosphorylation, O-GlcNAcylation is a dynamic and reversible post-translational modification. Its targets are involved in a wide range of biological processes such as transcription, translation, ubiquitin-proteasomal degradation, signal transduction, cell traffic and architecture, cell cycle, apoptosis or development. In parallel, binding of insulin or growth factor to their RTK leads to receptor activation and recruitment of IRS-1/2 and PI3K. PI3K produces PIP3 (from PIP2), which recruits AKT and PDK1 to the plasma membrane. PDK1 and mTOR in mTORC2 activate AKT through phosphorylation. mTORC1 is activated by AKT through TSC2 inhibition and upon amino acid stimulation and, is inhibited in response to low energy by AMPK. mTORC1 promotes protein synthesis via direct phosphorylation of p70S6K and 4E-BP1. By phosphorylating key substrates, AKT and mTORC1 regulate metabolism, cell cycle, proliferation, survival, growth, angiogenesis and autophagy. OGT localization and activity are regulated through phosphorylation by IR and AMPK. OGT stability is indirectly regulated at the protein synthesis level via mTORC1. Reciprocally, several actors of the PI3K/AKT/mTOR signaling pathway are modified by O-GlcNAcylation such as IRS-1, PI3K, PDK1, AKT, AMPK, p70S6K, and 4E-BP1.

## O-GLCNACYLATION AND PI3K/AKT/MTOR SIGNALING PATHWAY CROSS-DYSREGULATION IN HUMAN DISEASES

### Cancer

The Warburg effect is a metabolic reprogramming of the cell from oxidative phosphorylation to aerobic glycolysis that allows energy production and *de novo* macromolecule synthesis required to sustain cancer cells proliferation and growth. Enhanced glucose and glutamine uptake observed in the Warburg effect would lead to an increased flux through HBP and the hyper-O-GlcNAcylation that has been observed in many cancers (31). Aberrantly activated PI3K/AKT/mTOR signaling

pathway is known to play a central role in aerobic glycolytic reprogramming, tumor growth, and survival (32), and a cross-talk between PI3K/AKT/mTOR signaling and O-GlcNAcylation has been observed in several cancers.

Insulin or serum growth factors stimulation lead to increased OGT expression in a PI3K-dependent manner in HepG2 and MCF-7 cell lines (16, 33). Although it was not investigated in these studies, it is likely that this effect could be related to mTOR activation. Since it was observed that pharmacological inhibition of mTOR enhances proteasomal and autophagic degradation of OGT in HepG2 cells (34). We have also demonstrated that inhibition of mTOR affects OGT protein level and overall O-GlcNAcylation levels in HCT116 colon cancer cell line (35). In breast cancer cell lines the positive regulation of OGT

expression through mTOR is dependent on c-Myc-induced heat shock protein 90A (HSP90A) transcription (36). This chaperone binds to OGT and prevents its proteasomal degradation (36). Additionally, the transcriptional regulator Yes-associated protein (YAP) strongly activates the OGT promoter in hepatic cancer cell lines. In turn, O-GlcNAcylation of YAP promotes its stability, and its tumorigenic activity both *in vitro* and *in vivo* in liver cancer mouse models showing that a positive feedback is set up in liver tumorigenesis (37). YAP is activated by PI3K in hepatocellular (38) and mammary carcinoma (39), but has been shown to regulate PI3K/AKT/mTOR signaling in the MCF 10A human immortalized mammary epithelial cell line (40). These recent works highlight once more the tight link that exists in cancer cells between PI3K/AKT/mTOR axis and OGT activity.

O-GlcNAcylation impacts PI3K/AKT and mTOR axis in cancer cells. Pre-B acute lymphocytic leukemia (pre-B-ALL) cells overexpress OGT and exhibit a higher O-GlcNAcylation levels and an overactivation of PI3K, AKT and c-Myc compared to normal B cells. This dysregulation is associated with the overexpression of the transcription factor *HIF-1 $\alpha$*  and its target glycolytic genes such as *glucose transporter 1 (GLUT1)*, *hexokinase 2 (HK2)*, *phosphofructokinase-1 (PFK-1)* and *lactate dehydrogenase A (LDHA)*. OGT knockdown, in pre-B-ALL cells, decreases PI3K and AKT activation and glycolysis, resulting in a reduced cell proliferation and apoptosis. These inhibitory effects can be partly rescued by IGF-1 mediated stimulation of PI3K/AKT, indicating that effect of OGT on glycolysis is, in part, PI3K/AKT-dependent (41). Similarly, in 3D cultures of T4-2 breast cancer cells, OGT inhibition or silencing suppresses AKT signaling and glycolytic activity (42) (**Figure 2**).

In addition to glycolysis, regulation of PI3K/AKT signaling by O-GlcNAcylation was shown to modulate proliferation, growth and invasion properties of cancer cells (32, 42–47). We have demonstrated that knockdown or pharmacological inhibition of OGT decreases PI3K activation and prevents serum-stimulated cyclin D1 synthesis, leading to a delay in proliferation of MCF-7 cells (33). Since AKT prevents ubiquitin-mediated degradation of cyclin D1 by inhibiting GSK3 $\beta$  activity in the murine NIH/3T3 fibroblast cell line (43), it is likely that the decrease in cyclin D1 level could result from an increase of its proteasomal degradation under low O-GlcNAcylation levels. Reciprocally, enhanced O-GlcNAcylation level stimulates PIP3 production and AKT phosphorylation in MCF-7 cells (44). Similar results showed that hyper-O-GlcNAcylation induced by OGA down-regulation in 8305C thyroid anaplastic tumor cell line stimulates proliferation through increased phosphorylation of AKT at Ser473 and cyclin D1 amount (45). Additionally, glucose deprivation in osteosarcoma U2OS cell line attenuates protein O-GlcNAcylation, phosphorylation of IRS-1 and AKT, production of PIP3 and suppresses cell growth (46). Importantly, in these cell line, insulin signaling pathway, and tumor growth can be rescued by glucosamine-mediated increased HBP flux and O-GlcNAcylation (46). In parallel, increased O-GlcNAcylation promotes gastric and thyroid cancer cells invasion in a PI3K/AKT dependent manner, since the pro-invasion effect of O-GlcNAcylation is suppressed by PI3K inhibition or AKT silencing (47, 48).

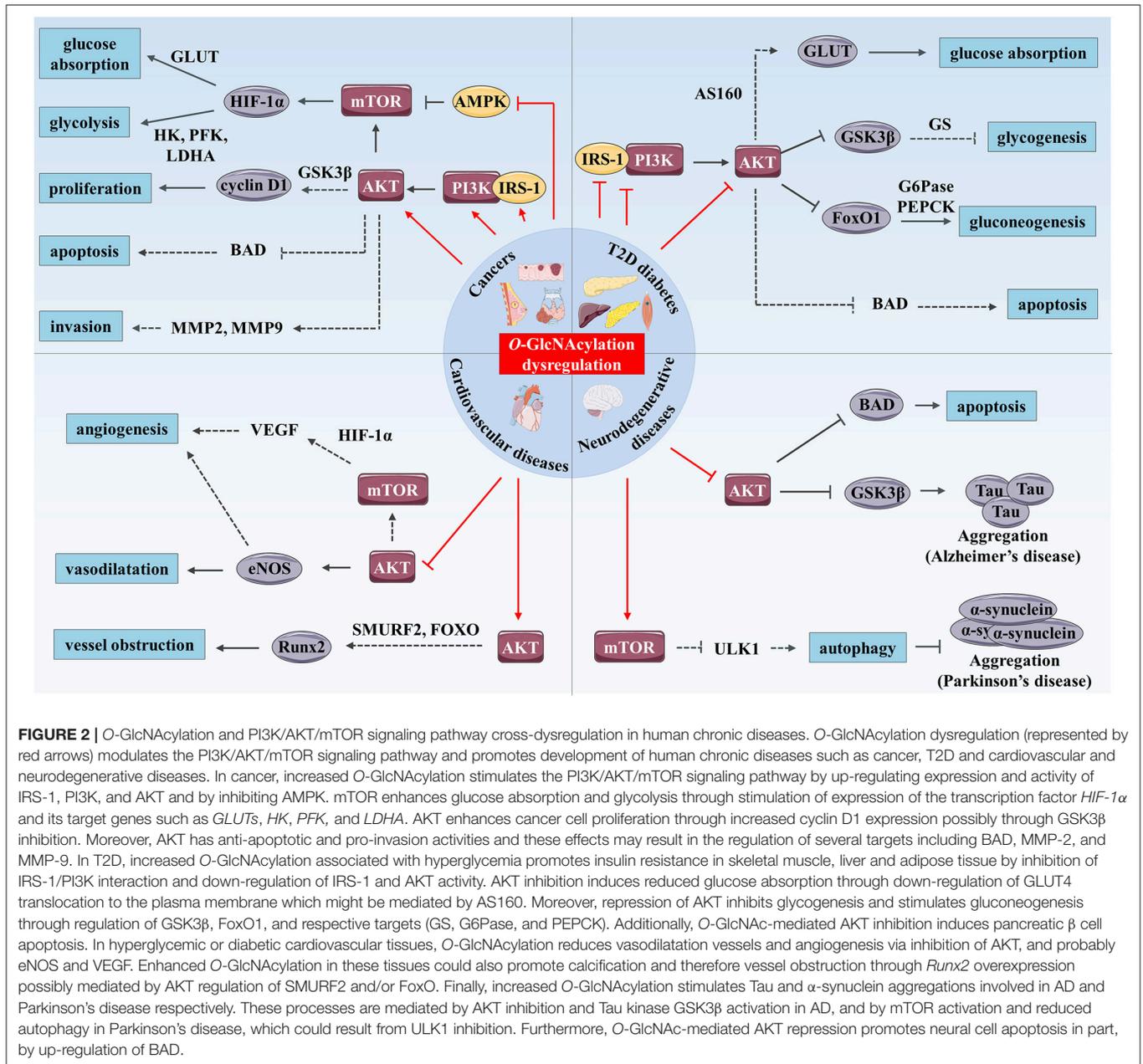
This may result from the regulation that O-GlcNAcylation exerts on AKT-mediated control of a myriad of downstream substrates, such as matrix metalloproteinase-2 (MMP-2) and MMP-9 (49) (**Figure 2**). However, other studies report contradictory results regarding the effect of O-GlcNAcylation on the activation of AKT signaling pathway (26, 50). OGA overexpression reduces AKT O-GlcNAcylation and promotes its activation, albeit in a PI3K-independent manner, both in HepG2 cells and in liver of euglycemic mice (50). Our group also demonstrated that OGT silencing prevents AKT Ser473 phosphorylation in HepG2 (16) and MCF-7 (33) cell lines. More recently, this effect has also been described in cholangiocarcinoma cell lines (51). Furthermore, AKT O-GlcNAcylation at Thr305 and Thr312 reduces MCF-7 cell proliferation and migration *via* inhibition of AKT phosphorylation at Thr308 and disruption of its interaction with PDK1 (26).

Finally, it was shown that O-GlcNAcylation regulates the mitogenic mTOR signaling pathway through targeting the mTOR inhibitor AMPK (35, 52, 53). Increased O-GlcNAcylation in colon cancer cells, either by OGT overexpression or OGA inhibition, reduces phosphorylation of AMPK at Thr172, activates mTOR and induces cell growth *in vitro* in LoVo cell line and *in vivo* in LoVo cell-derived tumors of BALB/c-nu/nu mice (52). We have confirmed that O-GlcNAcylation activates mTOR in HCT116 colon cancer cell line but not in CCD841CoN normal cells (35). Reciprocally, OGT silencing or inhibition increases phosphorylation of AMPK, decreases phosphorylation of mTOR downstream effectors 4E-BP1 and p70S6K, decreases *HIF-1 $\alpha$* , *GLUT1*, and *LDHA* expression and impairs glucose uptake and growth in breast cancer cell lines (53) (**Figure 2**).

Together, these studies establish the involvement of O-GlcNAcylation in cancer biology (increased glycolysis, proliferation, growth, and invasion) through direct activation of the PI3K/AKT/mTOR axis. One may consider this post-translational modification as a key node between metabolism and cell signaling. However, intricate ties linking metabolism and cancer are not completely elucidated and need further investigations. In parallel, anti-cancer inhibitors targeting mTOR axis are currently in clinical development and must be encouraged (54). Tumor cells resistant to GDC-0941, a PI3K inhibitor, exhibit an increased activation of the PI3K/AKT/mTOR signaling pathway and OGT expression in comparison to GDC-0941-sensitive cells. Interestingly, OGT silencing sensitizes these cells to GDC-0941 (55). In this sense, targeting OGT in cancer cells and/or adapting patients to low caloric diet could increase the efficiency of anti-PI3K/AKT/mTOR therapeutic strategies and foil drug resistance.

## Type 2 Diabetes

Insulin resistance, a hallmark of type 2 diabetes (T2D), refers to impaired insulin sensitivity and glucose uptake of target tissues (liver, skeletal muscle, and adipose tissue). PI3K/AKT signaling pathway plays a key role in the regulation of glucose homeostasis by inhibiting gluconeogenesis and activating glycogenesis *via* the inhibition of FoxO1 and GSK3 $\beta$  respectively



**FIGURE 2** | O-GlcNAcylation and PI3K/AKT/mTOR signaling pathway cross-dysregulation in human chronic diseases. O-GlcNAcylation dysregulation (represented by red arrows) modulates the PI3K/AKT/mTOR signaling pathway and promotes development of human chronic diseases such as cancer, T2D and cardiovascular and neurodegenerative diseases. In cancer, increased O-GlcNAcylation stimulates the PI3K/AKT/mTOR signaling pathway by up-regulating expression and activity of IRS-1, PI3K, and AKT and by inhibiting AMPK. mTOR enhances glucose absorption and glycolysis through stimulation of expression of the transcription factor *HIF-1α* and its target genes such as *GLUTs*, *HK*, *PFK*, and *LDHA*. AKT enhances cancer cell proliferation through increased cyclin D1 expression possibly through GSK3β inhibition. Moreover, AKT has anti-apoptotic and pro-invasion activities and these effects may result in the regulation of several targets including BAD, MMP-2, and MMP-9. In T2D, increased O-GlcNAcylation associated with hyperglycemia promotes insulin resistance in skeletal muscle, liver and adipose tissue by inhibition of IRS-1/PI3K interaction and down-regulation of IRS-1 and AKT activity. AKT inhibition induces reduced glucose absorption through down-regulation of GLUT4 translocation to the plasma membrane which might be mediated by AS160. Moreover, repression of AKT inhibits glycogenesis and stimulates gluconeogenesis through regulation of GSK3β, FoxO1, and respective targets (GS, G6Pase, and PEPCK). Additionally, O-GlcNAc-mediated AKT inhibition induces pancreatic β cell apoptosis. In hyperglycemic or diabetic cardiovascular tissues, O-GlcNAcylation reduces vasodilatation vessels and angiogenesis via inhibition of AKT, and probably eNOS and VEGF. Enhanced O-GlcNAcylation in these tissues could also promote calcification and therefore vessel obstruction through *Runx2* overexpression possibly mediated by AKT regulation of SMURF2 and/or FoxO. Finally, increased O-GlcNAcylation stimulates Tau and α-synuclein aggregations involved in AD and Parkinson's disease respectively. These processes are mediated by AKT inhibition and Tau kinase GSK3β activation in AD, and by mTOR activation and reduced autophagy in Parkinson's disease, which could result from ULK1 inhibition. Furthermore, O-GlcNAc-mediated AKT repression promotes neural cell apoptosis in part, by up-regulation of BAD.

(9) (Figure 2). Some studies have also established a link between dysregulation of O-GlcNAcylation cycling and insulin resistance.

Interestingly, single nucleotide polymorphisms (SNPs) on *OGA* (also called *MGEA5* for *meningioma expressed antigen 5*) gene and *GFPT2* (for *GFAT isomerizing 2*) gene, coding the glutamine fructose-6-phosphate amidotransferase (GFAT) rate-limiting enzyme controlling the production of UDP-GlcNAc, are associated with increased T2D risk in American-Mexican and Caucasian populations, respectively (56, 57). These mutations may lead to reduced *OGA* expression and increased *GFPT2* expression respectively (56, 57), and an up-regulation of cellular O-GlcNAcylation levels. O-GlcNAcylation levels are

significantly increased in skeletal muscle, liver, heart, colon-rectum, erythrocytes, and leukocytes of diabetic animals and humans (35, 58–62). Consistent with these epidemiologic data, *db/db* mice overexpressing *Oga* showed improved hepatic insulin sensitivity (63), whereas *Ogt* overexpression and subsequent elevation of global O-GlcNAcylation level inhibits insulin signaling pathway, both *in vitro* in 3T3-L1 adipocyte and Fao hepatic cell lines, and *in vivo* in skeletal muscle and adipose tissue in mice (15, 64, 65). Skeletal muscle-specific *Ogt* knockout mice have increased glucose uptake, insulin signaling and whole-body insulin sensitivity (62). Likewise, inhibition of *OGA* with *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-phenylcarbamate (PUGNAc) induces insulin resistance in

3T3-L1 and rat primary adipocytes by perturbing both insulin-signaling pathway and glucose absorption (17, 21). Indeed, blockade of OGA increases O-GlcNAcylation of IRS-1 and AKT while decreasing their phosphorylation (17, 21). Consequently, a reduction of insulin-stimulated PI3K/IRS-1 interaction, GSK3 $\beta$  phosphorylation (15, 17, 64) and GLUT4 translocation to the plasma membrane is observed (17, 21). This reduced translocation of GLUT4 might be related to the decrease in AKT phosphorylation since phosphorylation of AKT substrate of 160 kDa (AS160) is required for insulin-stimulated translocation of GLUT4 to the plasma membrane (66). Reciprocally, in euglycemic HepG2 and mice hepatic cells, the reduced global O-GlcNAcylation levels induced by OGA overexpression is associated with an increase of AKT activation but not of PI3K (50). This results in inhibition by phosphorylation of GSK3 $\beta$  (Ser9) and FoxO1 (Ser166), leading to a decrease of gluconeogenic genes transcription, including *glucose-6-phosphatase (G6Pase)* and *phosphoenolpyruvate carboxykinase (PEPCK)* (50). In addition, glycogen synthase (GS), substrate of GSK3 $\beta$ , is also O-GlcNAc-modified in 3T3-L1 cells and this modification blocks its activation, which is associated with insulin resistance (67). These data clearly establish the impact of O-GlcNAcylation in the etiology of insulin resistance and, thus, potentially in metabolic related diseases such as diabetes.

However, there are studies showing that OGA inhibition does not cause insulin resistance in 3T3-L1 adipocytes, rat liver and muscle (68, 69), while others show that OGA inhibition induces insulin resistance in rat skeletal muscle in an AKT and GSK3 $\beta$ -independent manner (70). In these studies, authors suggest that conflicting primary results might result from the use of the non-selective OGA inhibitor PUGNAc which has been shown to also inhibit lysosomal hexosaminidases and alter plasma membrane oligosaccharide structures that are critical in signal transduction (71, 72). These contradictory findings could also be due to supraphysiological concentrations of insulin (12 nmol/L) used for stimulation (70). These findings raise the question of whether high O-GlcNAcylation levels are responsible for insulin resistance and show to what extent the understanding of the role of O-GlcNAcylation in cell signaling regulation in such multifactorial disease needs to be deepened.

In addition to its role in insulin resistance, O-GlcNAcylation could also take part in pancreatic islet  $\beta$  cell dysfunction. Pancreatic  $\beta$  cells are the cells in the body in charge of producing, storing and releasing insulin upon increased blood glucose concentration; its dysregulation is a cause of diabetes. OGT and global O-GlcNAcylation levels are increased in pancreatic islets of Goto-Kakizaki diabetic rats (73). In murine pancreatic  $\beta$  cells, glucosamine-mediated hyperglycemia increases O-GlcNAcylation of AKT and concomitantly reduces its Ser473 phosphorylation (27). Glucosamine induces  $\beta$  cells apoptosis likely through O-GlcNAc-mediated inhibition of AKT (27) (Figure 2). In contrast,  $\beta$  cell-specific *Ogt* knockout mice develop  $\beta$  cell failure and diabetes. In this model, a reduction of AKT phosphorylation at Ser473 was observed (74). These data suggest that the phospho/O-GlcNAc interplay on AKT may play a pivotal role as a regulator of downstream signaling cascades in response to nutrient conditions. The impact of

O-GlcNAcylation dysregulation may be tissue-specific (75). In conclusion, increased O-GlcNAcylation in diabetes toward PI3K/AKT-mediated insulin resistance in target tissues could contribute to the maintenance of the pathology.

## Cardiovascular Diseases

Many studies suggest that elevated protein O-GlcNAcylation levels contribute to cardiovascular complications (76). Chronic hyperglycemia is a risk factor for cardiovascular diseases and patients with diabetes may develop atherosclerotic carotid plaques with a marked increase of O-GlcNAcylation levels (23). Aorta from streptozotocin-induced hyperglycemic mice exhibits high levels of O-GlcNAcylation and impaired vascular sprouting (77). Endothelial dysfunction is a feature of cardiovascular diseases that is characterized by reduced bioavailability of nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS). Endothelial production of NO plays indeed a key role in preventing vascular diseases by preventing thrombosis, inflammation, vascular tone, and remodeling (78). O-GlcNAc modification is known to modulate NO production in endothelial cells, promoting macro- and microvascular complications. In response to insulin, AKT induces vasodilatation in primary human aortic endothelial cells (HAEC), and it may exert anti-atherogenic effects by increasing activating phosphorylation of eNOS at Ser615 and Ser1177 (79) (Figure 2). Federici and collaborators showed that hyperglycemia or HBP activation decreases eNOS activity through a reduction of AKT and eNOS phosphorylation in human coronary artery endothelial cells (HCAEC) (23). *In vitro*, glucosamine-induced protein O-GlcNAcylation also modulates the angiogenic properties of EA.hy926 endothelial cells, most probably by a concomitant increase of AKT O-GlcNAcylation that leads to inhibition of its pro-angiogenic activity (77). AKT could directly up-regulate the production of the pro-angiogenic factor NO (80). In addition, PI3K/AKT/mTOR signaling pathway stimulates angiogenesis by increasing expression of *HIF-1 $\alpha$*  and its target, the *vascular endothelial growth factor (VEGF)* (80) (Figure 2). Elevated O-GlcNAcylation levels also induce vascular calcification *in vitro* in murine cells, and *in vivo* in aortic arc and descending aorta of diabetic mice. It has been shown, in primary mouse vascular smooth muscle cells (VSMC), that this process results from increased Thr430/Thr479-AKT O-GlcNAcylation, which promotes its activation and the expression of *osteogenic runt-related transcription factor 2 (Runx2)* (81). AKT-mediated Runx2 stabilization by degradation of E3 ubiquitin ligase SMURF2 or by the nuclear exclusion of its transcription regulators FoxO could take part in this mechanism (82) (Figure 2). Thus, angiogenesis impairment and vessel obstruction are among the biological effects related to aberrant O-GlcNAcylation of AKT-mediated signaling involved in cardiovascular complications associated with diabetes.

## Neurodegenerative Diseases

Dysregulated O-GlcNAcylation has been implicated in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (83). Neurofibrillary degeneration associated with aggregation of abnormal

hyperphosphorylated tubulin-associated unit (Tau) proteins is one of the features of AD. The latter undergoes a “Ying-Yang” competition mechanism between O-GlcNAcylation and phosphorylation (83). Using thiamet-G, a blood-brain barrier-permeable OGA inhibitor, several *in vivo* studies evidenced the ability of O-GlcNAcylation to protect against Tau aggregation (84–86). Increased levels of O-GlcNAcylation in mice brain by intracerebroventricular injection of thiamet-G is associated with Tau site-dependent increased and decreased phosphorylation further confirming the complex relation between modifications on Tau protein (87). Elevated phosphorylation of Tau at Ser199, Ser202, Ser396, and Ser422 is likely to result from the combination of increased Tau O-GlcNAcylation, PI3K-independent inhibition of Ser473-AKT phosphorylation and the subsequent over-activation of GSK3 $\beta$ , a key Tau kinase (87) (**Figure 2**). Elevated O-GlcNAcylation of proteins is found in Parkinson’s disease postmortem brains (88). In rat primary cortical neurons, thiamet-G treatment increases accumulation of  $\alpha$ -synuclein, a neuronal protein that aggregates in this pathology, through activation of mTOR and reduction of autophagy (88) (**Figure 2**). Conversely,  $\alpha$ -synuclein is O-GlcNAcylated at Thr72 and Ser87, leading to reduced aggregation *in vitro* (89, 90). But, these discrepancies could be due to different experimental approaches. Evidence that excessive O-GlcNAcylation is detrimental to neurons by increasing  $\alpha$ -synuclein accumulation was demonstrated *in vitro* and related to mTOR pathway (88), while O-GlcNAc-reduced aggregation of  $\alpha$ -synuclein was demonstrated by biochemical approaches (89, 90). Taken together, these results indicate that the mitigation of pathological aggregation of neuronal proteins by direct O-GlcNAc modification is a complex mechanism that could be indirectly counterbalanced by AKT/mTOR signaling pathway.

Another common pathological hallmark of neurodegenerative diseases is the loss of neurons as a consequence of neuronal cell death (91). Although not yet studied in such pathological conditions, indirect evidence suggests that O-GlcNAcylation could be involved in the regulation of neuronal apoptosis. Elevation of protein O-GlcNAcylation after cerebral ischemia is responsible for O-GlcNAc-mediated AKT inhibition, BAD activation and neuronal apoptosis in mice (25). An increase of O-GlcNAcylation levels is also associated with a default in Thr308-AKT phosphorylation and cellular apoptosis during cortical differentiation of human embryonic stem cells (hESC) (92) (**Figure 2**). These studies strongly support that O-GlcNAc-mediated AKT inhibition might be involved in neuronal cell loss of function and apoptosis in neurodegenerative diseases.

## CONCLUSION

Highlighted by the studies discussed above O-GlcNAcylation and the PI3K/AKT/mTOR signaling pathway appear to be intimately cross-linked. Both are considered as metabolic sensor that regulate folding, stability, subcellular localization, partner interaction, and therefore the activity of a plethora of targets

involved in key biological functions. Here, we summarized evidence that O-GlcNAcylation can modulate the activation of the PI3K/AKT/mTOR signaling pathway by targeting different signaling actors, and that, reciprocally; expression, localization and activation of OGT are regulated by these signaling pathways (**Figure 1**). Although, further works are required to clarify the roles of O-GlcNAcylation on PI3K/AKT/mTOR regulation under normal physiological context, their interplay is highlighted by their associated dysregulation in several types of cancer, T2D, and cardiovascular and neurodegenerative diseases (**Figure 2**). Under pathological glucose conditions, aberrant O-GlcNAcylation levels result in activation or inhibition PI3K/AKT/mTOR signaling pathway as found in cancer and diabetes, respectively. Because of the key role of the PI3K/AKT/mTOR signaling pathway in cellular metabolism and physiology, these regulatory mechanisms contribute to pathogenicity by promoting, on one hand, glycolysis, proliferation, growth and invasion of cancer cells, and on the other hand, insulin resistance in insulin target tissues and/or pancreatic  $\beta$  cell dysfunction and death. Moreover, O-GlcNAc-mediated disturbance of AKT activity in endothelial cells leads to impairment of angiogenesis and vessel obstruction, supporting cardiovascular diseases associated with T2D. Finally, O-GlcNAcylation regulation of the PI3K/AKT/mTOR signaling pathway can indirectly modulate aggregation of neuronal proteins, such as Tau and  $\alpha$ -synuclein that are involved in AD and Parkinson’s disease, respectively, as well as in neuronal cell death. Taken together, evidence presented here shows that targeting OGT or OGA with selective small molecules to inhibit their activity or their interaction with specific actors of the PI3K/AKT/mTOR signaling pathway, in association with an adapted diet, may be a promising combined therapeutic approach to treat chronic metabolic-related diseases.

## AUTHOR CONTRIBUTIONS

NV and IE conceived the plan and wrote the review. A-SV-E, TL, and SH revised it critically for important intellectual content.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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