Physiological relevance of protein-glycosylation to pathogenesis of diabetes

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Abstract
Pancreatic β cells express glucose transporter-2 (GLUT2) on the cell surface as a glucose sensor molecule for appropriate insulin secretion. Disappearance of GLUT2 from the β cell surface is one of the early markers of the onset of type 2 diabetes, though the molecular mechanism has not been well understood. Glycobiology and mouse genetics revealed that GnT-IV is a glycosyltransferase indispensable for increasing the N-glycan branch complexity of GLUT2, which provides carbohydrate epitopes bound to galectin-9 on the β cell surface. The engagement of galectin-9 with GLUT2 regulates remodeling of GLUT2 clusters among cell surface membrane sub-domains, to control glucose transport activities, and prevents endocytosis to increase cell surface residency of GLUT2 that contributes to sustaining the glucose sensor function of β cells. The pathophysiological pathway to diet- and obesity-associated diabetes has recently been revealed, in which a high-fat diet leading to diabetes recapitulated the free-fatty acid induced-oxidative stress in human and mouse pancreatic β cells that induced nuclear exclusion of transcription factors and, subsequently, attenuated GnT-IVa-dependent GLUT2 glycosylation. Engineering and characterizing of transgenic mice overexpressing GnT-IVa in pancreatic β cells revealed that maintenance of GnT-IVa-dependent protein glycosylation prevents high fat diet-induced β cell dysfunction and ameliorates the onset of type 2 diabetes. These findings indicate that GnT-IV-mediated organization of cell surface GLUT2 is a fundamental process to modulate insulin secretion responses to fluctuating extracellular glucose levels, a paradigm that can be practically applied to better understand the pathogenesis of type 2 diabetes and development of drugs.

Keywords: type 2 diabetes, glucose transporter, glycosylation, GnT-IVa, pancreatic β cells

Failure of glucose sensor function of pancreatic β cells in the pathogenesis of type 2 diabetes

Glucose is an essential substance for all organisms for the production of energy in the form of ATP, as well as a major component in the elaboration of proteins, lipids, and nucleotides. Therefore, maintaining of glucose homeostasis is important for biological activities that are sustained by endocrinological and neurological cooperation in response to dynamic physiological fluctuations. In vertebrates, cells specialized in glucose homeostasis commonly express a glucose sensor molecule, glucose transporter (GLUT), on their cell surface, which enables cells to uptake extracellular glucose from interstitial fluid. This uptake is achieved by passive and facilitative transport processes along with the downward gradient of glucose concentration across the cellular plasma membrane, initiating cellular metabolic responses. Therefore, GLUT-dependent cellular glucose uptake was thought to be a physiological mandatory process.

GLUT consists of 13 members, of which 11 are specific for sugar transport without any energy-requirements, such as ATP hydrolysis or H+ gradient. GLUT family proteins are structurally conserved, and share a structure of 12 membrane-spanning regions and a single N-glycan in either the first or the fifth extracellular loop domain141. It has been reported that N-glycosylation is important for the stable expression of GLUT on the cell surface57, implying that N-glycosylation of GLUT plays an important role in glucose homeostasis; and further suggests that dysglycosylation might be involved in the pathogenesis of glucose homeostasis disorders.

It has been well characterized that the glucose-stimulated insulin secretion of pancreatic β cells is impaired in the disease process of diabetes. Consistent with this finding, the glucose sensor function of pancreatic β cells is attenuated in human type 2 diabetes patients and in various diabetic animal models. In addition, a decreased total and cell surface expression of GLUT2 protein is found in pancreatic β cells in the early stage of diabetes that reflects
the failure of glucose-stimulated insulin secretion. Its molecular mechanism, however, is not well understood.

**Impaired GLUT2 glycosylation in pancreatic β cells in the pathogenesis of type 2 diabetes**

Human chromosomal susceptible regions to type 2 diabetes were analyzed by genetic linkage analyses among type 2 diabetes patients and their relatives, and a chromosomal loci, 2q11.5, identified. The region encompasses a gene of Mannosyl (α-1,3-)glycoprotein β-1,4-N-acetylglucosaminyltransferase (N-acetylglucosaminyltransferase-IV) isoenzyme A, abbreviated to GnT-IVa or MGAT4A, which catalyzes the transfer of GlcNAc (N-acetylglucosamine) from UDP-GlcNAc to the GlcNAcβ1-2Manα1,3 arm of the core structure of N-linked oligosaccharides (Man3GlcNAc2-Asn) via a β1-4 linkage. Coincident with these findings, DNA microarray analyses of gene expression profile of pancreatic β cells of type 2 diabetes patients revealed that the expression level of the MGAT4A gene is significantly reduced in type 2 diabetes patients. These findings suggest that the failure of GnT-IVa function is involved in the pathogenesis of type 2 diabetes.

GnT-IVa is an essential glycosyltransferase to form multiantennary (tri- or tetra-antennary branched) complex type N-glycans (Fig. 1A), which is highly expressed in pancreas (β cells), small intestine, colon, kidney, and brain, as well as many types of tumor cells. The expression of GnT-IVa seems to be regulated by multiple mechanisms. The tissue distribution of GnT-IVa largely overlaps with that of GLUT2. Sequence motif analyses among promoter regions of human and mouse MGAT4A and GLUT2 genes revealed that they share transcriptional cis-elements of FOXA2 and HNF-1α, which are well characterized to regulate function and development of pancreatic β cells. Moreover, it has been well-defined that GLUT2 expression is transcriptionally regulated by FOXA2 and HNF-1α, expressions of GnT-IVa and GLUT2 might be synchronously regulated, consequently enabling GLUT2 to acquire an N-glycan formed by GnT-IVa in a specific type of cells. Indeed, GLUT2 in pancreatic β cells in normal humans and mice has GnT-IVa-mediated multiantennary N-glycans. In contrast, GLUT2 in pancreatic β cells of type 2 diabetes and mice receiving a high fat diet has less branched N-glycans (Fig. 1B), which is consistent with reduced expression of MGAT4A in pancreatic β cells in type 2 diabetes patients, described above.

**GnT-IVa deficiency impairs function and in situ distribution of GLUT2**

Pathophysiological relevance of GnT-IVa deficiency in the disease process of type 2 diabetes has been well elucidated by studies of GnT-IVa deficient mice. GnT-IVa deficient mice exhibit signs of type 2 diabetes including moderate hyperglycemia, hypoinsulinemia, elevation of glucose-stimulated insulin secretion. Its molecular mechanism, however, is not well understood.

**Fig. 1** GLUT2 N-glycan structure in pancreatic β cells. (A) Newly synthesized proteins are transported to plasma membrane through the endoplasmic reticulum (ER) and Golgi apparatus, where they are N-glycosylated by GnT-IV and -V, and consequently acquire multiantennary N-glycans. (B) GLUT2 in normal pancreatic β cells has a tetra-antennary N-glycan, whereas that of type 2 diabetes has a biantennary N-glycan.
free fatty acids, triglycerides, ALT, and AST in serum chemistry, impaired glucose tolerance, hepatic steatosis, and diminished insulin action in muscle and adipose tissues\(^7\).

In vitro insulin secretion assay revealed that primary insulin secretion in response to glucose stimulation is lost in GnT-IVa deficient pancreatic β cells\(^7\). This insulin secretion pattern resembled that of GLUT2 deficiency that suggested that GnT-IVa deficiency impaired the function of GLUT2 in pancreatic β cells. In addition, analyses of glucose uptake kinetics of primary pancreatic β cells revealed that the affinity to glucose was not altered and glucose uptake speed was significantly reduced (~10% of wild type) in GnT-IVa deficient β cells\(^7\), implying that the attenuation of glucose uptake in GnT-IVa deficient β cells was attributed to the reduced cell surface expression of GLUT2, but not to the impairment of GLUT2 function. The reduced β cell surface residency of GLUT2 in GnT-IVa deficiency was revealed by flow cytometry, and confirmed by immunohistochemistry of pancreatic β cells, indicating that the deposition of GLUT2 in plasma membrane was significantly reduced and the greater part intracellularly sequestered in early endosome and lysosome\(^7\).

Pancreatic β cells have two glucose sensor molecules, GLUT2 and glucokinase (GK), which are rate-limiting molecules in glucose metabolism in β cells. Under normal conditions, the latter predominantly works as a glucose sensor in the insulin secretion process in β cells, whereas the former restrains glucose uptake and becomes the limiting step if the cell surface expression level of GLUT2 is lowered by 20% of normal. Indeed, GLUT2 deficient mice and GLUT2 knock down mice exhibit impaired glucose-stimulated insulin secretion\(^20\)-\(^22\).

GnT-IVa deficient β cells showed reduced branch formation of N-glycans, the majority of which was altered to a bi-antennary structure\(^7\). These results indicate that GnT-IVa-dependent GLUT2 glycosylation regulates its in situ distribution, and the dysglycosylation of GLUT2 impairs glucose sensor function in β cells.

**Mechanism of glycan-mediated cell surface expression of GLUT2**

The mechanism of the dysglycosylation-induced abnormal cellular localization of GLUT2 has not been well elucidated. For explaining the mechanism, two molecular functions of the GLUT2 N-glycan were looked at: 1) GLUT2 N-glycan determines the outcome of the intracellular sorting of newly synthesized GLUT2 protein, and 2) GLUT2 N-glycan regulates the stability and half-life of GLUT2 protein on the β cell surface. These were tested by pulse-chase labeling experiments of primary isolated pancreatic β cells that revealed that intracellular transport of newly synthesized GLUT2 protein is normally transported to the cell surface when there is a GnT-IVa deficiency, and that cell surface half life of GLUT2 was significantly shortened when there was a GnT-IVa deficiency\(^7\). These results indicate that the GnT-IVa-dependent GLUT2 N-glycan regulates the stability and residency of GLUT2 protein on the cell surface, but not the intracellular sorting system.

Moreover, the molecular mechanism of N-glycan-dependent stabilization of GLUT2 protein on the cell surface was discovered. Lectins are a major protein family, which recognize and bind to specific glycan structures, and play important roles in various biological systems. On the cell surface, glycoproteins bind to lectins via their glycans and form a lattice structure, and thereby, cell surface glycoproteins are organized to exert proper molecular functions\(^23\). Galectin-9 was identified as a lectin associating with GLUT2 in pancreatic β cells by exploring lectins selectively bind to the Galβ1-4GlcNAc structure, which is a terminal moiety of GLUT2 N-glycan branches. Galectin-9 is a member of the galectin family that selectively binds to β-galactosides with a relatively weak affinity, and has two carbohydrate recognition domains that enables it to work as a cross-linker of multiple glycoproteins bearing β-galactosides. Galectin-9 preferentially binds to tri-, and tetra-antennary N-glycan structures, rather than bi-antennary structures\(^24\) that suggests that the glycan-binding specificity of galectin-9 determines the molecular interaction between GLUT2 and galectin-9, and consequently controls the cell surface residency of GLUT2. Indeed, disruption of the GLUT2-galectin-9 binding on the β cell surface, by addition of synthetic glycan mimetics (Galβ1-4GlcNAc) to β cell culture, induced GLUT2 endocytosis and diminished cell surface expression levels of GLUT2\(^7\). Collectively, these findings indicate that GnT-IVa produces N-glycan epitopes on GLUT2 that bind to endogenous lectins, including galectin-9, leading to a reduction in the rate of GLUT2 endocytosis, and thereby maintaining glucose sensor function of glucose-stimulated insulin secretion (Fig. 2).

**Glucose transport activity of GLUT2 controlled by glycan-mediated membrane sub-domain distribution**

Analyses of membrane sub-domain distribution of GLUT2 in primary isolated pancreatic β cells revealed that GLUT2 almost exclusively resides in the non-lipid raft microdomain in β cell membrane\(^25\). Furthermore, the disruption of the GLUT2-Galectin lattice by genetic inactivation of GnT-IVa, or by treatment of pancreatic beta cells with competitive glycan mimetics, induced the redistribution of GLUT2 into the lipid-raft microdomain that was coincident with attenuation of cellular glucose transport activity. Moreover, disruption of the lipid-raft microdomain by methyl-β-cyclodextrin treatment released GLUT2 from lipid-rafts and reactivated cellular glucose transport in GnT-Iva-deficient β cells\(^25\). These results indicate that GLUT2 N-glycosylation is involved in
membrane sub-domain distribution and glucose transport activity.

It has been well established that the re-distribution of GLUT1 among membrane sub-domains is associated with cellular glucose transport activity\(^{26}\), since GLUT1 associates with Stomatin in lipid-rafts. Stomatin erythrocyte membrane protein 7.2b is a 31 kDa integral membrane protein residing in lipid-rafts and controls the function of ion channels and transporters\(^{27,28}\). GLUT1 and GLUT2 exhibit a high degree of sequence similarity, and their hydropathy plots are virtually superimposable, suggesting these proteins are likely to adopt similar global structures within the membrane\(^{4}\). These findings imply that the glucose transport activity of GLUT2 is also regulated in the same manner. Indeed, experiments of intracellular protein cross-linking of pancreatic β cells demonstrated that the disruption of GLUT2-galectin interaction triggered membrane sub-domain re-distribution, and then allowed GLUT2 to associate with Stomatin that was coincident with the attenuation of the glucose transport activity of GLUT2\(^{25}\).

These findings indicate that the lipid raft-microdomain residency of GLUT2 causes the Stomatin interaction, and thereby suppresses the transport activity of GLUT2,
further suggesting that the glycosylation-mediated membrane sub-domain distribution of GLUT2 is important for the regulation of the glucose sensor function for glucose stimulated-insulin secretion of pancreatic β cells (Fig. 3).

Process of high-fat diet induced impairment of β cell glucose sensor function

As described above, expression of GnT-IVa and GLUT2 is transcriptionally co-regulated by HNF-1α and FOXA2, suggesting that the glucose sensor function of pancreatic β cells should be exclusively controlled by these transcription factors. However, the physiological mechanism of how metabolic fluctuation compromises transcriptional regulation and, subsequently, the glucose sensor function of β cells are not well understood.

Mice receiving a high-fat diet is a useful animal model recapitulating the onset of obesity-associated type 2 diabetes, exhibiting attenuation of glucose-stimulated insulin secretion and the development of insulin resistance[29,30]. Pancreatic β cells of high fat diet-administrated mice showed diminished GLUT2 glycosylation and intracellular accumulation of GLUT2 associated with reduced expression of GnT-IVa and GLUT2[7,18,29]. This was coincident with reduced histone acetylation levels of MGAT4A and GLUT2 gene promoters, reflecting inactivation of these genes[18]. These results were consistent with decreased promoter binding of HNF-1α and FOXA2, which are capable of recruiting histone acetyltransferase in high fat diet-administrated mouse β cells[18]. These results indicate that impairment of the glucose sensor function of β cells is deeply associated with defective HNF-1α and FOXA2-mediated transcription. In agreement with this, analyses of in situ distribution of these transcription factors revealed that they localize in the nucleus under normal conditions, whereas they localize in cytoplasm in high fat diet-administrated mouse β cells[18]. Furthermore, studies of in vitro β cell cultures demonstrated that the nuclear exclusion of these transcription factors is induced by treatment with free fatty acids ameliorated by treatment with antioxidants[19]. These findings indicate that elevation of the free fatty acid level, associated with high fat diet administration, induces oxidative stress in β cells that evokes the nuclear exclusion of HNF-1α and FOXA2, and thereby diminishes MGAT4A and GLUT2 expression and impairs glucose sensor function.

Prevention of β cell failure under high fat diet conditions due to overexpression of GnT-IVa

Based on the above findings, it has been speculated that supplementation of GnT-IVa activity in pancreatic β cells improves their glucose sensor function and maintains partial glucose homeostasis under high fat diet conditions. Engineering and characterizing of transgenic mice over-expressing GnT-IVa in pancreatic β cells demonstrated that their pancreatic β cells maintained cell surface residency of GLUT2 and glucose-stimulated insulin secretion (GSIS) under high fat diet conditions that contributed to improving long-term blood glucose levels, glucose tolerance, and peripheral insulin sensitivities[19]. These findings indicate that maintenance of the GnT-IVa-dependent protein glycosylation prevents high fat diet-induced β cell dysfunction and ameliorates the onset of type 2 diabetes, further suggesting that glycans and glycosyltransferases can be targeted by antidiabetic drugs in the future.

References
