O-GlcNAcylation: a bridge between glucose and cell differentiation

Chao Sun a,#, Jin Shang a,#, Yuan Yao a, Xiaohong Yin b, Minghan Liu a, Huan Liu a,*, Yue Zhou a,*

a Department of Orthopedics, Xinqiao Hospital, Third Military Medical University, Chongqing, China
b Center for Evidence-based and Translational Medicine, Zhongnan Hospital, Wuhan University, Wuhan, China

Received: October 12, 2015; Accepted: January 8, 2016

Abstract

Glucose is the major energy supply and a critical metabolite for most cells and is especially important when cell is differentiating. High or low concentrations of glucose enhances or inhibits the osteogenic, chondrogenic and adipogenic differentiation of cell via the insulin, transforming growth factor-β and peroxisome proliferator-activated receptor γ pathways, among others. New evidence implicates the hexosamine biosynthetic pathway as a mediator of crosstalk between glucose flux, cellular signalling and epigenetic regulation of cell differentiation. Extracellular glucose flux alters intracellular O-GlcNAcylation levels through the hexosamine biosynthetic pathway. Signalling molecules that are important for cell differentiation, including protein kinase C, extracellular signal-regulated kinase, Runx2, CCAAT/enhancer-binding proteins, are modified by O-GlcNAcylation. Thus, O-GlcNAcylation markedly alters cell fate during differentiation via the post-transcriptional modification of proteins. Furthermore, O-GlcNAcylation and phosphorylation show complex interactions during cell differentiation: they can either non-competitively occupy different sites on a substrate or competitively occupy a single site or proximal sites. Therefore, the influence of glucose on cell differentiation via O-GlcNAcylation offers a potential target for controlling tissue homeostasis and regeneration in ageing and disease. Here, we review recent progress establishing an emerging relationship among glucose concentration, O-GlcNAcylation levels and cell differentiation.

Keywords: glucose O-GlcNAcylation cell differentiation osteogenic differentiation chondrogenic differentiation adipogenic differentiation

Introduction

Glucose is a central source of energy and an important metabolite for all organisms. Other simple sugars and related molecules derived from sugars provide sources of energy for cells. Glucose also participates in the biosynthesis of polysaccharides, lipids, proteins and nucleic acids and the glucose concentration in the microenvironment, both in vitro and in vivo, markedly affects cell gene expression, proliferation, apoptosis and differentiation [1–3].

#Chao Sun and Jin Shang are co-first authors. They contributed equally to this work.
*Correspondence to: Huan LIU
E-mail: 20016040@163.com
Yue ZHOU.
E-mail: happyzhou@vip.163.com2

© 2016 The Authors.
Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

doi: 10.1111/jcmm.12807
homeostasis and other cellular processes. The addition and removal of O-GlcNAc is sensitive to metabolic status [5, 6], altering the level of uridine 5'-diphosphate-GlcNAc (UDP-GlcNAc) to activate O-GlcNAcylation via the hexosamine biosynthetic pathway (HBP). In addition to the metabolic status, the removal of O-GlcNAc is also regulated by the response of OGA to O-GlcNAcylation levels (Fig. 1). O-GlcNAcylation contributes to diverse intracellular functions via an assortment of targeted isoforms of enzymes in O-GlcNAc and is critical to transcription, proliferation, differentiation and apoptosis [7]. The O-GlcNAc pathway regulates many important cellular pathways, including the insulin, transforming growth factor β (TGF-β) and mitogen-activated protein kinase (MAPK) signalling pathways. Lastly, O-GlcNAcylation controls cells differentiation through the proteins and signalling pathways mentioned above in response to stress or changes nutrient levels.

The concentrations of glucose in common culture media range from 1.0 to 4.5 g/l (5.6–25 mM) [2]. Specifically, based on conventional serum glucose levels, a glucose concentration of 5.5 mM is equal to approximately 0.99 g/l. Glucose concentrations of 11 mmol/l (1.98 g/l) or above are considered hyperglycaemic conditions. However, the higher end of the glucose concentration range (20–30 mM) is nearly equivalent to glucose levels of 3.6–5.4 g/l in clinical measurements. Regarding glucose concentrations related to cell culture medium, 5.5 mM is generally considered a low-glucose culture medium, whereas 25 mM is considered a high-glucose culture medium. The concentrations of glucose that is most often recommended and used for maintaining stem cell in culture is 5.5 mM, and is also called normal glucose [8].

During chondrogenic differentiation, cells migrate into the limb field and undergo a phenomenon termed ‘pre-cartilaginous condensation’. The chondrocytes in the centre of the cartilaginous templates are stimulated to proliferate and then proceed through stages of maturation and hypertrophy. In the region of hypertrophy the chondrocytes are replaced by invading osteoblasts and the tissue is replaced by bone and bone marrow [9]. And the stage of maturation of an osteocyte, which includes pre-osteoblast proliferation, matrix formation and maturation and extracellular matrix mineralization from
Chondrogenic differentiation

Glucose concentration affects chondrogenic differentiation

The growth, development and structural integrity of joint are dramatically affected by the transport of glucose into chondrocytes and through articular cartilage [12, 13]. In chondrogenic differentiation, glucose is the main precursor and a critical energy source for the synthesis of the extracellular matrix (ECM) and glycosaminoglycans [14–16]. Thus, the concentration of glucose is essential for chondrocyte matrix synthesis, viability and differentiation. Studies have demonstrated that high concentrations of glucose reduce the chondrogenic potential of human mesenchymal stem cells (MSCs) [17], muscle-derived stem cells [18], and adipose tissue-derived MSCs (ASCs) [19]. And, low concentrations of glucose have been reported to increase the chondrogenic potential of MSCs [20].

Studies in vitro and vivo have linked hyperglycaemia with local and systemic toxicities relevant to OA, caused by high-glucose concentration [21]. Hyperglycaemia decreases transport of dehydroascorbate into chondrocytes, compromising the synthesis of type II collagen and increasing levels of reactive oxygen species (ROS) and inflammatory mediators to mediate cartilage destruction [22, 23]. Insulin-like growth factor-1 (IGF-1) and insulin play an important role in chondrogenic differentiation. Insulin-like growth factor-1 stimulates the chondrogenic differentiation of MSC into chondrocyte pre-hypertrophic and hypertrophic chondrocytes by stimulating proliferation, regulating cell apoptosis, inducing expression of chondrocyte markers and enhancing extracellular matrix biosynthesis [24, 25]. Insulin is structurally similar to IGF-1 and can activate the IGF-1 receptor, and insulin has been shown to be an essential additive for chondrogenic differentiation of mesenchymal progenitor cells [26]. Previous studies have shown that there is an accumulation of O-GlcNAcylated proteins in the cartilage of human osteoarthritic patients [27]. It has been shown that the expression and activity of matrix metalloprotease (MMP) 2 and MMP9 [28] and the progression of chondrogenic differentiation [29] are enhanced by OGA inhibition, which could increase the intracellular level of O-GlcNAcylation. In addition, it has been reported that insulin and thiamet-G (an inhibitor of OGA) produce a obvious difference in the activation proteoglycan synthesis although little difference in the extent of differentiation markers inductions in ATDC5 cells. Then, the mechanisms by which glucose and O-GlcNAcylation influences chondrogenic differentiation are discussed below.

Activation of TGF-β signalling pathway is critical for chondrogenic differentiation of MSCs [30]. High-glucose culture induces hypertrophy of mouse embryonic fibroblasts and rat kidney epithelial cells by up-regulating TGF-β signalling pathway [31]. High-glucose culture also modulates PKC activity to up-regulate the expression of TGF-β receptor expression of vascular smooth muscle cells [32]. Mesenchymal stem cells cultured in high glucose prior to differentiation show decreased chondrogenesis [19]. High-glucose expansion culture reduces PKC activity to chondrogenic induction, resulting in down-regulating the expression of TGFβRII in MSCs. Then TGF-β signalling upon the activation of TGF-β ligand was decreased by the reduced TGFβRII, further leading to reduced chondrogenesis [17] (Fig. 1).

However, in another report, high concentrations of glucose was shown to enhance chondrogenesis in chick mesenchymal cells. High glucose has been shown to up-regulate p38 and down-regulate extracellular signal-regulated kinase (ERK) activity through PKCα, priming the stimulation of chondrogenic differentiation by modulating the expression of adhesion molecules [33] (Fig. 2). In addition, chondrogenesis might be modulated by complex protein kinase signalling cascades, including those downstream of ERK [34], PKC [35] and p38 [36]. The expression levels of cell adhesion molecules, including fibronectin, N-cadherin and α5β1 integrin are positively regulated by PKC in mesenchymal cells [34–36]. Extracellular signal regulated kinase negatively modulates chondrogenesis by altering the expression of cell adhesion molecules, whereas p38 plays an opposite role at the post-pre-cartilage condensation stage [36] (Fig. 2). Activation of p38 is necessary for the accumulation of sulphated proteoglycans and cellular condensation. In addition, long-term effect of high-glucose concentration on human media artery smooth muscle cells down-regulates of basal RAC-α serine/threonine-protein kinase (Akt) phosphorylation, while acute stimulation of cells in high glucose with insulin-activated Akt [37]. The different effect of high glucose on MSCs and chick mesenchymal cells may be caused by different cell and induction (TGF-β/insulin). And it remains to be further exploring.

At last, high concentration glucose can also increase the formation of advanced glycation end-products (AGEs) in diabetes or in vitro models [38]. It is reported that the proteoglycan synthesis and degradation of articular cartilage were negatively affected by an increase in AGE levels in OA patients [39]. And chondrogenic differentiation in AGE-2–treated or AGE-3–treated MSCs were inhibited [40].

O-GlcNAcylation affects chondrogenic differentiation

Early hypertrophic chondrocytes accumulate glycogen occurs during the maturation phase, and it seems plausible that proteins can be O-GlcNAcylated during chondrogenic differentiation [12]. Recent findings demonstrate MAPK, ERK1/2 and p38 could be O-GlcNAcylated [41, 42]. Insulin and thiamet-G can induce increases in p-MAPK, p-ERK1/2 and p-p38 in some cell types [43, 44]. However, thiamet-G and other OGA inhibitors failed to induce Akt phosphorylation [45]; intriguingly, some studies have shown that Akt phosphorylation is critical for insulin-induced proteoglycan synthesis in chondrocytes [46] (Fig. 2).

Previous studies show there is an extensive cross-talk between O-GlcNAcylation and the phosphorylation of Akt, with both modulating its...
function [47, 48], and it has been proposed that Akt O-GlcNAcylation and phosphorylation can be simultaneously induced [45]. Same site competition, proximal site competition and proximal site occupation are interrelationships between O-GlcNAcylation and phosphorylation. The balance between O-GlcNAcylation and phosphorylation can change the cellular function of the protein [49]. O-GlcNAcylation of Akt has no effect or a stimulating effect on its enzymatic activity but did not inhibit its phosphorylation [48]. Furthermore, Akt O-GlcNAcylation was even more intense when Akt phosphorylation was activated in insulin-induced chondrogenic differentiation. However, some reports have shown that decreases in Akt phosphorylation and/or Akt activity is correlation with an increase in Akt O-GlcNAcylation [50–52] (Fig. 2). O-GlcNAcylation not only modulates Akt activity but also modulates the cellular distribution of the enzyme. Such processes may induce further changes in the targets of Akt [45]. PKC also plays key regulatory roles in major signal transduction pathways controlling a wide range of biological responses including gene expression, cell morphology, proliferation and differentiation [53]. It is reported that all PKC isozymes are dynamically modified by O-GlcNAc, and O-GlcNAc modifications correlate negatively with PKCα activity in rat hepatocytes [54].

### Osteogenic differentiation

**Glucose concentration affects osteogenic differentiation**

Bone is affected by diabetes in both humans and animal models, leading to osteoporosis and osteopaenia [55–58]. Diabetes alters biochemical markers [59] and mineral density of bone in humans, and the poor glycaemic control in diabetes mellitus contributes to reduced bone mass and frequently to fractures. We therefore attribute this complication to the high blood glucose concentrations in...
diabetic patients. Indeed, glucose is reported to have a direct activating effect on osteoclasts and acts as a principal energy source for osteoclastic bone resorption [60]. It is reported that glucose inhibits collagen fibril formation and subsequent cross-linking in human osteoblast-like cells in vitro [61]. Furthermore, the osteoclastic cell proliferation-induced production of IGF-1 and the basal and osteocalcin secretion-induced production of 1,25(OH)2D are inhibited in human MG-63 cells in a high-glucose environment in vitro [62, 63].

In recent decades, scientists have paid increasing attention to the influence of glucose on cells. It has been reported that the proliferation and differentiation of MSCs, which are the common starting point in the development of osteoblasts, are down-regulated in the streptozotocin-induced diabetic mouse [30, 64, 65]. Furthermore, high-glucose concentrations reduce the osteogenic potential of human MSCs [20], mouse bone marrow-derived MSCs [66-68] and ASCs [19], along with subsequent diminished mineralization. In addition, low-glucose media leads to a higher degree of differentiation by human bone marrow MSCs and mouse MSCs compared with osteocytes in normal- and high-glucose media [69, 70]. Another report demonstrated that glucose restriction increases the osteogenic capacity of mouse MSCs in vitro.

High concentrations of glucose alter the differentiation of MSCs into osteoblast lineages and their mineralization into nodules. High glucose also interferes with the formation and mineralization of the extracellular matrix. The deleterious effect of high glucose on BMSC-derived osteoblast proliferation and function can be ameliorated by insulin [66], which controls blood glucose levels and maintains the levels of vitamin 1, 25(OH)2D, IGF-1, and parathyroid hormone (PTH) to indirectly regulate bone development and formation in patients and rats in vivo [71–73]. Furthermore, insulin treatment of human and mouse osteoblasts down-regulates apoptosis, increases the presence of transporter molecules, induces the synthesis of collagen and insulin-like growth factor-binding protein-3 (IGFBP-3), increases proliferation and sensitizes cells to PTH [74–79]. Finally, glucose regulates the distribution pattern of insulin receptors in MSCs during osteogenic differentiation.

Runt-related transcription factor-2 (Runx2) is a member of the runt-domain gene family of DNA-binding proteins (Runx1, Runx2, Runx3), which control the expression of numerous genes involved in cell growth, proliferation and determination of cell lineage [80]. OSE2 is the specific DNA-binding site for Runx2 [81]. It is reported that high-glucose (11 mmol/l) stimulates Runx2 expression, while higher glucose (44 mmol/l) inhibits Runx2 expression [82]. And high glucose can also enhance phosphorylation of CREB [83]. Long-term incubation of human and mouse osteoblasts with AGEs decreases cellular activity, proliferation, the expression of collagen type I, osteocalcin and IGF-1, alkaline phosphatase (ALP) activity, and the formation and mineralization of the ECM [84–86]. Advanced glycation end-products increase ALP activity and intracellular calcium content.

| Table 1 | Expression of proteins in MSCs under normal glucose (5.5 mM) and low glucose (1.4 mM) conditions during osteogenic differentiation |
|---------|-----------------------------------------------------------------------------------------------------------------|
| Protein | MW (kD) | Characteristic | Regulation | Reference |
| Aldehyde dehydrogenase | 57.6 | Protecting or detoxifying enzyme; preserves stem cells from cytotoxic effects | Up | [70] |
| Prolyl 4-hydroxylase alpha subunit | 61.1 | Intracellular enzyme; required for synthesis and formation of all known types of collagen | Up | [71] |
| Laminin binding protein | 31.9 | Extracellular protein; affects cell-substratum attachment, spreading, migration, differentiation, proliferation, and neurite outgrowth | Down | [63] |
| Mutant beta-actin | 42.1 | Cytoskeletal protein; participates in muscle contraction, cell motility, cytokinesis, vesicle and organelle movement, cell signalling, establishment and maintenance of cell junctions and cell shape | Down | [64] |
| Sec 12 protein | 80 | Guanine nucleotide exchange factor; promotes the recruitment of COPII vesicle coats and cargo selection | Down | [65] |
| Alpha soluble N-ethylmaleimide sensitive fusion protein | 33.7 | Homohexameric AAA ATPase; a central component of the cellular machinery in the transfer of membrane vesicles from one membrane compartment to another | Down | [66] |
| Manganese superoxide dismutase | 24.9 | Vesicle coats and cargo selection | Down | [67] |
| Proteasome alpha 1 subunit | 29.8 | Intracellular protein; modifies proteasome | Down | [68] |
| Ribosomal protein S12 | 14.9 | Locates in the cytoplasm; belongs to the S12E family of ribosomal proteins | Down | [69] |

© 2016 The Authors. Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.
while decrease mineralization and mature bone nodule formation in MSCs differentiation [40].

Hyun-Jung et al. used two-dimensional electrophoresis for a proteomic analysis of proteins in MSCs affected by calorie restriction [70] and found seven proteins to be down-regulated: laminin-binding protein [87], mutant beta-actin [88], Sec-12 protein [89], alpha soluble N-ethylmaleimide-sensitive fusion protein (SNAP) [90], manganese superoxide dismutase (MnSOD) [91], proteasome alpha 1 subunit [92] and ribosomal protein S12 [93]. These authors also observed the up-regulation of three other proteins: aldehyde dehydrogenase (ALDH) [94] and the prolyl 4-hydroxylase alpha (P4HA) [95] subunit, under normal-glucose and low-glucose conditions (Table 1). These proteins are critical for cell division, development, differentiation, protein synthesis, protein folding and assembly and the stress response. The potential of MSCs to differentiate into osteocytes may be influenced by differentially expressed proteins under low concentrations of glucose.

O-GlcNAcylation affects osteogenic differentiation

An increasing number of studies report that the skeleton can act as a nutrient stress sensor that associates bone metabolism, bone mineral homeostasis and whole-body nutrient status through bone-specific endocrine signals or other signalling pathways [96–100]. Among them, the role of protein glycosylation in osteoblast function may indicate that whole-body glucose homeostasis can affects bone metabolism [96, 97, 99, 101, 102].

It has been proposed that dynamic O-GlcNAcylation is sensitive to nutrient status, including extracellular glucose flux, via the HBP [103]. O-GlcNAcylation may act as a nutrient-responsive regulatory mechanism in the skeleton because insulin receptor substrates are O-GlcNAcylated [104], and insulin receptor substrates are critical mediators of insulin/IGF-1 signalling. It has also been reported that many proteins are O-GlcNAcylated in osteoblasts and

Fig. 3 A schematic model illustrating influence of O-GlcNAcylation on osteogenic differentiation. Elevated O-GlcNAc increases osteocalcin transcription via OSE2 and Runx2. IL-1, TGF and BMPs influence the O-GlcNAcylation of Runx2, CBP and CREB via the TAK1 complex to increase BMP2 transcription, with all enhancing osteogenic differentiation.
that the extent of protein O-GlcNAcylation varies during osteoblast differentiation [105]. O-GlcNAcylation of protein may induce osteocalcin. It is evidently based on an observed increase in global protein O-GlcNAc modification, including CREB and TAK1 signalling complex, in osteoblasts cultured in high concentrations of glucose compared to low concentrations of glucose [106]. Thus, O-GlcNAcylation may offer a potential target for controlling bone development at the osteoblast level.

It has been reported that the transcriptional activity of Runx2 is enhanced in osteoblast differentiation via PTH stimulation with an OGA inhibitor [105]. Furthermore, previous studies have demonstrated that elevated O-GlcNAcylation of proteins enhances the expression of differentiation markers in pre-osteoblasts and have suggested that O-GlcNAcylation of Runx2 and osteoblast-specific cis-element 2 (OSE2) contributes to osteoblast differentiation. OSE2 region of the osteocalcin promoter is important for elevated O-GlcNAcylation, priming inducing osteocalcin [107]. Runx2 transcriptional activity is modified by elevated O-GlcNAcylation, and the transcription of osteoblast-specific markers (such as osteocalcin) can be stimulated by the binding of Runx2 to specific enhancer regions of the gene (OSE2). Thus, the transcription of osteocalcin is increased by elevated O-GlcNAcylation and mediated by Runx2 and OSE2 [81, 108] (Fig. 3).

It has been proposed that osteoblast function is regulated by the O-GlcNAcylation of TGF-β-activated kinase-1/MAP3K7-binding protein-1 and -2 (TAB 1/TAB 2), which are associated with the TGF-β-activated kinase 1 (TAK1) signalling node [109]. It is intriguing that TAK1 interferes with osteoblast differentiation by regulating Runx2 activation and its association with the cAMP response element-binding protein (CREB)-binding protein (CBP) [110], a transcriptional co-activator and histone acetyltransferase, plays a crucial role in osteoblast differentiation. CBP is O-GlcNAcylated at its C-terminal domain, at Ser-2360, which is also a phosphorylation site [111, 112]. O-GlcNAcylation and phosphorylation thus may interact at Ser-2360 to affects CBP function. Osteoblast differentiation, mineralization and skeletal development are influenced by TAK1-modulated transcription by enhancing the association between Runx2 and CBP [110].

![Fig. 4](image_url) A schematic model illustrating influence of glucose and O-GlcNAcylation on adipogenic differentiation. High concentrations of glucose enhances adipogenic differentiation through the ERK-mediated PI3K/Akt pathway or the ROS/PKCβ pathway. O-GlcNAcylation of C/EBPα promotes adipogenic differentiation, but O-GlcNAcylation of C/EBPβ inhibits autophosphorylation thereby delaying adipogenic differentiation.
findings demonstrate that TAB 1 [113-115], TAB 2 [109], TAB 3 [113, 114] and TAK1 [113] are O-GlcNAcylated and that TAB 2 is essential for osteogenic differentiation [114]. IL-1, TGF and BMPs stimulate the TAK1 complex (Fig. 3).

It has been proposed that CBP interacts with and regulates the transcriptional activity of Runx2 and CREB and that it also enhances CREB-mediated BMP2 [116]. Post-translational modifications modulate the activity and protein interactions of CBP; as one class of PTM, O-GlcNAcylation modifies CBP, CREB and CREB-regulated transcription coactivator-2 (CRTC2) [117] and OGT has also been shown to co-localize with CREB at unique promoter regions [118]. Although the O-GlcNAcylation of signalling regulator such as CBP is regarded as a mechanism controlling the fate of osteoblast, CBP is also affected by PTH [119], insulin/IGF-1 [116], BMPs [110] and Wnts [120] (Fig. 3).

**Adipogenic differentiation**

**Glucose concentration affects adipogenic differentiation**

Increased adipose accumulation in marrow has recently been shown in a streptozotocin-induced insulin-dependent diabetes mellitus mouse model [121]. As a high level of glucose in the blood is a major characteristic of diabetes, the glucose concentration may have an important influence on adipogenic differentiation. It has been reported that in comparison to a low-glucose culture medium, a high-glucose medium enhances the adipogenesis of mouse muscle-derived stem cells, mouse bone marrow-derived MSCs [122] and human ASCs [18, 19]. And, in another report adipogenic capacity was impaired by transfer to a low-glucose medium [20].

PKC activation and ROS production are crucial steps in adipogenesis, and both processes are induced by high glucose. The neoformation of adipose cells is enhanced by ROS via downstream signalling molecules particularly PKCζ [18], and previous studies have demonstrated that PKC plays a critical role in adipogenic differentiation and diabetes. Additionally, there are close relationship among ROS production, PKC and adipogenesis [65] (Fig. 4). Peroxisome proliferator-activated receptor (PPAR) and CCAAT/enhancer-binding proteins (C/EBPs) are also crucial for adipogenic differentiation [123-125]. A recently study demonstrates that the mRNA and protein levels of C/EBPs and PPARγ were increased during adipocyte differentiation [126]. C/EBPs is key to the production of specific adipogenic genes, and its expression is induced by PPARγ, which is regulated by MEK/ERK signalling pathway and by C/EBPβ during adipogenic differentiation [127], in late-stage adipogenesis. The ERK signalling pathway has both positive and negative functions in the adipocytic differentiation of MSCs. Adipocyte differentiation is regulated at each step by the MAPK signalling pathway [128]. Furthermore, activation of insulin receptor substrate-1 (IRS-1)/phosphatidylinositol 3-hydroxy kinase (PI3K)/Akt plays a crucial role in lipid synthesis stimulated by insulin [129]. The expression of the forkhead transcription factor gene Foxc2 is induced by tumour necrosis factor-α (TNF-α) and insulin via PI3K/ERK1/2 signalling pathways in 3T3-L1 adipocytes [130]. Therefore, high concentrations of glucose enhance the accumulation of lipid in adipogenesis via an ERK1/2-activated PI3K/Akt-regulated PPARγ signalling pathway in mouse bone marrow-derived MSCs [122] (Fig. 4).

| Protein | MW (kD) | Characteristic | Role | Reference |
|---------|---------|----------------|------|-----------|
| Vimentin | 53.7 | Major intermediate filament protein | The arrangement of vimentin intermediate filament changes dynamically from an extended fibrillar state to a complex cage formation tightly associated with the forming lipid droplets during adipocyte differentiation | [118] |
| Pyruvate carboxylase | 130.3 | Enzyme that catalyzes the irreversible carboxylation of pyruvate to form oxaloacetate | Plays a crucial role in gluconeogenesis and lipogenesis, in the biosynthesis of neurotransmitters, and in glucose-induced insulin secretion by pancreatic islets | [119] |
| Ewing sarcoma protein | 68.6 | A member of the TET (TLS/EWS/TAF15) family of RNA- and DNA-binding proteins whose expression is altered in cancer | Affects transcription and RNA processing and plays a role in homologous recombination, DNA damage response and maintenance of genome integrity | [120] |
| Long-chain fatty acid-CoA ligase 1 | 78.9 | Isozyme of the long-chain fatty-acid-coenzyme A ligase family | Plays a key role in lipid biosynthesis and fatty acid degradation | [116] |
| Nucleoporin p62/p98 | 53.2/97.9 | Proteins which are the constituent building blocks of the nuclear pore complex | Mediates transport of macromolecules between the cell nucleus and cytoplasm in eukaryotes | [117] |

© 2016 The Authors.
O-GlcNAcylation affects adipogenic differentiation

Glucose uptake, lipid storage and insulin sensitivity are affected by the activation of HBP via the administration of glucosamine or the overexpression of glutamine 6 fructose phosphate transaminase 1 (GFAT-1) in adipocytes [131–134], and the O-GlcNAcylation of proteins may be intimately connected to this phenomenon. Indeed, recent findings demonstrate that O-GlcNAc-modified proteins are modulated throughout development in a complex pattern. Aberrant O-GlcNAcylation may affect cell differentiation, which may lead to developmental abnormalities [135]. It has been observed that protein O-GlcNAcylation dynamically increases when 3T3-L1 pre-adipocytes are induced to differentiate, and O-GlcNAcylation of protein may play an important role in adipocyte differentiation with this elevation persisting for the entire differentiation period [126]. Furthermore, the formation of lipids in adipocytes is prevented by GFAT-1 siRNA and GFAT-1 inhibitors although a reduction in protein O-GlcNAcylation. The expression of C/EBPβ and PPARγ was reduced by GFAT-1 siRNA treatment in adipocytes, suggesting that the HBP may regulate adipocyte differentiation partly by altering the expression of C/EBPβ and PPARγ. Such findings show that the timing of the increase in O-GlcNAcylation is associated with the timing of C/EBPexpression in adipogenesis and that an inhibitor of GFAT-1 can block the O-GlcNAcylation-induced adipocyte differentiation. Thus, O-GlcNAcylation may play an important role in adipogenic differentiation by altering C/EBPβ expression [136].

Recently, it has been proposed that C/EBPβ O-GlcNAcylation delays adipocyte differentiation [137]. C/EBPβ is sequentially phosphorylated on Thr188/Ser184/Thr179; and C/EBPβThr188 phosphorylation primes phosphorylations on Ser184/Thr179. Phosphorylations on Thr188/Ser184/Thr179 of C/EBPβ are key to the binding activity between C/EBPβ and DNA. C/EBPβ is itself O-GlcNAcylated at Ser180 and Ser181, and the phosphorylation and O-GlcNAcylation sites are very close, both being located in the regulatory domain. O-GlcNAcylation of C/EBPβ inhibits the phosphorylations of itself, but it does not affect its DNA-binding activity. Elevated O-GlcNAcylation of C/EBPβ markedly reduces both the phosphorylation and DNA-binding activity of itself. As a result, elevated C/EBPβ O-GlcNAcylation delays the adipocyte differentiation programme. Furthermore, mutations on Ser180 and Ser181 significantly enhance the transactivation activity of C/EBPβ, indicating that the blockade of O-GlcNAcylation promotes this phosphorylation. In conclusion, O-GlcNAcylation and phosphorylation compete for occupation of adjacent sites to influence C/EBPβ [137] (Fig. 4). Finally, it has also been reported that PPARγ is O-GlcNAcylated during adipocyte differentiation [126]; however, the site of O-GlcNAcylation has not yet been identified. The function of the O-GlcNAcylation of the key regulators in adipocyte differentiation should be studied further.

At last, the O-GlcNAcylation of proteins is global increased in adipogenic differentiation [136], including vimentin, pyruvate carboxylase, ewing sarcoma protein, long-chain fatty acid-CoA ligase 1 [138] and nucleoporin p62/p98 [139], Vimentin [140], pyruvate carboxylase [141] and Ewing sarcoma protein [142] are heavily O-GlcNAcylated during adipocyte differentiation (Table 2). Further studies should be performed to expand our knowledge of the roles of the O-GlcNAcylation of these proteins in adipocyte differentiation.

Conclusions and perspectives

The microenvironment, including glucose level, pH and oxygen level, determines the fate of these cells, and glucose concentration regulates differentiation proficiency. Increasing evidence suggests that O-GlcNAcylation acts as a nutrient sensor that associates the glucose metabolic status with cellular regulation of signal transduction, transcription, protein function and differentiation. The O-GlcNAcylation of signalling molecules involved in glucose metabolism and cell differentiation has recently received greater appreciation, and the roles of this modification to signalling molecules in the cytoplasm, nucleus, and mitochondria in regulating cell differentiation with glucose metabolism constitutes an intriguing area of research. Because glucose concentrations, protein O-GlcNAcylation and cell differentiation affect ageing and diseases, uncovering the underlying functions and mechanisms will be very important for exploring glucose or O-GlcNAcylation as a therapeutic target for diseases.

Acknowledgements

This work was supported by grants from the Natural Science Foundation of China (No’s. 81271982, 81472076 and 81401801). Yue Zhou and Huan Liu outlined the main topics of this article. Xiaohong Yin, Yuan Yao and Minghan Liu, collected the needed articles. Chao Sun and Jin Shang collected and analyzed the needed articles and wrote the manuscript.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

References

1. Hsieh TJ, Fustier P, Zhang SL, et al. High glucose stimulates angiotensinogen gene expression and cell hypertrophy via activation of the hexosamine biosynthesis pathway in rat kidney proximal tubular cells. Endocrinology. 2003; 10: 4338–49.
2. Li YM, Schilling T, Benisch P, et al. Effects of high glucose on mesenchymal stem cell proliferation and differentiation. Biochem Biophys Res Commun. 2007; 1: 209–15.
3. Horie N, Moriya T, Mitome M, et al. Lowered glucose suppressed the proliferation and increased the differentiation of murine neural stem cells in vitro. FEBS Lett. 2004; 1: 3: 237–42.
4. Love DC, Hanover JA. The hexosamine signaling pathway: deciphering the “O-GlcNAc code”. Sci STKE. 2005; 312: re13.
alter the regenerative potential of mesenchymal stem cells. Stem Cells Dev. 2010; 12: 1875–84.
20. Lo T, Ho JH, Yang MH, et al. Glucose reduction prevents replicative senescence and increases mitochondrial respiration in human mesenchymal stem cells. Cell Transplant. 2011; 8: 813–25.
21. Berenbaum F, Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype. Postgrad Med J. 2012; 1038: 240–2.
22. McNulty AL, Stabler TV, Vail TP, et al. Dehydroascorbate transport in human chondrocytes is regulated by hypoxia and is a physiologically relevant source of ascorbic acid in the joint. Arthritis Rheum. 2005; 9: 2567–85.
23. Hiraisha H, Sakai T, Mitsuyama H, et al. Inflammatory effect of advanced glycation end products on human meniscal cells from osteoarthritic knees. Inflamm Res. 2011; 11: 1039–48.
24. Longobardi L, Granero-Mollo F, O’Rear L, et al. Subcellular localization of IRS-1 in IGF-I-mediated chondrogenic differentiation, hypertrophy and bone marrow mesenchymal stem cells. Growth Factors. 2009; 3: 309–20.
25. Longobardi L, O’Rear L, Azaka S, et al. Effect of IGF-I in the chondrogenesis of bone marrow mesenchymal stem cells in the presence or absence of TGF-beta signaling. J Bone Miner Res. 2006; 4: 626–36.
26. Mueller MB, Blunk T, Appel B, et al. Insulin is essential for in vitro chondrogenesis of mesenchymal progenitor cells and influences chondrogenesis in a dose-dependent manner. Int Orthop. 2013; 1: 153–8.
27. Tardio L, Andres-Bergos J, Zachara NE, et al. O-linked N-acetylgalactosamine (O-GlcNAc) protein modification is increased between cartilage in patients with knee osteoarthritis. Osteoarthritis Cartilage. 2014; 2: 259–63.
28. Andres-Bergos J, Tardio L, Larranaga-Vera A, et al. The increase in O-linked N-acetylgalactosamine protein modification stimulates chondrogenic differentiation both in vitro and in vivo. J Biol Chem. 2012; 40: 33615–28.
29. Yuzwa SA, Macauley MS, Heinonen JE, et al. A potent mechanism-inspired O-GlcNACase inhibitor that blocks phosphorylation of tau in vivo. Nat Chem Biol. 2008; 8: 483–90.
30. Pittenger MF, Mackay AM, Beck SC, et al. Multipotential potential of adult human mesenchymal stem cells. Science. 1999; 5411: 143–7.
glomerular mesangial cells. Am J Physiol Endocrinol Metab. 2011; 4: E713–26.

44. Tallent MK, Varghis N, Skorobogatko Y, et al. In vivo modulation of O-GlcNAc levels regulates hippocampal synaptic plasticity through interplay with phosphorylation. J Biol Chem. 2009; 1: 174–81.

45. Gandy JC, Rountree AE, Bijur GN. Akt1 is dynamically modified with O-GlcNAc following treatments with PUGNAc and insulin-like growth factor-1. FEBS Lett. 2006; 13: 3051–8.

46. Starkman BG, Cravero JD, Delcarlo M, et al. IGF-1 stimulation of proteoglycan synthesis by chondrocytes requires activation of the PI 3-kinase pathway but not ERK MAPK. Biochem J 2005; 389: 723–9.

47. Wang S, Huang X, Sun DN, et al. Extensive crosstalk between O-GlcNAcylation and phosphorylation regulates Akt signaling. PLoS ONE. 2012; 5: e37427.

48. Park S, Park S-H, Baek JY, et al. Protein O-GlcNAcylation regulates Drosophila growth through the insulin signaling pathway. Cell Mol Life Sci. 2012; 20: 3377–84.

49. Butikinaree C, Park K, Hart GW. O-linked beta-N-acetylglucosamine (O-GlcNAc): extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress. Biochim Biophys Acta. 2010; 2: 96–106.

50. Vosseller K, Wells L, Lane MD, et al. Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. Proc Natl Acad Sci USA. 2002; 8: 5313–8.

51. Yang XY, Ongusaha PP, Miles PD, et al. Phosphoinositide signalling links O-GlcNAc transferase to insulin resistance. Nature. 2008; 7181: 964–U1.

52. Wang S, Huang X, Sun D, et al. Extensive crosstalk between O-GlcNAcylation and phosphorylation regulates Akt signaling. PLoS ONE. 2012; 5: e37427.

53. Ohno S, Nishizuka Y. Protein kinase C isoforms and their specific functions: prologue. J Biochem. 2002; 2: 509–11.

54. Robles-Flores M, Melendez L, Garcia W, et al. Posttranslational modifications on protein kinase C isoforms. Effects of epinephrine and phorbol esters. Biochim Biophys Acta. 2008; 5: 695–712.

55. Stromeyer ES, Cauley JA, Orchard TJ, et al. Middle-aged premenopausal women with type 1 diabetes have lower bone mineral density and calcaneal quantitative ultrasound than nondiabetic women. Diabetes Care. 2006; 2: 306–11.

56. Hadjidakis DJ, Raptis A, Stakianakis M, et al. Bone mineral density of both genders in Type 1 diabetes according to bone composition. J Diabetes Complications. 2006; 5: 302–7.

57. Botolin S, McCabe LR. Bone loss and increased bone adiposity in spontaneous and pharmacologically induced diabetic mice. Endocrinology. 2007; 1: 198–205.

58. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. Osteoporos Int. 2007; 4: 427–44.

59. Starup-Linde J. Diabetes, biochemical markers of bone turnover, diabetes control, and bone. Front Endocrinol. 2013; 4: 21.

60. Williams JP, Blair HC, McDonald JM, et al. Regulation of osteoclastic bone resorption by glucose. Biochem Biophys Res Commun. 1997; 3: 646–51.

61. Lien Y-H, Stern R, Fu J, et al. Inhibition of collagen fibril formation in vitro and subsequent cross-linking by glucose. Science. 1984; 4669: 1489–91.

62. Inaba M, Terada M, Koyama H, et al. Influence of high glucose on 1,25-dihydroxyvitamin D3-induced effect on human osteoblast-like MG-63 cells. J Bone Miner Res. 1995; 7: 1050–6.

63. Terada M, Inaba M, Yano Y, et al. Growth-inhibitory effect of a high glucose concentration on osteoblast-like cells. Bone. 1998; 1: 17–23.

64. Weiss RE, Reddi AH. Influence of experimental diabetes and insulin on matrix-induced cartilage and bone differentiation. Am J Physiol. 1990; 8: E200–7.

65. Stolzing A, Sellers D, Llewelyn O, et al. Diabetes induced changes in rat mesenchymal stem cells. Cells Tissues Organs. 2010; 6: 453–65.

66. Gopalakrishnan V, Vignesh RC, Arunan J, et al. Oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. Diabetes. 2008; 6: 1526–35.

67. Jager M, Wild A, Lensing-Hoeh S, et al. Influence of different culture solutions on osteoblastic differentiation in cord blood and bone marrow derived progenitor cells. Einfluß verschiedener Kulturvorgänge von Osteoblasten in Knochenmark und Nabelschnurblut. Biomedizinische Technik/Biomedical Engineering. 2003; 9: 241–4.

68. Kim HJ, Ji BR, Kim JS, et al. Proteomic analysis of proteins associated with cellular senescence by calorie restriction in mesenchymal stem cells. In Vitro Cell Dev Biol Anim. 2012; 3: 186–95.

69. Hough S, Russell JE, Teitelbaum SL, et al. Calcium homeostasis in chronic streptozotocin-induced diabetes mellitus in the rat. Am J Physiol. 1982; 6: E451–6.

70. Ikeda K, Matsumoto T, Morita K, et al. The role of insulin in the stimulation of renal 1,25-dihydroxyvitamin D synthesis by parathyroid hormone in rats. Endocrinology. 1987; 5: 1721–6.

71. Bereket A, Lang CH, Biethen SL, et al. Insulin treatment normalizes reduced free insulin-like growth factor-I concentrations in diabetic children. Clin Endocrinol (Oxf). 1996; 5: 321–6.

72. Rosen DM, Luben RA. Multiple hormonal mechanisms for the control of collagen synthesis in an osteoblast-like cell line, MMB-1+. Endocrinology. 1983; 3: 992–9.

73. Hickman J, McElruff A. Insulin sensitizes a cultured rat osteogenic sarcoma cell line to hormones which activate adenyly cyclase. Calcif Tissue Int. 1996; 6: 401–5.

74. Felsenfeld AJ, Iida-Klein A, Hahn TJ. Interrelationship between parathyroid hormone and insulin: effects on DNA synthesis in UMR-106-01 cells. J Bone Miner Res. 1992; 11: 1319–25.

75. Conover CA, Lee PD, Riggs BL, et al. Insulin-like growth factor-binding protein-1 expression in cultured human bone cells: regulation by insulin and glucocorticoid. Endocrinology. 1996; 8: 3295–301.

76. Thomas DM, Maher F, Rogers SD, et al. Expression and regulation by insulin of GLUT 3 in UMR 106-01, a clonal rat osteosarcoma cell line. Biochem Biophys Res Commun. 1996; 3: 789–93.

77. Hill PA, Tumber A, Meikle MC. Multiple extracellular signals promote osteoblast survival and apoptosis. Endocrinology. 1997; 9: 3849–58.

78. Martin JW, Zielenska M, Stein GS, et al. The role of RUNX2 in osteosarcoma oncogenesis. Sarcoma. 2011; 2011: 2972.
81. Komori T. Mechanism of transcriptional regulation by Runx2 in osteoblasts. Clin Calcium. 2006; 5: 801–7.

82. Zhen D, Chen Y, Tang X. Metformin reverses the deleterious effects of high glucose on osteoblast function. J Diabetes Complications. 2010; 5: 334–44.

83. Visavadiya NP, Li Y, Wang S. High glucose upregulates upstream stimulatory factor 2 in human renal proximal tubular cells through angiotensin II-dependent activation of CREB. Nephron Exp Nephrol. 2011; 3: e52–70.

84. McCarthy A, Etchevery S, Cortizo A. Effect of advanced glycation endproducts on the secretion of insulin-like growth factor-1 and its binding proteins: role in osteoblast development. Acta Diabetol. 2001; 3: 113–22.

85. Yamamoto T, Ozono K, Miyauchi A, et al. Role of advanced glycation end products in adynamic bone disease in patients with diabetic nephropathy. Am J Kidney Dis. 2001; 4: S161–4.

86. Sanguineti R, Storace D, Monacelli F, Komori T. Role of advanced glycation end products in osteoblasts in vitro. Ann N Y Acad Sci. 2008; 1126: 166–72.

87. Mecham RP. Receptors for laminin on mammalian cells. FASEB J. 1991; 11: 2538–46.

88. Proccaccio V, Salazar G, Ono S, et al. A mutation of beta-actin that alters depolymerization dynamics is associated with autosomal dominant developmental malformations, deafness, and dystonia. Am J Hum Genet. 2006; 6: 947–60.

89. Simmer F, Moorman C, van der Linden AM, et al. Genome-wide RNAi of C-elegans crx gene mutants: a tissue-specific atlas of mouse. Nucleic Acids Res. 2010; 7: 2457–73.

90. Hornbeck PV, Kornhauser JM, Tachoev S, et al. PhosphoSitePlus: a comprehensive resource for investigating the structure and function of experimentally determined post-translational modifications in man and mouse. Nucleic Acids Res. 2012; 40: D261–70.

91. Nagel AK, Schilling M, Come-Walters S, et al. Identification of O-linked N-acetylglucosamine (O-GlcNAc)-modified osteoblast proteins by electron transfer dissociation tandem mass spectrometry reveals proteins critical for bone formation. Mol Cell Proteomics. 2013; 4: 945–55.

92. Shui C, Spelsberg TC, Riggs BL, et al. Changes in Runx2/Cbfal expression and activity during osteoblastic differentiation of human bone marrow stromal cells. J Bone Miner Res. 2003; 2: 213–21.

93. Cheng Q, Lau WM, Chew SH, et al. Identification of molecular markers for the early detection of human squamous cell carcinoma of the uterine cervix. Br J Cancer. 2002; 2: 274–81.

94. Xu D, Guthrie JR, Mabry S, et al. Mitochondrial aldehyde dehydrogenase attenuates hyperoxia-induced cell death through activation of ERK/MAPK and PI3K-Akt pathways in lung epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2006; 5: L966–75.

95. Grimmer C, Balbas N, Lang U, et al. Regulation of type II collagen synthesis during osteoarthritis by prolyl-4-hydroxylases - Possible influence of low oxygen levels. Am J Pathol. 2006; 2: 491–502.

96. Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. Cell. 2007; 3: 456–69.

97. Ferron M, Wei J, Yoshizawa T, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell. 2010; 2: 296–308.

98. Fulzele K, Riddle RC, DiGirolamo DJ, et al. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. Cell. 2010; 2: 309–19.

99. Yoshikawa Y, Kode A, Xu L, et al. Genetic evidence points to an osteoclast-independent influence of osteoblasts on energy metabolism. J Bone Miner Res. 2011; 9: 2012–25.

100. Fukumoto S, Martin TJ. Bone as an endocrine organ. Trends Endocrinol Metab. 2009; 5: 230–6.

101. Rached M-T, Kode A, Silva BC, et al. FoxO1 expression in osteoblasts regulates glucose homeostasis through regulation of osteocalcin in mice. Journal of clinical investigation. 2010; 1: 357–68.

102. Yoshizawa T, Hinoi E, Jung DY, et al. The transcription factor ATF4 regulates glucose metabolism in mice through its expression in osteoblasts. Journal of clinical investigation. 2009; 9: 2807–17.

103. Zachara NE, Hart GW. O-GlcNACa sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress. Biochim Biophys Acta. 2004; 1: 13–28.

104. Klein AL, Berkaw MN, Buse MG, et al. O-linked N-acetylgalactosamine modification of insulin receptor substrate-1 occurs in close proximity to multiple SH2 domain binding motifs. Mol Cell Proteomics. 2009; 12: 2733–45.

105. Kim SH, Kim YH, Song M, et al. O-GlcNac modification modulates the expression of osteocalcin via OSE2 and Runx2. Biochem Biophys Res Commun. 2007; 2: 325–9.
117. Altarejos JY, Montminy M. CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. Nat Rev Mol Cell Biol. 2011; 3: 141–51.

118. Lamarrre-Vincent N, Hsieh-Wilson LC. Dynamic glycosylation of the transcription factor CREB: a potential role in gene regulation. J Am Chem Soc. 2003; 22: 6612–3.

119. Tyson DR, Swarthing JT, Partridge NC. Increased osteoblastic c-fos expression by parathyroid hormone requires protein kinase A phosphorylation of the cyclic adenosine 3',5'-monophosphate response element-binding protein at serine 133. Endocrinology. 1999; 3: 1255–61.

120. Rodriguez-Carballo E, Ulsamer A, Susperregui AR, et al. Conserved regulatory motifs in osteogenic gene promoters integrate cooperative effects of canonical Wnt and BMP pathways. J Bone Miner Res. 2011; 4: 718–29.

121. Botolin S, Faugere MC, Malluche H, et al. Increased bone adiposity and peroxisomal proliferator-activated receptor-gamma2 expression in type I diabetic mice. Endocrinology. 2005; 8: 3622–31.

122. Chuang CC, Yang RS, Tsai KS, et al. Hyperglycemia enhances adipogenic induction of lipid accumulation: involvement of extracellular signal-regulated protein kinase 1/2, phosphoinositide 3-kinase/Akt, and peroxisomal proliferator-activated receptor gamma signaling. Endocrinology. 2007; 9: 4267–75.

123. Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. Physiol Rev. 1998; 3: 783–809.

124. Selvarajan S, Lund LR, Takeuchi T, et al. A plasma kallikrein-dependent plasminogen cascade required for adipocyte differentiation. Nat Cell Biol. 2001; 3: 267–75.

125. Farmer SR. Transcriptional control of adipocyte formation. Cell Metab. 2006; 4: 263–73.

126. Hsieh TJ, Lin T, Hsieh PC, et al. Suppression of Glutamine:fructose-6-phosphate amidotransferase-1 inhibits adipogenesis in 3T3-L1 adipocytes. J Cell Physiol. 2012; 1: 108–15.

127. Farmer S. Regulation of PPARγ activity during adipogenesis. International Journal of Obesity. 2005; 29: S13–6.

128. Best F, Aouadi M, Caron L, et al. The role of MAPKs in adipocyte differentiation and obesity. Biochimie. 2005; 5: 51–6.

129. Valverde AM, Lorenzo M, Navarro P, et al. Phosphatidylinositol 3-kinase is a requirement for insulin-like growth factor I-induced differentiation, but not for mitogenesis, in fetal brown adipocytes. Mol Endocrinol. 1997; 5: 595–607.

130. Grenning LM, Cederberg A, Miura N, et al. Insulin and TNFα induce expression of the forkhead transcription factor gene Foxc2 in 3T3-L1 adipocytes via PI3K and ERK 1/2-dependent pathways. Mol Endocrinol. 2002; 4: 873–83.

131. McClain DA, Hazel M, Parker G, et al. Adipocytes with increased hexosamine flux exhibit insulin resistance, increased glucose uptake, and increased synthesis and storage of lipid. Am J Physiol Endocrinol Metab. 2005; 5: E673–9.

132. Buse MG. Hexosomes, insulin resistance, and the complications of diabetes: current status. Am J Physiol Endocrinol Metab. 2006; 1: E1–8.

133. Copeland RJ, Bullen JW, Hart GW. Cross-talk between GlcNAcylation and phosphorylation: roles in insulin resistance and glycose toxicity. Am J Physiol Endocrinol Metab. 2008; 1: E17–28.

134. Teo CF, Wollaston-Hayden EE, Wells L. Hexosamine flux, the O-GlcNAc modification, and the development of insulin resistance in adipocytes. Mol Cell Endocrinol. 2010; 1: 44–53.

135. Dehennaut V, Lefebvre T, Leroy Y, et al. Survey of O-GlcNAc level variations in Xenopus laevis from oogenesis to early development. Glycoconj J. 2009; 3: 301–11.

136. Ishihara K, Takahashi I, Tsuchiya Y, et al. Characteristic increase in nucleocytoplasmic protein glycosylation by O-GlcNAc in 3T3-L1 adipocyte differentiation. Biochem Biophys Res Commun. 2010; 3: 489–94.

137. Li X, Molina H, Huang H, et al. N-acetylgalactosamine modification on CCAAT enhancer-binding protein beta: role during adipocyte differentiation. J Biol Chem. 2009; 29: 19248–54.

138. Hisanaga Y, Ago H, Nakagawa N, et al. Structural basis of the substrate-specific two-step catalysis of long chain fatty acyl-CoA synthetase dimer. J Biol Chem. 2004; 30: 3717–26.

139. Davis Li, Blobel G. Nuclear pore complex contains a family of glycoproteins that includes p62: glycosylation through a previously unidentified cellular pathway. Proc Natl Acad Sci USA. 1987; 21: 7552–4.

140. Franke WW, Herget M, Grund C. Rearrangement of the vimentin cytoskeleton during adipose conversion: formation of an intermediate filament cage around lipid globules. Cell. 1987; 1: 131–41.

141. Hu Y, Suarez J, Fricovsky E, et al. Increased enzymatic O-GlcNAcylation of mitochondrial proteins impairs mitochondrial function in cardiac myocytes exposed to high glucose. J Biol Chem. 2009; 1: 547–55.

142. Bachmaier R, Aryee DNT, Jug G, et al. O-GlcNAcylation is involved in the transcriptional activity of EWS-FLI1 in Ewing’s sarcoma. Oncogene. 2009; 9: 1280–4.

© 2016 The Authors.
Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.