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GM3 and diabetes

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Abstract We demonstrated the molecular pathogenesis of type 2 diabetes and insulin resistance focusing on the interaction between insulin receptor and GM3 ganglioside in adipocytes and propose a working hypothesis "metabolic disorders, such as type 2 diabetes, are membrane microdomain disorders caused by aberrant expression of gangliosides". It is expected that the development of novel diagnosis of metabolic syndrome by identifying the specific ganglioside species and a therapeutic strategy "membrane microdomain ortho-signaling therapy".

Keywords Ganglioside GM3 · Insulin resistance · Diabetes · Lipid rafts · Membrane microdomains · Diversity of ceramide structure

Introduction

"Gangliosides expressed in a cell-type specific manner" in the outer leaflet of the cell membranes are expected to interact with various molecules on plasma membranes based on different potentials of non-covalent bonding such as electrostatic and hydrophobic interactions and thereby ganglioside family could participate in various aspects of cellular activities by forming dynamic functional complexes (membrane microdomains or lipid rafts) [1, 2]. Expression levels of cellular gangliosides are known to be influenced by various extracellular stimuli including inflammatory cytokines. Namely, the presence of gangliosides in membrane microdomains is reflecting the characteristics of individual cells under various physiological and pathological environments. Since I was involved in gene cloning of "GM3 synthase" in 1988 [3], much efforts of our research group have been dedicated to the elucidation of biological function of GM3. [4] GM3 synthase (CMP-*N*-acetylneuraminate:lactosylceramide-α-2,3-*N*-acetylneuraminyltransferase; EC 2.4.99.9), also known by the names GM3S (used here), SAT-I, ST3GalV, and Siat 9. This review describes our findings on the pathophysiological significance of gangliosides especially for GM3 in diabetes.

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GM3 is an inducer of insulin resistance

Insulin elicits a wide variety of biological activities, which can be categorized into metabolic and mitogenic actions. The binding of insulin to insulin receptor (IR) activates IR internal-tyrosine kinase activity. The activated tyrosine-phosphorylated IR was able to recruit and phosphorylate adaptor proteins such as insulin receptor substrate (IRS). The phosphorylated IRS activates PI3-kinase (PI3K). The activated PI3K translocates to lipid rafts and converts PIP_2 to PIP_3, and then PIP_3 recruits PDK1 to phosphorylates Akt. The full activation of Akt might be required for phosphorylation of the other site by mTORC2 (mTOR complex 2) [5]. This IR–IRS–PI3K-Akt signaling cascade is the representative metabolic pathway triggered by insulin, resulting in the translocation of glucose transporter 4 (GLUT-4) to plasma membrane to facilitate glucose uptake. When mouse adipocytes were cultured in low concentrations of TNFα which do not cause generalized suppression of adipocyte gene expression including IRS-1 and GLUT-4, interference of insulin action by TNFα occurred [6]. This requires prolonged treatment (at least 72 h), unlike many acute effects of this cytokine. The slowness of the effect suggests that insulin resistance in adipocytes treated with 0.1 nM TNFα was accompanied by progressive increases in cellular GM3 content, GM3 synthase activity and GM3 synthase mRNA content, indicating that TNFα upregulates GM3

synthesis at the transcriptional level in cultured adipocytes (Fig. 1a right panel) [7, 8]. On the other hand, ceramide levels were transiently increased up to 6 h upon TNFα treatment and returned to normal by 24 h (Fig. 1a left panel). This observation suggests distinct and independent roles of GM3 and ceramides in the development of insulin resistance in adipocytes. To elucidate whether the increased GM3 in 3 T3-L1 adipocytes treated with TNFα is involved in insulin resistance, we used an inhibitor of glucosylceramide synthase, D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1propanol (D-PDMP) [9] to deplete cellular glycosphingolipids derived from glucosylceramide. D-PDMP proved able to counteract TNFα-induced increase of GM3 content in adipocytes and completely normalize the TNFα-induced defect in tyrosine phosphorylation of IRS-1 in response to insulin stimulation [8]. These findings are supported by the observation that knockout mouse lacking GM3 synthase exhibits enhancement of insulin signaling [10]. It has been reported that treatment of adipocytes with TNFα induces an increase in the serine phosphorylation of IRS-1 [11]. This phosphorylation is an important event since immunoprecipitated IRS-1, which has been serine phosphorylated in response to TNFα, is a direct inhibitor of insulin receptor tyrosine kinase activity. We have shown that TNFα induced serine phosphorylation of IRS-1 in adipocytes was completely suppressed by inhibition of GM3 biosynthesis with D-PDMP treatment, suggesting that the elevated GM3 synthesis induced by TNFα caused the upregulation of serine phosphorylation of IRS-1 [8]. An improved PDMP analog [12] and another type of glucosylceramide synthase inhibitor [13], were proven to have therapeutic value by oral administration in diabetic rodent models. Zucker *fa/fa* rat produced significant levels of TNFα [11]. Much less expression was seen in adipose tissues obtained from the lean control animals. Interestingly, these obese-diabetic animals did not show evidence of altered expression of other cytokines, such as IL-1 or IFNγ [11]. Thus, we were interested in measuring the expression of GM3 synthase mRNA in the epididymal fat of Zucker *fa/fa* rats and *ob/ob* mice. Northern blot analysis of GM3 synthase mRNA contents in the adipose tissues from these two typical models of insulin resistance exhibited significantly high levels compared to their lean counterparts (Fig. 1b) [8]. Comparison of the mobility of GM3 bands on TLC between the lean animals and *ob/ob* mice and Zucker fatty rats indicates the appearance of GM3 species showing low mobility (more hydrophilic) in both obese and diabetic animals (Fig. 1b).

Insulin resistance as a membrane microdomain disorder

In a state of insulin resistance induced in adipocytes by TNFα we presented evidence that the transformation to a resistant state may depend on increased ganglioside GM3 biosynthesis following upregulated GM3 synthase gene expression. Additionally, GM3 may function as an inhibitor of insulin signaling during chronic exposure to TNFα [8]. Since GSL, including GM3, are important components of lipid rafts, we pursued the possibility that increased GM3 levels in lipid rafts confer insulin resistance upon TNFα-treated adipocytes. We

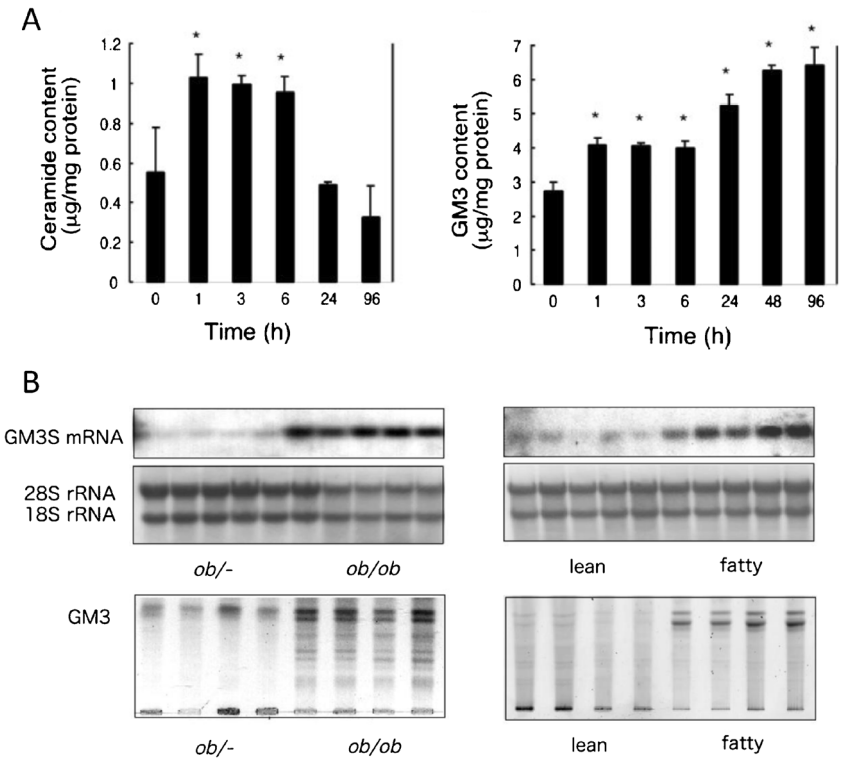

Fig. 1 Alteration of GM3 and ceramide levels in adipocytes acquired insulin resistance. **a** GM3 and ceramide levels in 3T3L-1 adipocytes are differentially regulated by TNFa, [7]. **b** Significant increase of GM3 biosynthesis in the epididymal fats in *ob/ob* mice and Zucker fatty rats [8]

examined GM3–protein interactions occurring within the plasma membrane of living cells by performing a cross-linking assay using a photoactivatable radioactive derivative of GM3. Adipocytes were preincubated with [^{3}H]GM3(N3), then irradiated to induce cross-linking of GM3. Target proteins were then separated by SDS/PAGE and visualized by autoradiography. A specific radioactive band corresponding to the 90-kDa IRβ-subunit was immunoprecipitated with anti-IRβ antibodies, confirming the direct association of GM3 and IR. Therefore, we found that IR form complexes with caveolin-1 and GM3 independently in 3 T3-L1 adipocytes [14]. Lipids are asymmetrically distributed in the outer and inner leaflets of plasma membranes. In typical mammalian cells, most acidic phospholipids are located in the inner leaflet, and only acidic glycosphingolipids such as sulfatides and gangliosides are in the outer. The binding of proteins to lipid membranes is often mediated by electrostatic interactions between the proteins' basic domains and acidic lipids. Gangliosides, which bear sialic acid residues, exist ubiquitously in the outer leaflet of the vertebrate plasma membrane. GM3 is the most abundant ganglioside, and the primary ganglioside found in adipocytes [15]. Glycosphingolipids, including gangliosides, is oriented at a defined angle to the axis of the ceramide [16]. In addition, GM3 spontaneously forms clusters with its own saturated fatty acyl chains, regardless of any repulsion between the negatively charged units in the sugar chains [17]. Thus, GM3 clusters with other cell surface gangliosides such as glycosphingolipid-enriched microdomains (GEM) generate a negatively charged environment just above the plasma membrane. Conversely, IR has a sequence in its transmembrane domain, homologous among mammals, that allows presentation of the basic amino acid lysine (IR944) just above transmembrane domain. Therefore, during lateral diffusion an electrostatic interaction between the lysine residue at IR944 and the GM3 cluster could occur due to their proximity on the plasma membrane. Our study has proven a mechanism in which the dissociation of the IR-caveolin-1 complex is caused by the interaction of a lysine residue, located just above the transmembrane domain in IRβ-subunit and the increased GM3 clustered at the cell surface by live cell studies using FRAP techniques [14]. Based on this evidence we propose a mechanism behind the shift of IR from the caveolae to the GEM in adipocytes during a state of insulin resistance (Fig. 2).

Reportedly, insulin signaling in skeletal muscle of GM3S KO mice was enhanced compared to wild type B6 mice [10]. However, it has been recently reported that the inhibition of insulin signaling of C2C12 myotubes exposed to saturated fatty acid, was not able to reverse by the treatment with a glucosylceramide synthase inhibitor (D-PDMP analog). [18] Thus, the involvement of GM3 for pathophysiology of insulin resistance in skeletal muscle needs for further study.

The role of the ceramide acyl chain length in insulin signaling was explored by using a ceramide synthase 2 (CerS2) null mouse, which is unable to synthesize very long acyl chain (C22-C24) ceramides [19]. In CerS2 null mice, IR and Akt phosphorylation in response to insulin were abrogated in liver. The lack of insulin receptor phosphorylation in liver correlated with its inability to translocate into detergent-resistant membranes (DRMs). Moreover, DRMs in CerS2 null mice displayed properties significantly different from those in wild-type mice, suggesting that the altered sphingolipid acyl chain length directly affects IR translocation to lipid rafts and subsequent signaling.

Serum GM3 as a new biomarker of metabolic syndrome

GM3 is the major ganglioside present in serum and is known to be associated with serum lipoproteins [20]. We examinined a relationship between serum GM3 levels and adiposity indices, as well as between serum GM3 levels and metabolic risk variables [21]. Serum GM3 levels were significantly increased in type 2 diabetic patients with severe obesity (visceral fat area >200 cm2, BMI >30). The GM3 level was positively correlated with LDL-c (0.403, P=0.012) in type 2 diabetes mellitus, but not affected by blood pressure. In addition, the high levels of small dense LDL (>10 mg/dL) were associated with the elevation of GM3. Serum GM3 levels were affected by glucose and lipid metabolism abnormalities and by visceral obesity. Interestingly, small dense LDL is reportedly associated with the development of atherosclerosis [22–24], and GM3 has been detected in atherosclerotic lesions [25, 26]. Thus, our findings provide evidence that GM3 may be a useful marker for the management of metabolic syndrome including insulin resistance, as well as for the early diagnosis of atherosclerosis (Fig. 3). The structural diversity of ceramide species is generated by various factors in the N-acyl chains; 1) the length (C16-24), 2) alpha hydroxylation and 3) desaturation; and in the sphingoid bases; 1) d18:1, d18:0; 2) hydroxylation at C4, resulting in a substantial number of combinations. We are currently performing LC-MS/MS analyses to identify the GM3 species which are specifically involved in metabolic syndrome.

Perspective

Since gangliosides expose sialic acid residues to the outside of outer leaflet membranes, it is very interesting to know their true physiological counterparts for electrostatic interactions in microdomains. GM3 is dominantly expressed in insulin responsive organs such as skeletal muscle liver and adipose tissue as well as lymphocytes in human. Thus, the presence of GM3-dependent membrane microdomains (lipid rafts) is

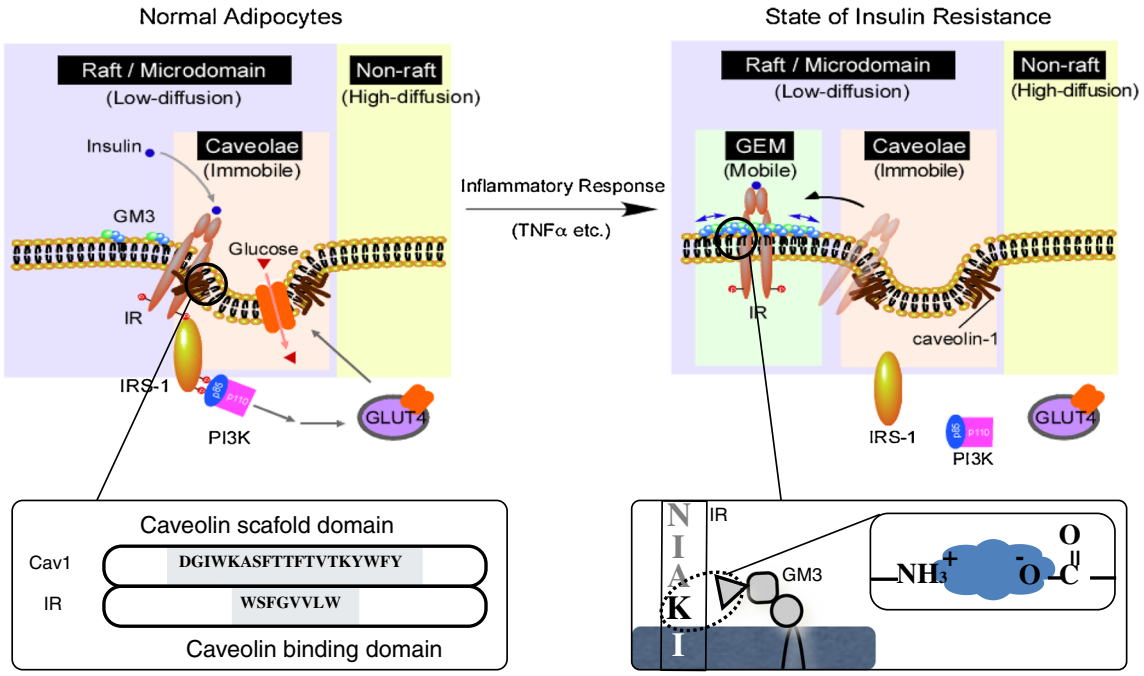

Fig. 2 Proposed mechanism behind the shift of insulin receptors from the caveolae to the glycosphingolipid-enriched microdomains (GEM) in adipocytes during a state of insulin resistance. A schematic representation of raft/microdomains comprising caveolae and non-caveolae rafts such as GEM. Caveolae and GEM reportedly can be separated by an anti-CAV1 antibody. IR may be constitutively resident in caveolae via its binding to the scaffolding domain of CAV1 through the caveolin-binding domain in its cytoplasmic region. Binding of IR and CAV1 is necessary for successful insulin metabolic signaling. In adipocytes the localization of IR in the caveolae is interrupted by elevated levels of the endogenous ganglioside GM3 during a state of insulin resistance induced by TNFα [7]. This study has proven a mechanism, at least in part, in which the dissociation of the IR/CAV1 complex is caused by the interaction of a lysine residue at IR944, located just above the transmembrane domain, and the increased GM3 clustered at the cell surface

reflecting characteristics of individual cells. In order to accumulate gangliosides in lipid rafts, hydrogen donor and acceptor, and saturated and relatively long acyl chains compared to those of phospholipids should be existing in their ceramide backbone to accelerate their self aggregation. The structural diversity of the sphingoid base and the N-acyl chain of ceramide moiety is key issue to define the behavior of gangliosides in living cell membrane and localization of lipid rafts. Comprehensive study to know the precise information of ceramide structures by "Sphingolipidomics" is essential to

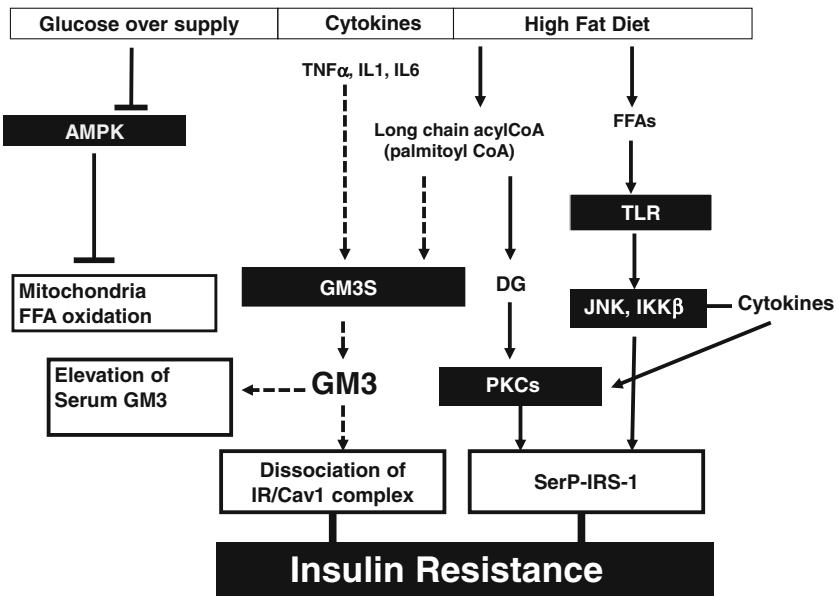

Fig. 3 Insulin resistance as a membrane microdomain disorder. *Arrows* indicate the classic intracellular pathway leading to induce insulin resistance and dotted arrows show the proposed pathway based on our working hypothesis

elucidate the functional supra-biomolecular complex consisting of gangliosides and functional proteins in microdomains.

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