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Critical Review

Hyperglycemia and Glucosamine-induced Mesangial Cell Cycle Arrest and Hypertrophy: Common or Independent Mechanisms?

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Summary

The Hexosamine Pathway (HP) is one hypothesis proposed to explain glucose toxicity and the alterations observed during the course of diabetic microvascular complication development. Glucosamine is a precursor of UDP-*N*-Acetylglucosamine (UDP-GlcNAc), the main product of the HP that has often been used to mimic its activation. The transfer of a UDP-GlcNAc residue onto proteins (O-GlcNAc modification) represents the final step of the HP and is considered as a major mechanism by which this pathway exerts its signalling effects. While it is well accepted that the HP promotes extracellular matrix accumulation in the context of diabetic nephropathy, its involvement in the perturbations of cell cycle progression and hypertrophy of renal cells has been poorly investigated. Nevertheless, in a growing number of studies, the HP and O-GlcNAc modification are emerging as important regulators of cell cycle progression. This review will focus on the role of glucosamine and O-GlcNAc modification in cell cycle regulation in the context of diabetic nephropathy. Special emphasis will be given into the role of the HP as a potential mediator of the effects of high glucose on the perturbations of renal cell growth.

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Keywords Glucosamine; hexosamine pathway; O-GlcNAc modification; diabetic nephropathy; cell-cycle; cyclin-dependent kinase inhibitor.

Abbreviations CDK, Cyclin-dependent Kinase; CDKI, Cyclin-dependent Kinase Inhibitor; DCCT, Diabetes Control and Complications Trial; DON, 6-diazo-5-oxo-L-norleucine; ECM, Extracellular Matrix; F-6-P, Fructose-6-Phosphate; GD3, Disialoganglioside 3;

GFAT, Glutamine: F-6-P Amidotransferase; GlcN-6-P, Glucosamine-6-Phosphate; GlcNAc, *N*-Acetylglucosamine; GM1, 2, 3, Monosialoganglioside 1, 2, 3; HP, Hexosamine Pathway; IRS, Insulin Receptor Substrate; OGT, O-GlcNAc Transferase; PAI-1, Plasminogen Activator Inhibitor-1; PDGF, Platelet Derived Growth Factor; PUGNAc, O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenylcarbamate; STZ, Streptozotocin; TGF, Transforming Growth Factor; UKPDS, United Kingdom Prospective Diabetes Study.

INTRODUCTION

The cell cycle of higher eukaryotic cells consists of a succession of four phases: the G₁ phase, when the cell grows and prepares to synthesize DNA by stimulating protein synthesis; the S phase, when DNA synthesis takes place and replicates the whole set of chromosomes; the G₂ phase, in which the cell prepares for division; and the mitotic M phase, in which the division of the original cell into two daughter cells occurs. After division, the cells may reenter the G₁ phase and keep cycling or remain quiescent in a stage called the G₀ phase. Numerous proteins interact in critical ratios to determine the passage from one phase to another and regulate the cell cycle progression. Cyclins, whose protein levels fluctuate throughout the cell cycle, interact with their partners, the cyclin dependant kinases (CDK) to form a complex which subsequently phosphorylates downstream effectors involved in cell cycle transition such as cell-cycle regulated transcription factors, pRb (retinoblastoma protein) or p53. The formation of these complexes is prevented by small proteins called cyclin dependent kinase inhibitors (CDKI) such as p21^{Waf1/Cip1} and p27^{Kip1}.

Cell growth, which is determined by the progression of each cell through the cell cycle, is particularly affected during diabetic nephropathy. Most renal cells, and particularly mesangial cells, undergo growth perturbations, characterized by cell cycle arrest at the G₀/G₁ phase and subsequent hypertrophy (1). These cellular perturbations are accompanied by a progressive accumulation of extracellular matrix (ECM)

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components and contribute to glomerulosclerosis and a decline in renal function. Induction of the cyclin dependant kinase inhibitors p21^{Waf1/Cip1} and p27^{Kip1} is thought to play a crucial role in mesangial cell cycle arrest and hypertrophy. Increased expression of these two proteins has been observed in glomeruli of diabetic db/db (2) and STZ mice (3). Moreover, it has been shown that diabetic mice deficient for p21^{Waf1/Cip1} (4) or p27^{Kip1} (5) genes are protected against the development of glomerular hypertrophy. Several factors such as high glucose levels, TGF β 1, PDGF or angiotensin II have been reported to induce the expression of these inhibitors and cause cell cycle arrest and hypertrophy in renal mesangial cells (1). However, the mechanisms accounting for the perturbations of cell cycle progression induced by hyperglycemia are not fully understood.

Numerous hypotheses have been proposed to explain the toxic effects of hyperglycemia and cellular alterations characteristic of diabetic microvascular complications, including the Hexosamine Pathway (HP). Initially, Marshall et al. reported the HP as a unique metabolic pathway that mediates the glucose-induced desensitization of the insulin-sensitive glucose transport system (6). In fact, after entering the cells, glucose is rapidly phosphorylated and converted to fructose-6-phosphate (F-6-P), which is mainly metabolized through the glycolytic pathway. However, under chronic hyperglycemic

conditions of a diabetic state, a greater part of F-6-P is diverted by the action of glutamine: F-6-P amidotransferase (GFAT), the first and rate-limiting enzyme of the hexosamine biosynthetic pathway, and converted into glucosamine-6-phosphate (GlcN-6-P). GlcN-6-P is then metabolized into various hexosamine products, including the main product UDP-*N*-Acetylglucosamine (UDP-GlcNAc) (7), as illustrated in Fig. 1.

The HP can exert its effects via two described mechanisms. First, hexosamine biosynthesis products provide glycosidic precursors for the synthesis of glycoproteins, glycolipids and proteoglycans. Second, UDP-GlcNAc can be used to covalently modify proteins and affect their functions, through a single *O*-linked glycosylation, referred to as *O*-GlcNAc modification or *O*-GlcNAcylation. These targeted proteins play roles in the control of gene expression, cell growth and division, enzyme activity, or structural integrity of the cytoskeleton (8). Since its first discovery, the role of the HP as a glucose sensor implicated in insulin resistance has been supported by both *in vivo* and *in vitro* studies (9–15), but this concept remains controversial (16).

Additionally, several studies have demonstrated a role of the HP in ECM protein accumulation, characteristic of diabetic nephropathy and a major cause of the decline in renal function. Thus, the expression of several proteins with profibrotic activity and participating in ECM accumulation,

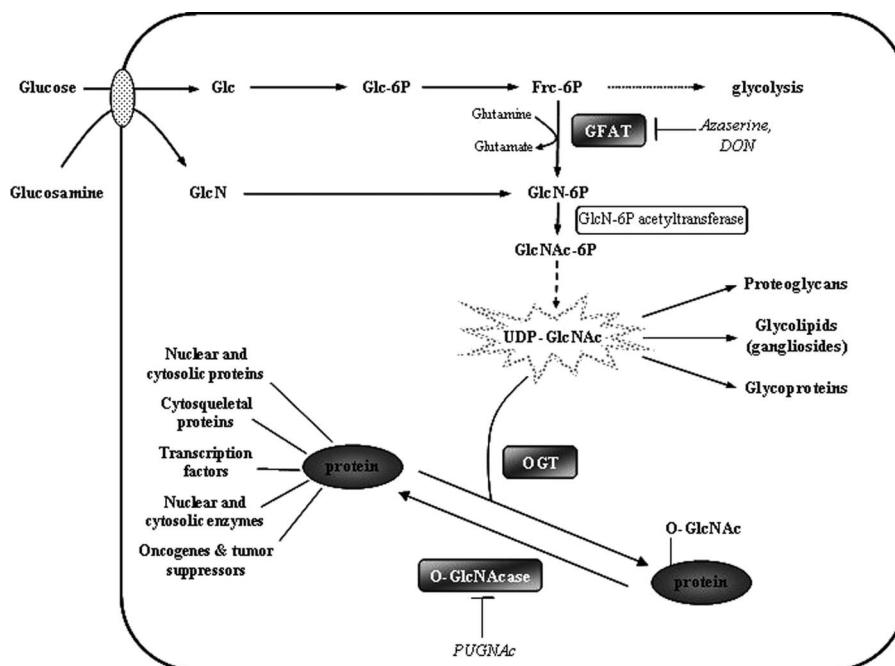


Figure 1. Schematic representation of the Hexosamine Pathway and *O*-GlcNAc modification. DON, 6-diazo-5-oxo-L-norleucine; Frc-6P, fructose 6-phosphate; GFAT, glucosamine: fructose-6-phosphate amidotransferase; Glc, glucose; Glc-6P, glucose 6-phosphate; GlcN, glucosamine; GlcN-6P, glucosamine 6-phosphate; GlcNAc, *N*-acetylglucosamine; OGT, *O*-GlcNAc transferase; PUGNAc, *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-*N*-phenylcarbamate. Dashed arrow indicates that multiple steps are involved in the conversion.

e.g., TGF β ₁ or PAI-1, as well as matrix proteins, such as fibronectin, have been found to be increased in response to the activation of HP (17–20). Moreover, HP inhibition decreases high glucose-induced TGF β expression and the consequent ECM production in renal mesangial cells, which strongly suggests that the HP is a mediator of high glucose effects on ECM accumulation (19).

Finally, recent studies have demonstrated the role of HP and O-GlcNAc modification in cell cycle progression. Although the contribution of high glucose to the regulation of cell proliferation and hypertrophy in the context of diabetic nephropathy is well known, very few studies focused on the involvement of the HP in these events. In fact, one could speculate that the effects of high glucose on cell growth and hypertrophy may actually be mediated, in part, by the HP and glucosamine, as is the case for the much more well documented ECM accumulation. In this brief review we will discuss recent literature that may help answer this question.

CELL CYCLE EVENTS IN RESPONSE TO HIGH GLUCOSE

Two major epidemiological studies (DCCT and UKPDS) have clearly demonstrated that high glucose is a major cause of diabetic complications, including nephropathy, since strict control of the glycemia can delay their onset and progression. It is thought that early glomerular and tubular hypertrophy is critical to the subsequent development of renal histopathological alterations of diabetic nephropathy but the mechanisms that initiate the renal cell changes in response to hyperglycemia are not completely characterized. Nevertheless, it is now well known that glomerular mesangial cells, like most renal cells, undergo a biphasic growth in response to high glucose *in vitro* as well as in the diabetic environment *in vivo* (1). First, these cells, which exhibit a very low proliferation rate under normal conditions, actively enter the cell cycle to complete one or two rounds of cell cycle progression. In a second phase, cells are arrested in G₁ where they increase their size, protein and mRNA content but do not duplicate and thus undergo hypertrophy. A large body of literature indicates that the cyclin-dependent kinase inhibitors p21^{Waf1/Cip1} and p27^{Kip1} play a crucial role in the high glucose-induced cell-cycle arrest and resultant hypertrophy of mesangial cells. Increased expression of both p21^{Waf1/Cip1} (3) and p27^{Kip1} (21) has been associated with mesangial cell hypertrophy in response to high glucose *in vitro*. Moreover, Fan et al. reported that attenuation of p21^{Waf1/Cip1} expression using antisense oligodeoxynucleotides in mesangial cells caused a reduction of high glucose-induced cellular hypertrophy (22). A study from Wolf et al. also indicated that high glucose failed to induce cell cycle arrest and hypertrophy in mesangial cells obtained from p27^{Kip1} knockout mice compared to wild type (23).

Some evidence has suggested that TGF β is a mediator in mesangial cell hypertrophy induced by high glucose, since the

addition of a neutralizing anti-TGF β antibody blunted these effects (24). Moreover, TGF β has been implicated in the high glucose-stimulated p27^{Kip1} expression (21).

Of note, cell cycle arrest at the G₁ phase and hypertrophy in response to high glucose have also been shown in proximal tubular cells. These cells are involved in diabetic nephropathy, by contributing to tubulo-interstitial fibrosis (25).

CELL CYCLE EVENTS IN RESPONSE TO GLUCOSAMINE

In Mesangial Cells

Increasing evidence suggests that glucosamine and the HP may be causally involved in the development of diabetic nephropathy, by promoting ECM protein accumulation. However, the potential role of glucosamine and the HP in the regulation of mesangial cell growth in response to high glucose has been poorly studied.

In a recent study, we investigated the effect of HP activation on mesangial cell growth and hypertrophy (26). Glucosamine is widely used to mimic HP activation by high glucose because of its demonstrated ability to readily enter the cells by the glucose transport system and selectively enter the HP, bypassing the rate-limiting enzyme, GFAT (see Fig. 1). First, we observed in time-course experiments that 0.5 mM glucosamine treatment significantly decreased proliferation and induced hypertrophy of mesangial cells as indicated by an increase of the total protein/cell number ratio starting at 48 h. Given that hypertrophy is marked by increased protein content combined with absent cell division, we next investigated the effect of glucosamine treatment on cell-cycle progression. Flow cytometry analyses indicated that 0.5 mM glucosamine caused an accumulation of mesangial cells at the G₀/G₁ phase, thereby suggesting a blockade of the cell-cycle progression at this phase. These effects are similar to those observed in cells treated with high glucose or in diabetic animal models (1). Finally, we studied the expression of two CDKI, p21^{Waf1/Cip1} and p27^{Kip1}, since these proteins have been implicated in G₀/G₁ arrest and hypertrophy of cultured mesangial cells following exposure to high ambient glucose concentrations as well as in different diabetic animal models (2–5, 21–23). Results indicated that p21^{Waf1/Cip1} expression was approximately increased by 3-fold in response to glucosamine treatment, thereby suggesting that p21^{Waf1/Cip1} may be involved in the cell-cycle arrest observed in mesangial cells exposed to glucosamine. In contrast, p27^{Kip1} expression was found to be diminished in response to glucosamine treatment. While increased expression of p27^{Kip1} has been more frequently reported in mesangial cells treated with high glucose or in glomeruli of diabetic animals, decreased or unchanged expression has also been shown (3). Because glucosamine and high ambient glucose effects are similar, one might speculate that the effects of high glucose on mesangial cell growth and hypertrophy are, at least in part,

mediated through a HP-induced p21^{Waf1/Cip1} pathway. Nevertheless, complementary experiments are needed to provide unequivocal evidence that high glucose effects are actually mediated by the HP.

In Other Cell Types

Hsieh et al. studied the effect of high glucose and glucosamine on the hypertrophy of rat kidney proximal tubular cells (27). The authors reported that both high glucose (25 mM) and glucosamine (1 μ M) treatment for 48 h induced cellular hypertrophy as assessed by a shift in cell size measured by flow cytometry and an increase in total protein synthesis and in p27^{Kip1} expression. Furthermore, it was shown that azaserine, a GFAT inhibitor, prevented the effects of high glucose but not those of glucosamine, thereby suggesting that the hypertrophic action of high glucose on proximal tubular cells is mediated via an activation of the HP. However, these results must be interpreted with caution considering that azaserine is a glutamine analog that can potentially inhibit all glutamine-requiring enzymes. The use of this non-specific inhibitor of GFAT may thus give misleading results regarding the HP.

Additional data obtained in different cell lines have also supported a crucial role for glucosamine in the regulation of cell growth and cell-cycle progression. For instance, Shen et al. reported that treatment of Chinese hamster ovary cells with 10 mM glucosamine led to a decrease in cell proliferation, as assessed by thymidine uptake, and a redistribution of the cells from the S phase into the G₀/G₁ phase (28). Tomida et al. also reported an arrest in the cell-cycle at the G₁ phase in A2780 and HT-29 human cancer cells following glucosamine treatment (29). Furthermore, their results indicated that this blockade was mediated by changes in the expression of two cell-cycle regulating proteins. The expression of cyclin D1 was decreased and the expression of the CDKI p21^{Waf1} was increased and associated with the appearance of the hypophosphorylated growth suppressing form of the retinoblastoma protein (pRb). Nakamura et al. demonstrated that exposure of a model of retinal neurons to high glucose or 1.5 mM glucosamine attenuated the ability of insulin to rescue the cells from apoptosis induced by serum deprivation. Additionally, glucose effects were blocked by azaserine, suggesting that high glucose may impair the protective effects of insulin, at least in part, via excessive flux through the HP (30).

Together, these observations suggest that the effects of high glucose on modulation of cell cycle events could in fact be mediated through activation of the HP.

POTENTIAL MECHANISMS OF ACTION OF GLUCOSAMINE ON CELL CYCLE

O-GlcNAc Modification: The Underlying Mechanism

The HP connects a nutrient-sensing system to a signaling pathway by providing glucosamine-6 phosphate (GlcN-6-P), the key precursor of UDP-N-Acetylglucosamine

(UDP-GlcNAc), the substrate for O-GlcNAc modification. Indeed, O-GlcNAcylation results from the addition of a single UDP-GlcNAc residue on cytosolic and nuclear proteins. This dynamic post-translational modification is rapidly responsive to hormones, nutrients, and cellular stress. It is reciprocal with phosphorylation on some well-studied proteins and is thought to play an analogous role to this modification in cellular regulation (31). It is under the control of two enzymes: the O-GlcNAc transferase (OGT) which adds a GlcNAc residue and O-GlcNAcase which removes it (see Fig. 1). O-GlcNAc modification alters protein-protein interactions, intracellular localization, protein turnover and activity (32). The list of proteins targeted by O-GlcNAcylation is ever growing and includes key intracellular proteins such as transcription factors, cytoskeletal proteins, tumor suppressor and oncogenes, nuclear and cytosolic proteins and enzymes (8).

Accumulating data suggest that global O-GlcNAc protein modification is regulated in a cell-cycle dependent manner and that any disruption of this cycling results in major cell-cycle defects. In this connection, a recent study by Slawson et al. showed that global O-GlcNAc levels decreased during the M phase. In addition, raising the global levels of O-GlcNAc proteins in multiple cell lines using the O-GlcNAcase inhibitor PUGNAc, caused growth delay linked to a blockade of G₂/M progression (33). While excessive O-GlcNAc modification alters cell-cycle progression, intact O-GlcNAcylation is nevertheless necessary for proper cell cycle function. Thus, in the same study, the authors also reported that lowering O-GlcNAc levels with DON, an inhibitor of GFAT, resulted in defective G₁ phase progression. However, non-specific effects of this inhibitor, which is a glutamine analog like azaserine, shouldn't be ruled out. The essential role of O-GlcNAc is supported by the findings of O'Donnell et al. which demonstrated that OGT deletion in fibroblasts caused growth arrest (34). Boehmelt et al. reported a decrease in proliferation in mouse embryonic fibroblasts with decreased UDP-GlcNAc levels as a result of a deficiency of glucosamine-6-phosphate acetyltransferase, an enzyme of the HP (35). The same authors showed that a mutation in this gene is lethal at embryonic day 7.5. These results are also in agreement with the loss of embryonic stem cell viability observed by Shafi et al. following the deletion of the OGT gene (36).

O-GlcNAc modification could be the underlying mechanism explaining cell-cycle progression defects induced by HP activation, through an alteration of protein function, enzyme activity or gene expression by targeting transcription factors. For instance, transcriptional activation of Sp1, a ubiquitous transcription factor, has been reported several times to be induced (37, 38), or inhibited (39) by O-GlcNAcylation. Other transcription factors have also been shown to be modified by O-GlcNAc including cAMP response element-binding protein (CREB) (40) and pancreatic duodenal homeobox (PDX-1) (41). Moreover, O-GlcNAcylation has also been described to target several important participants or regulators of the cell

cycle such as RNA polymerase II, the tumor suppressor p53, pRb, cytoskeletal proteins (8) and IRS (42). O-GlcNAc modification of some proteins is highly transient and can affect their proteasomal degradation (43) and/or modulate their phosphorylation status, two key processes in the regulation of cyclic level and activity of various proteins implicated in the cell-cycle progression. Consistent with this idea, overexpression of both O-GlcNAcase and OGT has been described to disrupt periodic cyclin expression and mitotic phosphorylation, particularly of pRb. Furthermore, overexpression of O-GlcNAcase induces changes in the cellular morphology with excessive condensation of the DNA and dramatic perturbations in the α -tubulin network (33).

Ganglioside Pathway

Glucosamine-6-phosphate can be converted to N-Acetylneuraminic acid and N-Acetylgalactosamine, two precursors of ganglioside biosynthesis. Gangliosides are membrane glycosphingolipids that have been described to play major roles in the regulation of cell proliferation. Therefore, the effects of glucosamine and the HP may involve other mechanisms, such as altered ganglioside synthesis. Using renal mesangial cells, we showed that cell cycle arrest at G₀/G₁ phase, hypertrophy and increased p21^{Waf1/Cip1} expression in response to glucosamine treatment are associated with significant modifications of the pattern of gangliosides. Thus, we observed a massive increase in the levels of two ganglioside species, GM2 (Monosialoganglioside 2) and GM1 (Monosialoganglioside 1) (26). Using radioactively-labelled glucosamine, we showed that exogenous glucosamine or its metabolites are incorporated into the ganglioside biosynthesis pathway. Thus, activation of the HP appears to be responsible for these ganglioside pattern changes by providing a high level of precursors for ganglioside biosynthesis. However, other indirect mechanisms should not be excluded. Indeed, the promoter of several ganglioside biosynthetic enzymes and in particular, GM2 synthase, contains a putative binding site for Sp1, whose activity is regulated by O-GlcNAc modification as discussed above (44).

We further investigated the possibility that GM2 and GM1 could be involved in mediating the glucosamine effects on mesangial cell growth and observed that addition of exogenous GM2 and GM1 caused cell cycle arrest at the G₀/G₁ phase, hypertrophy and an increase of p21^{Waf1/Cip1} expression. These results suggest that glucosamine and gangliosides inhibit mesangial cell proliferation, apparently through similar mechanisms and that GM2 and GM1 could mediate, at least partly, the glucosamine effects. In concurrence with our findings, Lee et al. reported a decrease in cell proliferation and hypertrophy of human mesangial cells in response to exogenous gangliosides (45). Moreover this hypothesis is supported by published data indicating that gangliosides are able to modulate cell-cycle progression. For instance, Nakatsuji et al. showed that ganglioside GM3 (Monosialoganglioside 3) induced cell-cycle arrest of astrocytes, in part through an

increase of p27^{Kip1} expression (46). Overexpression of the GD3 (Disialoganglioside 3) synthase gene has also been shown to cause a G₁ cell cycle arrest in vascular smooth muscle cells via increased p21^{Waf1/Cip1} expression and decreased expression of cyclin E and cyclin-dependent kinase 2 (47).

TGF β

Finally, it has been demonstrated in renal mesangial cells that glucosamine treatment and HP activation induced TGF β 1 expression and subsequent ECM protein synthesis (19, 20). In addition, TGF β appears to be implicated in high glucose-induced mesangial cell cycle arrest and hypertrophy as discussed above. Thus, these data raise the possibility that glucosamine could be involved in high glucose-induced cell growth arrest and hypertrophy via induction of TGF β .

Potential Toxic Action of Glucosamine

When using glucosamine as an experimental reagent, one should not forget that it is a 'pharmacological' agent that can have significant adverse effects. These effects, which are dependent on the dose, duration of treatment and model considered, could mask or mimic specific effects and should be considered in the interpretation of the results.

Hsieh et al. demonstrated that treatment of renal proximal tubular cells for 24 h with glucosamine concentrations from 10⁻⁸ to 10⁻⁴ M stimulated angiotensin II and renin mRNA expression, similarly to the results obtained with high glucose. In contrast, they observed that higher glucosamine concentrations inhibited angiotensin II and renin mRNA expression. Moreover, they showed that these higher glucosamine concentrations increased cell death from 5 to 12%, which suggested that glucosamine, at these concentrations, may be toxic to renal proximal tubular cells (27).

In our recent study, we performed cell cycle analyses of renal mesangial cells treated with increasing glucosamine concentrations (26). Our results indicated that 0.5 mM glucosamine induced an accumulation of cells at the G₀/G₁ phase, similar to the effects that have already been observed in response to high glucose concentrations in other studies. In contrast, treatment of cells with 5 mM glucosamine resulted in a cell cycle blockade at the G₂/M phase. A dose-response study revealed that glucosamine concentrations lower than 1 mM arrest mesangial cell cycle at the G₀/G₁ phase, whereas higher glucosamine concentrations (2–5 mM) induce G₂/M phase arrest. This suggests that different pathways are induced in mesangial cells depending on glucosamine concentration.

Observations from Marshall et al. could explain some of the discrepancies observed between different glucosamine concentrations or with high glucose levels. Indeed the authors showed that treatment of adipocytes with a high glucosamine concentration (2 mM) caused, in addition to an UDP-GlcNAc increase, a rapid and massive accumulation of the hexosamine intermediate product GlcN-6-P that was accompanied by ATP depletion. In contrast, these effects were not observed in

response to low glucosamine (0.25 mM) or to high glucose concentrations (20 mM), which both markedly elevated UDP-GlcNAc without increasing GlcN-6-P or depleting ATP levels (48). ATP depletion is a toxic adverse effect of glucosamine that could account for some of the observations made in response to glucosamine treatment, particularly at high concentrations.

CONCLUDING REMARKS AND PERSPECTIVES

The Hexosamine Pathway and O-GlcNAc modification are emerging fields and their involvement is being reported in more and more diverse processes. While phosphorylation has been a well-known mechanism that regulates cell cycle progression, it is now evident from a growing number of studies that activation of the HP and O-GlcNAc modification play an important role in the regulation of the cell cycle. In addition, recent data reported in this review suggest that this pathway could also mediate the cell cycle arrest and hypertrophy induced by high glucose, particularly in mesangial cells, where it has been shown to mediate ECM production. However, this hypothesis needs to be confirmed by complementary experiments. Indeed, the validity of using glucosamine to investigate the role of the HP has been questioned, especially on the basis of observations suggesting that glucosamine and high glucose act via different mechanisms to induce insulin resistance (16). Glucosamine is a very useful tool to study the HP by mimicking its activation because of its ability to be easily taken up by the cells and selectively enter the HP bypassing the rate-limiting enzyme GFAT. Nevertheless, this pharmacological agent may have toxic effects, such as ATP depletion, depending on the dose and treatment time. Moreover, the compounds commonly used to inhibit the HP, DON and azaserine, are non-specific inhibitors of GFAT. Thus, gene overexpression and deletion experiments should be preferred to get more reliable data on the biological roles of the HP in various cells and tissues. In addition, transgenic animal models would provide very helpful tools to sustain the pathophysiological role of this pathway *in vivo*. In fact, targeted overexpression of GFAT or OGT in mice provided strong evidence on the role of this pathway in insulin resistance (11, 14, 49, 50). Such studies are still needed to fully support the implication of the HP in the development of diabetic nephropathy and to understand its implications in cell cycle progression defects. Alternatively, down-regulation of the enzymes of the HP would also be required. Since complete depletion of some of these genes (OGT and GlcN6P acetyltransferase) results in embryonic lethality (35, 36), conditional knock-out or knock-down approaches may be needed.

Determining the contribution of the HP and O-GlcNAc modification in the dysregulation of cell cycle progression observed in response to hyperglycemia during the course of diabetic microvascular complications is very challenging. Since cell cycle arrest and hypertrophy of renal mesangial cells, among other cells, are causal factors leading to

glomerulosclerosis and renal failure, the understanding of the underlying molecular processes is essential for the development of novel therapeutic strategies. The HP may emerge as a potential target to therapeutically influence the cell cycle and to prevent diabetic nephropathy.

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REFERENCES

1. Wolf, G. (2000) Cell cycle regulation in diabetic nephropathy. *Kidney Int.* **77** (Suppl.), S59–S66.
2. Wolf, G., Schroeder, R., Thaiss, F., Ziyadeh, F. N., Helmchen, U., and Stahl, R. A. (1998) Glomerular expression of p27Kip1 in diabetic db/db mouse: role of hyperglycemia. *Kidney Int.* **53**, 869–879.
3. Kuan, C. J., al Douahji, M., and Shankland, S. J. (1998) The cyclin kinase inhibitor p21WAF1, CIP1 is increased in experimental diabetic nephropathy: potential role in glomerular hypertrophy. *J. Am. Soc. Nephrol.* **9**, 986–993.
4. al Douahji, M., Brugarolas, J., Brown, P. A., Stehman-Breen, C. O., Alpers, C. E., and Shankland, S. J. (1999) The cyclin kinase inhibitor p21WAF1/CIP1 is required for glomerular hypertrophy in experimental diabetic nephropathy. *Kidney Int.* **56**, 1691–1699.
5. Wolf, G., Schanze, A., Stahl, R. A., Shankland, S. J., and Amann, K. (2005) p27(Kip1) Knockout mice are protected from diabetic nephropathy: evidence for p27(Kip1) haplotype insufficiency. *Kidney Int.* **68**, 1583–1589.
6. Marshall, S., Bacote, V., and Traxinger, R. R. (1991) Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. *J. Biol. Chem.* **266**, 4706–4712.
7. Marshall, S. (2002) The hexosamine signaling pathway: a new road to drug discovery. *Curr. Opin. Endocrinol. Diabetes* **9**, 160–167.
8. Comer, F. I., and Hart, G. W. (2000) O-Glycosylation of nuclear and cytosolic proteins. Dynamic interplay between O-GlcNAc and O-phosphate. *J. Biol. Chem.* **275**, 29179–29182.
9. Baron, A. D., Zhu, J. S., Zhu, J. H., Weldon, H., Maianu, L., and Garvey, W. T. (1995) Glucosamine induces insulin resistance *in vivo* by affecting GLUT 4 translocation in skeletal muscle. Implications for glucose toxicity. *J. Clin. Invest.* **96**, 2792–2801.
10. Chen, G., Liu, P., Thurmond, D. C., and Elmendorf, J. S. (2003) Glucosamine-induced insulin resistance is coupled to O-linked glycosylation of Munc18c. *FEBS Lett.* **534**, 54–60.
11. Hebert, L. F., Jr., Daniels, M. C., Zhou, J., Crook, E. D., Turner, R. L., Simmons, S. T., Neidigh, J. L., Zhu, J. S., Baron, A. D., and McClain, D. A. (1996) Overexpression of glutamine: fructose-6-phosphate amidotransferase in transgenic mice leads to insulin resistance. *J. Clin. Invest.* **98**, 930–936.
12. Patti, M. E., Virkamaki, A., Landaker, E. J., Kahn, C. R., and Yki-Jarvinen, H. (1999) Activation of the hexosamine pathway by glucosamine *in vivo* induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle. *Diabetes* **48**, 1562–1571.
13. Rossetti, L., Hawkins, M., Chen, W., Gindi, J., and Barzilai, N. (1995) *In vivo* glucosamine infusion induces insulin resistance in normoglycemic but not in hyperglycemic conscious rats. *J. Clin. Invest.* **96**, 132–140.
14. Tang, J., Neidigh, J. L., Cooksey, R. C., and McClain, D. A. (2000) Transgenic mice with increased hexosamine flux specifically targeted to beta-cells exhibit hyperinsulinemia and peripheral insulin resistance. *Diabetes* **49**, 1492–1499.

15. Vosseller, K., Wells, L., Lane, M. D., and Hart, G. W. (2002) 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* **99**, 5313–5318.
16. Buse, M. G. (2006) Hexosamines, insulin resistance, and the complications of diabetes: current status. *Am. J. Physiol. Endocrinol. Metab.* **290**, E1–E8.
17. Goldberg, H. J., Scholey, J., and Fantus, I. G. (2000) Glucosamine activates the plasminogen activator inhibitor 1 gene promoter through Sp1 DNA binding sites in glomerular mesangial cells. *Diabetes* **49**, 863–871.
18. James, L. R., Fantus, I. G., Goldberg, H., Ly, H., and Scholey, J. W. (2000) Overexpression of GFAT activates PAI-1 promoter in mesangial cells. *Am. J. Physiol. Renal Physiol.* **279**, F718–F727.
19. Kolm-Litty, V., Sauer, U., Nerlich, A., Lehmann, R., and Schleicher, E. D. (1998) High glucose-induced transforming growth factor beta1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. *J. Clin. Invest.* **101**, 160–169.
20. Weigert, C., Friess, U., Brodbeck, K., Haring, H. U., and Schleicher, E. D. (2003) Glutamine: fructose-6-phosphate aminotransferase enzyme activity is necessary for the induction of TGF-beta1 and fibronectin expression in mesangial cells. *Diabetologia* **46**, 852–855.
21. Wolf, G., Schroeder, R., Ziyadeh, F. N., Thaiss, F., Zahner, G., and Stahl, R. A. (1997) High glucose stimulates expression of p27Kip1 in cultured mouse mesangial cells: relationship to hypertrophy. *Am. J. Physiol.* **273**, F348–F356.
22. Fan, Y. P., and Weiss, R. H. (2004) Exogenous attenuation of p21(Waf1/Cip1) decreases mesangial cell hypertrophy as a result of hyperglycemia and IGF-1. *J. Am. Soc. Nephrol.* **15**, 575–584.
23. Wolf, G., Schroeder, R., Zahner, G., Stahl, R. A., and Shankland, S. J. (2001) High glucose-induced hypertrophy of mesangial cells requires p27(Kip1), an inhibitor of cyclin-dependent kinases. *Am. J. Pathol.* **158**, 1091–1100.
24. Wolf, G., Sharma, K., Chen, Y., Ericksen, M., and Ziyadeh, F. N. (1992) High glucose-induced proliferation in mesangial cells is reversed by autocrine TGF-beta. *Kidney Int.* **42**, 647–656.
25. Wolf, G., and Ziyadeh, F. N. (1999) Molecular mechanisms of diabetic renal hypertrophy. *Kidney Int.* **56**, 393–405.
26. Masson, E., Wiernsperger, N., Lagarde, M., and El Bawab, S. (2005) Glucosamine induces cell-cycle arrest and hypertrophy of mesangial cells: implication of gangliosides. *Biochem. J.* **388**, 537–544.
27. Hsieh, T. J., Fustier, P., Zhang, S. L., Filep, J. G., Tang, S. S., Ingelfinger, J. R., Fantus, I. G., Hamet, P., and Chan, J. S. (2003) High glucose stimulates angiotensinogen gene expression and cell hypertrophy via activation of the hexosamine biosynthesis pathway in rat kidney proximal tubular cells. *Endocrinology* **144**, 4338–4349.
28. Shen, J. W., Subject, J. R., Lock, R. B., and Ross, W. E. (1989) Depletion of topoisomerase II in isolated nuclei during a glucose-regulated stress response. *Mol. Cell Biol.* **9**, 3284–3291.
29. Tomida, A., Suzuki, H., Kim, H. D., and Tsuruo, T. (1996) Glucose-regulated stresses cause decreased expression of cyclin D1 and hypophosphorylation of retinoblastoma protein in human cancer cells. *Oncogene* **13**, 2699–2705.
30. Nakamura, M., Barber, A. J., Antonetti, D. A., LaNoue, K. F., Robinson, K. A., Buse, M. G., and Gardner, T. W. (2001) Excessive hexosamines block the neuroprotective effect of insulin and induce apoptosis in retinal neurons. *J. Biol. Chem.* **276**, 43748–43755.
31. Love, D. C., and Hanover, J. A. (2005) The hexosamine signaling pathway: deciphering the 'O-GlcNAc code'. *Sci. STKE*. 2005, re13.
32. Zachara, N. E., and Hart, G. W. (2002) The emerging significance of O-GlcNAc in cellular regulation. *Chem. Rev.* **102**, 431–438.
33. Slawson, C., Zachara, N. E., Vosseller, K., Cheung, W. D., Lane, M. D., and Hart, G. W. (2005) Perturbations in O-linked beta-N-acetylglucosamine protein modification cause severe defects in mitotic progression and cytokinesis. *J. Biol. Chem.* **280**, 32944–32956.
34. O'Donnell, N., Zachara, N. E., Hart, G. W., and Marth, J. D. (2004) Ogt-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability. *Mol. Cell Biol.* **24**, 1680–1690.
35. Boehmelt, G., Wakeham, A., Elia, A., Sasaki, T., Plyte, S., Potter, J., Yang, Y., Tsang, E., Ruland, J., Iscove, N. N., Dennis, J. W., and Mak, T. W. (2000) Decreased UDP-GlcNAc levels abrogate proliferation control in EMeg32-deficient cells. *EMBO J.* **19**, 5092–5104.
36. Shafi, R., Iyer, S. P., Ellies, L. G., O'Donnell, N., Marek, K. W., Chui, D., Hart, G. W., and Marth, J. D. (2000) The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. *Proc. Natl. Acad. Sci. USA* **97**, 5735–5739.
37. Du, X. L., Edelstein, D., Rossetti, L., Fantus, I. G., Goldberg, H., Ziyadeh, F., Wu, J., and Brownlee, M. (2000) Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc. Natl. Acad. Sci. USA* **97**, 12222–12226.
38. Goldberg, H. J., Whiteside, C. I., Hart, G. W., and Fantus, I. G. (2005) Posttranslational, reversible O-glycosylation is stimulated by high glucose and mediates plasminogen activator inhibitor-1 (PAI-1) gene expression and Sp1 transcriptional activity in glomerular mesangial cells. *Endocrinology* **147** (1), 222–231.
39. Yang, X., Su, K., Roos, M. D., Chang, Q., Paterson, A. J., and Kudlow, J. E. (2001) O-linkage of N-acetylglucosamine to Sp1 activation domain inhibits its transcriptional capability. *Proc. Natl. Acad. Sci. USA* **98**, 6611–6616.
40. Lamarre-Vincent, N., and Hsieh-Wilson, L. C. (2003) Dynamic glycosylation of the transcription factor CREB: a potential role in gene regulation. *J. Am. Chem. Soc.* **125**, 6612–6613.
41. Gao, Y., Miyazaki, J., and Hart, G. W. (2003) The transcription factor PDX-1 is post-translationally modified by O-linked N-acetylglucosamine and this modification is correlated with its DNA binding activity and insulin secretion in min6 beta-cells. *Arch. Biochem. Biophys.* **415**, 155–163.
42. Ball, L. E., Berkaw, M. N., and Buse, M. G. (2006) Identification of the major site of O-linked beta-N-acetylglucosamine modification in the C terminus of insulin receptor substrate-1. *Mol. Cell Proteomics* **5**, 313–323.
43. Han, I., and Kudlow, J. E. (1997) Reduced O glycosylation of Sp1 is associated with increased proteasome susceptibility. *Mol. Cell Biol.* **17**, 2550–2558.
44. Furukawa, K., Soejima, H., Niikawa, N., and Shiku, H. (1996) Genomic organization and chromosomal assignment of the human beta1, 4-N-acetylgalactosaminyltransferase gene. Identification of multiple transcription units. *J. Biol. Chem.* **271**, 20836–20844.
45. Lee, S., Ahn, S. H., Baek, S. H., Song, J. H., Choo, Y. K., Kwon, O. D., Choi, B. K., and Jung, K. Y. (2005) Modulation of cell proliferation and hypertrophy by gangliosides in cultured human glomerular mesangial cells. *Arch. Pharm. Res.* **28**, 948–955.
46. Nakatsuji, Y., and Miller, R. H. (2001) Selective cell-cycle arrest and induction of apoptosis in proliferating neural cells by ganglioside GM3. *Exp. Neurol.* **168**, 290–299.
47. Moon, S. K., Kim, H. M., Lee, Y. C., and Kim, C. H. (2004) Disialoganglioside (GD3) synthase gene expression suppresses vascular smooth muscle cell responses via the inhibition of ERK1/2 phosphorylation, cell cycle progression, and matrix metalloproteinase-9 expression. *J. Biol. Chem.* **279**, 33063–33070.

48. Marshall, S., Nadeau, O., and Yamasaki, K. (2004) Dynamic actions of glucose and glucosamine on hexosamine biosynthesis in isolated adipocytes: differential effects on glucosamine 6-phosphate, UDP-N-acetylglucosamine, and ATP levels. *J. Biol. Chem.* **279**, 35313–35319.
49. McClain, D. A., Lubas, W. A., Cooksey, R. C., Hazel, M., Parker, G. J., Love, D. C., and Hanover, J. A. (2002) Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. *Proc. Natl. Acad. Sci. USA* **99**, 10695–10699.
50. Veerababu, G., Tang, J., Hoffman, R. T., Daniels, M. C., Hebert, L. F., Jr., Crook, E. D., Cooksey, R. C., and McClain, D. A. (2000) Overexpression of glutamine: fructose-6-phosphate amidotransferase in the liver of transgenic mice results in enhanced glycogen storage, hyperlipidemia, obesity, and impaired glucose tolerance. *Diabetes* **49**, 2070–2078.