FEBS Letters 584 (2010) 1864–1871

journal homepage: www.FEBSLetters.org

Review

Membrane microdomains and insulin resistance

Jin-ichi Inokuchi *

Division of Glycopathology, Institute of Molecular Biomembranes and Glycobiology, Tohoku Pharmaceutical University, 4-4-1, Komatsushima, Aoba-ku, Sendai 981-8558, Miyagi, Japan

ARTICLE INFO

Article history:
Received 7 July 2009
Revised 3 October 2009
Accepted 6 October 2009
Available online 12 October 2009

Edited by Sandro Sonnino

Keywords:
Ganglioside
GM3
Insulin resistance
Membrane microdomain (lipid raft)
Insulin receptor

ABSTRACT

A new concept, that "metabolic disorders, such as type 2 diabetes, are membrane microdomain disorders caused by aberrant expression of gangliosides", has arisen. By examining this working hypothesis, we demonstrate the molecular pathogenesis of type 2 diabetes and insulin resistance focusing on the interaction between insulin receptor and gangliosides in microdomains and propose the new therapeutic strategy "membrane microdomain ortho-signaling therapy".

1. Introduction

Gangliosides expressing cell-type specific manner 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 various aspects of cellular activities by forming dynamic functional complexes (membrane microdomains or lipid rafts) in living cell membranes. 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 pathophysiological environment.

Critical dependence of the insulin metabolic signal transduction on caveolae/microdomains in adipocytes has been demonstrated. These microdomains can be biochemically isolated at cold with non-ionic detergents from cell membranes on the basis of their lower density by sucrose gradient centrifugation, and were designated as detergent resistant membrane microdomains (DRM). DRM are considered the experimental evidence of the existence of lipid rafts including caveolae and glycolipid-enriched membrane microdomains (GEM). We observed the striking increase of cellular ganglioside GM3 in the state of insulin resistance of mouse 3T3-L1 adipocytes induced by TNFα and in the adipose tissues of obese/diabetic rodent models such as Zucker *fa/fa* rats and *ob/ob* mice [1]. We examined the effect of TNFα on the composition and function of DRM in adipocytes and demonstrated that increased GM3 levels result in the elimination of insulin receptor (IR) from the DRM while caveolin and flotillin remain in the DRM, leading to the inhibition of insulin's metabolic signaling [2]. These findings are further supported by the report that mice lacking GM3 synthase exhibit enhanced insulin signaling [3]. To gain insight into molecular mechanisms behind interactions of IR, caveolin-1 (Cav1) and GM3 in adipocytes, we performed immunoprecipitations, cross-linking studies of IR and GM3, and live cell studies using fluorescence recovery after photobleaching (FRAP) technique. We found that (i) IR form complexes with Cav1 and GM3 independently; (ii) in GM3-enriched membranes the mobility of IR is increased by dissociation of the IR-Cav1 interaction; (iii) the lysine residue localized just above the transmembrane domain of the IRβ-subunit is essential for the interaction of IR with GM3. Since insulin metabolic signal transduction in adipocytes is known to be critically dependent on caveolae, we propose a new pathological feature of insulin resistance in adipocytes caused by dissociation of the IR-Cav1 complex by the interactions of IR with GM3 in microdomains [4].

Abbreviations: DRM, detergent resistant membrane microdomains; Cav1, caveolin-1; IR, insulin receptor; IRS, insulin receptor substrate; SAT-I, GM3 synthase; FRAP, fluorescence recovery after photobleeching; GEM, glycosphingolipid-enriched microdomains

* Fax: +81 22 727 0076.
E-mail address: jin@tohoku-pharm.ac.jp

0014-5793/$36.00
doi:10.1016/j.febslet.2009.10.012

2. Ganglioside GM3 is an inducer of insulin resistance

Insulin elicits a wide variety of biological activities, which can be globally 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, resulting in the translocation of glucose transporter 4 (GLUT-4) to plasma membrane to facilitate glucose uptake. This IR–IRS–PI3-kinase signaling cascade is the representative metabolic pathway of insulin. On the other hand, the mitogenic pathway in insulin signaling initiates phosphorylation of Shc by the activated IR and then activates Ras-MAPK signaling.

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 [5]. This requires prolonged treatment (at least 72 h), unlike many acute effects of this cytokine. The slowness of the effect suggests that TNFα induces the synthesis of an inhibitor that is the actual effector. We demonstrated that the state of insulin resistance in adipocytes treated with 0.1 nM TNFα was accompanied by a progressive increase in cell surface GM3. This was reflected by 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 [1]. To elucidate whether the increased GM3 in 3T3-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-1-propanol (D-PDMP) [6] to deplete cellular glycosphingolipids derived from glucosylceramide. D-PDMP proved able to counteract TNF-induced increase of GM3 content in adipocytes and completely normalize the TNF-induced defect in tyrosine phosphorylation of IRS-1 in response to insulin stimulation (Fig. 1A). These findings are supported by the recent observation that knockout mouse lacking GM3 synthase exhibits enhancement of insulin signaling [3].

Hotamisligil et al. reported that treatment of adipocytes with TNFα induces an increase in the serine phosphorylation of IRS-1 [7]. 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 [1] (Fig. 1B). Since TNF-induced serine phosphorylation of IRS-1 may occur through the activation of a variety of kinases including protein kinase C, c-Jun NH2-terminal kinase, p44/42 kinase, and PI3-kinase, it is important to identify the actual kinase(s) activated by endogenous GM3.

It was shown that adipose tissues of the obese-diabetic *db/db, ob/ob*, KK-A^{y} mice and the Zucker *fa/fa* rat produced significant levels of TNFα [7]. 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 [7,8]. 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. 2) [1].

3. Caveolae microdomains and insulin signaling

Caveolae are a subset of membrane microdomains particularly abundant in adipocytes [9,10]. Critical dependence of the insulin metabolic signal transduction on caveolae/microdomains in adipocytes has been demonstrated [11,12]. Disruption of microdomains by cholesterol extraction with methyl-β-cyclodextrin resulted in progressive inhibition of tyrosine phosphorylation of IRS-1 and activation of glucose transport in response to insulin although autophosphorylation of IR and activation of MAP kinase were not impaired [9]. Similarities between these cell culture results and the findings in many cases of clinical insulin resistance [13] suggest a potential role for microdomains in the pathogenesis of this disorder.

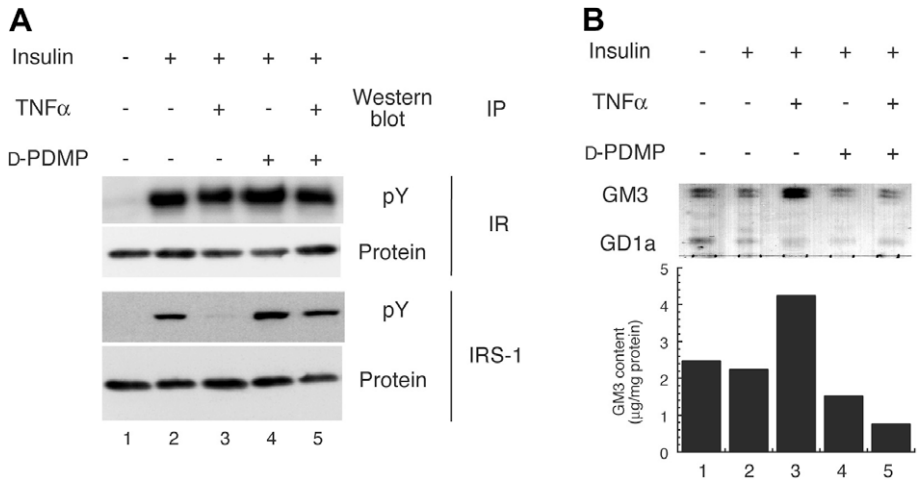

Fig. 1. TNFα increases the expression of GM3 and prevention of GM3 synthesis reverses TNFα induced suppression insulin signaling in adipocytes (A) 3T3-L1 adipocytes were cultured in maintenance medium without (lanes 1, 2 and 4) or with (lanes 3 and 5) 0.1 nM TNFα for 96 h and in order to deplete GM3, 20 μM D-PDMP was also included (lanes 4 and 5). Before insulin stimulation (100 nM for 3 min), cells were starved in serum-free media containing 0.5% bovine serum albumin in the absence or presence of TNFα and D-PDMP as above for 8 h. Proteins in cell lysates were immunoprecipitated with antiserum to IR and IRS-1, fractionated by SDS–PAGE, and transferred to Immobilon-P. Western blot was then proved with anti-phosphotyrosine monoclonal antibody, stripped and reproved with antiserum to IR and IRS-1 (B) 3T3-L1 adipocytes were incubated in the absence or presence of TNFα and D-PDMP as in (A) and the ganglioside fraction was visualized by resorcinol staining on HPTLC [1].

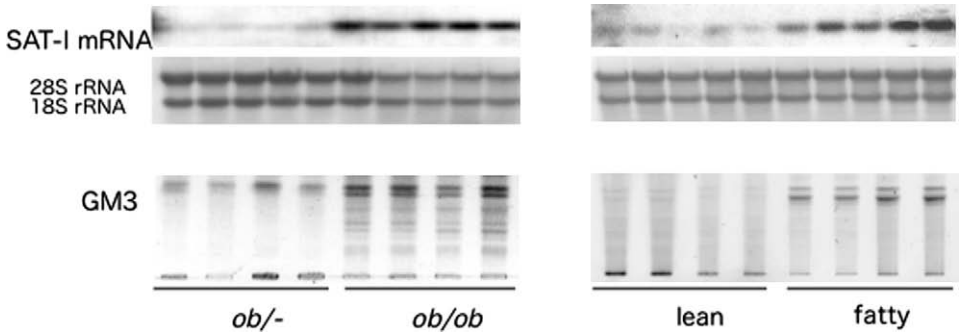

Fig. 2. Increased GM3 synthase mRNA in adipose tissue of typical rodent models of insulin resistance. Northern blot analysis of GM3 synthase mRNA was performed using total mRNA from adipose tissues of *ob/ob* mice and Zucker *fa/fa* rats, and their lean counterparts [1].

Couet et al. demonstrated the presence of a caveolin binding motif (fXXXXfXXf [f: an aromatic amino acid, X: any amino acid] in the b subunit of IRs that could bind to the scaffold domain of caveolin [14]. Moreover, mutation of this motif resulted in the inhibition of insulin signaling [15]. Indeed, mutations of the IRβ-subunit have been found in type 2 diabetes patients [16]. Lisanti's laboratory reported that caveolin-1-null mice developed insulin resistance when placed on a high fat diet [17] Interestingly, insulin signaling, as measured by IR phosphorylation and its downstream targets, was selectively decreased in the adipocytes of these animals while signaling in both muscle and liver cells was normal. This signaling defect was attributed to a 90% decrease in IR protein content in the adipocytes, with no changes in mRNA levels, indicating that caveolin-1 serves to stabilize the IR protein [12,17]. These studies clearly indicate the critical importance of the interaction between caveolin and IR in executing successful insulin signaling in adipocytes.

Although the direct interaction between caveolin-1 and IR has been shown as described above, studies of the presence of IRs in DRM have provided conflicting data [18–22]. Saltiel and colleagues found that insulin stimulation of 3T3-L1 adipocytes was associated with tyrosine phosphorylation of caveolin-1 [23]. However, since only trace levels of IR were recovered in the DRM in assays with a buffer of 1% Triton X-100, they speculated on the presence of intermediate molecule(s) bridging IR and caveolin [21]. Gustavsson et al. also observed the dissociation of IRs from caveolin-containing DRM after treatments of 0.3% and 0.1% Triton X-100 [18]. It has been reported that a comparison of protein and lipid contents of DRM prepared with a variety of detergents indicated considerable differences in their ability to selectively solubilize membrane proteins and to enrich sphingolipids and cholesterol over glycerophospholipids and that Triton X-100 was the most reliable detergent [24]. Therefore, we performed a flotation assay with a wide range of Triton X-100 concentrations to identify the protein of interest which might weakly associate with DRM. In an assay system containing less than 0.08% Triton X-100, we were able to show that in normal adipocytes IRs can localize to DRM. Thus, by employing low detergent concentrations we were able to demonstrate, for the first time, the presence of IR in DRM [2]. As summarized in Table 1, there are many evidence demonstrating that the localization of IRs in caveolae microdomains are essential for successful metabolic signaling of insulin.

4. 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 [1]. Since GSL, including GM3, are important components of DRM, we have pursued the possibility that increased GM3 levels in DRM confer insulin resistance upon TNFα-treated adipocytes. We examined the effect of TNFα on the composition and function of DRM in adipocytes and demonstrated that increased GM3 levels result in the elimination of IRs from the DRM while raft marker proteins such as caveolin and flotillin remain in the DRM [2]. Although the localization of IRs to DRM may be maintained by the association with caveolin-1 as mentioned above, the excess accumulation of GM3 in the DRM may weaken IR–caveolin interaction. Therefore, to examine interactions among IR, caveolin-1, and GM3 in 3T3-L1 adipocytes, we initially performed coimmunoprecipitation assays. caveolin-1 has a scaffolding domain to which IR and other functional transmembrane proteins bind through a caveolin binding domain in their cytoplasmic region [14,15]. As expected from another study [15], IR was co-precipitated with Cav1 (Fig. 3A). GM3 was co-precipitated with IR but not with Cav1 (Fig. 3B, upper panel). In addition, IR but not Cav1 was co-precipitated with GM3 (Fig. 3B, lower panel). Thus, IR can bind both Cav1 and GM3, but there is no interaction

Table 1
Localization of insulin receptor in caveolae microdomains is essential for the metabolic signaling of insulin.

Function	Evidence	Reference
Direct biding of IR and caveolin-1	IR has caveolin binding domain	[14]
	Coimmunoprecipitation of IR and caveolin	[15]
Colocalization of IR and caveolin-1	IR and caveolin in light-density fractions by sucrose density floatation assay	[2]
	Fluorescence microscope	[18]
	Electron microscope	[40,41]
Signaling	Stimulation of caveolin-1 tyrosine phosphorylation by insulin	[23,20]
	Caveolin deficient mice show insulin resistance due to accelerated degradation of IR in adipose tissue	[17,20]
	Cholesterol depletion disrupts caveolae and metabolic signaling of insulin	[9]
	Increased GM3 eliminates IR from DRM and inhibits IR-IRS-1 signaling	[2,4]

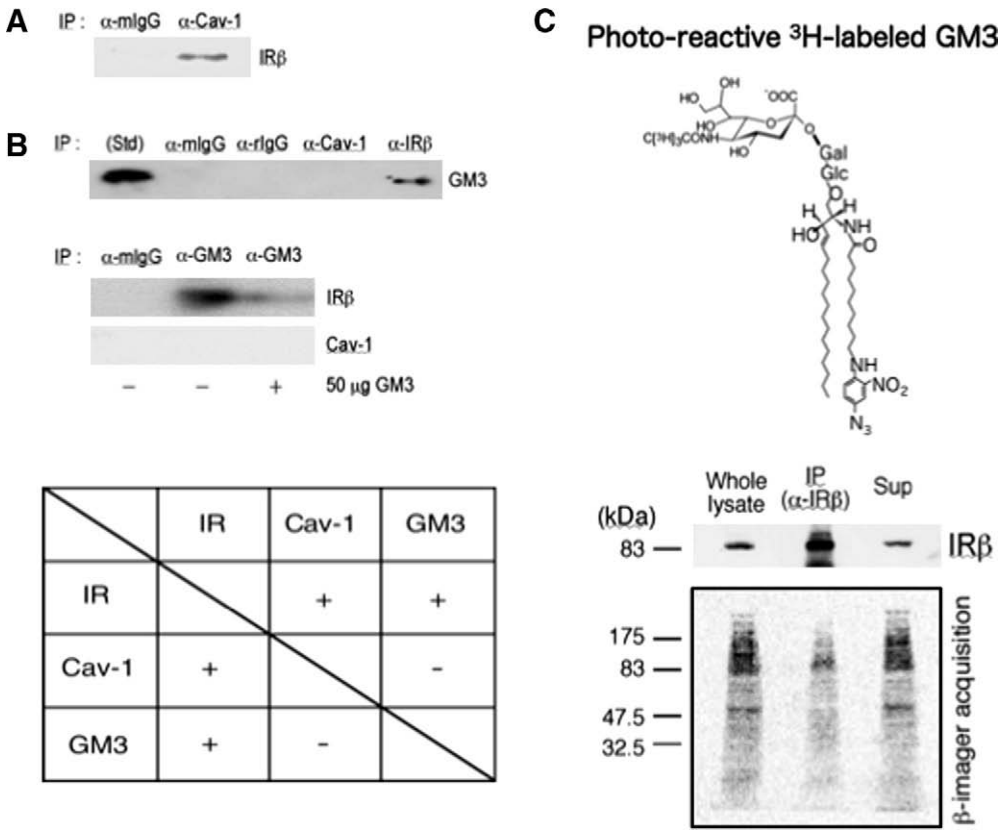

Fig. 3. The insulin receptor forms distinct complexes with Cav1 and GM3 in adipocytes. (A) Coimmunoprecipitation assay of Cav1 and IR. Post nuclear supernatants (PNS) of whole cell lysates were immunoprecipitated with an anti-Cav1 antibody or anti-mouse IgG (−), and the precipitates were subjected to SDS–PAGE followed by immunoblotting with an anti-IRβ antibody. (B) GM3 associates with IR but not with Cav1. Upper panel: PNS were immunoprecipitated with an anti-Cav1 antibody, an anti-IRβ antibody, or an anti-mouse or anti-rat IgG (−). The precipitates were subjected to TLC followed by immunostaining with the anti-GM3 antibody M2590 as described in Section 2. Lower panel: Immunoprecipitation was performed with the anti-GM3 antibody DH2, in the presence or absence of 50 μg GM3, or with anti-mouse IgG (−). The precipitates were then subjected to SDS–PAGE followed by immunoblotting with an anti-IRβ or anti-Cav1 antibody. (C) Cross-linking assay of GM3 and IR. Adipocytes were treated with photoactivatable ^{3}H-labeled GM3, washed, and then irradiated. Cells were then lysed and subjected to immunoprecipitation with an anti-IRβ antibody. PNS, anti-IRβ immunoprecipitates (IP), and the supernatant from the immunoprecipitation (Sup) were subjected to SDS–PAGE, followed by immunoblotting with an anti-IRβ antibody and autoradiography [4].

between GM3 and Cav1, suggesting that IR can form distinct complexes with each. The association between IR and GM3 was abolished by the presence of GM3, confirming the specific binding ability of the anti-GM3 antibody to GM3 in the immunoprecipitation medium (Fig. 3B, lower panel).

We next 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 (Fig. 3C). Adipocytes were preincubated with [^{3}H]GM3(N_3), then irradiated to induce cross-linking of GM3. Target proteins were then separated by SDS/PAGE and visualized by autoradiography. A broad range of radioactivity reflecting GM3–protein complexes could be detected from 80 kDa to 200 kDa, suggesting a close association between GM3 and a variety of cell surface proteins, including IR. Moreover, 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 3T3-L1 adipocytes [4].

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 [25]. Glycosphingolipids, including gangliosides, share a common minimum energy conformational structure in which the oligosaccharide chain is oriented at a defined angle to the axis of the ceramide [26]. 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 [27]. Thus, GM3 clusters with other cell surface gangliosides such as 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 (Fig. 4A).

We previously developed GM3-reconstituted cells by stably transfecting the GM3 synthase (SAT-I) gene into GM3-deficient cells [28] (Fig. 4B, left panel). Using the fluorescence recovery after photobleaching (FRAP) technique, we examined the mobility of IR in the plasma membranes of GM3-reconstituted (GM3 (+)) cells and mock (GM3 (−)) cells expressing equal levels of Cav1 (Fig. 4B, right panel inset). The mobility of IR-GFP expressed in the GM3 (+) cells was statistically (10%) higher than that in the GM3 (−) cells (Fig. 4B, right panel), providing further evidence that

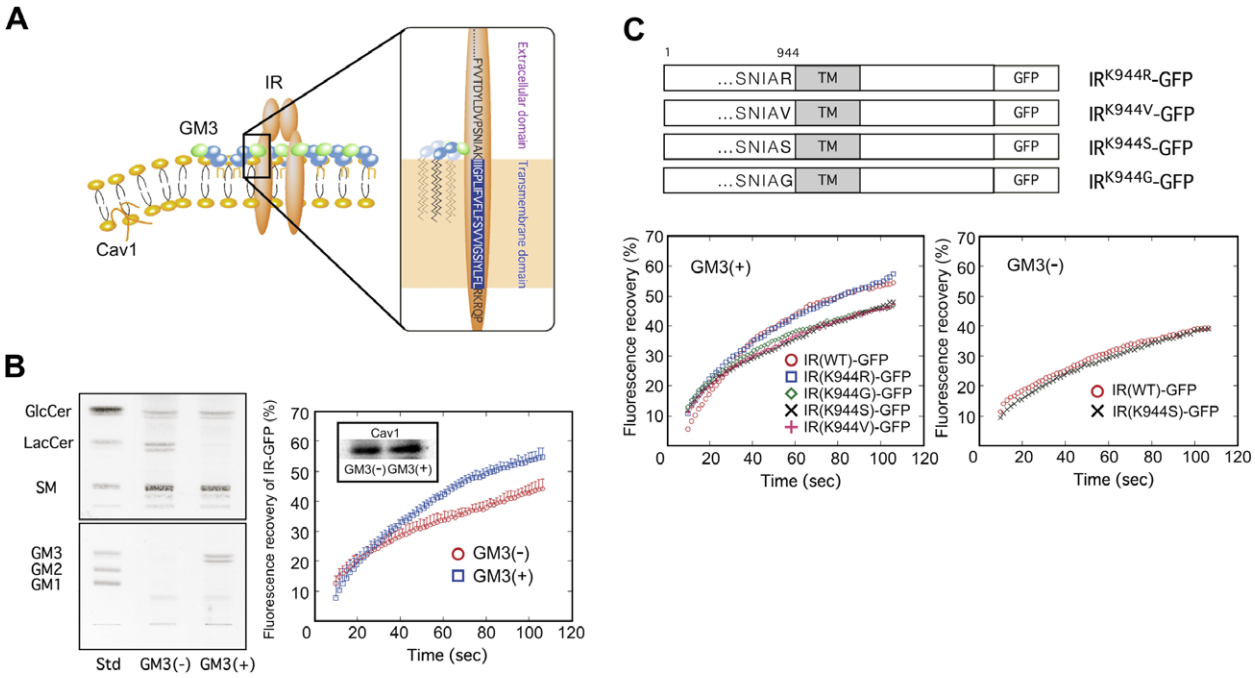

Fig. 4. The lysine residue IR944 is essential for the interaction of IR with GM3. (A) Schematic representation of the proposed interaction of a lysine residue at IR944, which is located just above the transmembrane domain, and GM3 at the cell surface. (B) Enhanced mobility of IR in GM3-enriched membrane. Left panel: Glycosphingolipid (GSL) analysis of GM3-reconstituted cells (GM3 (+)) and mock cells (GM3 (−)). GSLs extracted from these cells, corresponding to 1 mg of cellular protein, were separated on HPTLC plates and stained with resorcinol–HCl reagent, to visualize gangliosides, or with cupric acetate–phosphoric acid reagent for neutral GSLs. Right panel: FRAP analyses. Fluorescence recovery of IR-GFP in GM3 (−) and GM3 (+) cells expressing equal levels of Cav1(inset). (C) Specificity of the interaction between lysine at IR944 and GM3 by FRAP analyses. Upper panel: Schematic structure of IR-GFP mutants in which the lysine at IR944 is replaced with basic and neutral amino acids. Lower panel: Fluorescence recovery of IR-GFP mutants in GM3(+) cells [4].

GM3 is able to enhance IR mobility by dissociating the Cav1 and IR complex in living cells.

The binding of between IR and Cav1 has been studied in detail [15]. To similarly understand interactions between IR and GM3 have not been analyzed. We constructed several mutants of IR in which the lysine at IR944 was replaced with the basic amino acid, arginine, or with the neutral amino acid valine, serine, or glutamine. The fluorescence recovery of IR(K944G), IR(K944S), and IR(K944V) 100 s after bleaching was decreased by 10% compared to those of IR(WT) and IR(K944R) in GM3 (+) cells. However, in GM3 (−) cells, no such difference in the mobility between IR (wt) and IR (K944S) was observed. This demonstrates that the lysine in the wildtype is essential for its binding to GM3 due to its basic charge [4].

IR may be constitutively resident in caveolae via its binding to the scaffolding domain of caveolin-1 through the caveolin binding domain in its cytoplasmic region [14]. In fact, there are many supporting data showing that binding of IR and caveolin-1 is necessary for successful insulin metabolic signaling (Table 1). Iwabuchi et al. demonstrated that caveolae and GEM could be separated by an anti-caveolin-1 antibody [29]. 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 inflammatory response (e.g. TNFα) [1]. Our latest study has proven a mechanism, at least in part, 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 [4] (Fig. 4C). Here, we proposed mechanism behind the shift of IR from the caveolae to the GEM in adipocytes during a state of insulin resistance. Shown is a schematic representation of raft/microdomains comprising caveolae and non-caveolae rafts such as GEM (Fig. 5).

5. Serum GM3 levels as a new biomarker of metabolic disorder

GM3 is the major ganglioside present in serum and is known to be associated with serum lipoproteins [30]. However, there have been no studies examining a relationship between serum GM3 levels and diabetes or abdominal obesity. So, we investigated the relationship between serum GM3 levels and adiposity indices, as well as between serum GM3 levels and metabolic risk variables [31]. Serum GM3 levels were higher in hyperglycemic patients (1.4-fold), hyperlipidemic patients (1.4-fold) and hyperglycemic patients with hyperlipidemia (1.6-fold), than in normal subjects. In addition, serum GM3 levels were significantly increased in type 2 diabetic patients with severe obesity (visceral fat area >200 cm^2, 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/gl) were associated with the elevation of GM3. Serum GM3 levels was affected by glucose and lipid metabolism abnormalities and by visceral obesity. Interestingly, small dense LDL is reportedly associated with the development of atherosclerosis [32–34], and GM3 has been detected in atherosclerotic lesions [35,36]. 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.

6. A possible therapeutic intervention of metabolic disorder by inhibiting ganglioside synthesis

Critical involvement of ganglioside GM3 in insulin resistance and metabolic syndrome including type 2 diabetes has now became evident based on the following key observations; (i) TNFα

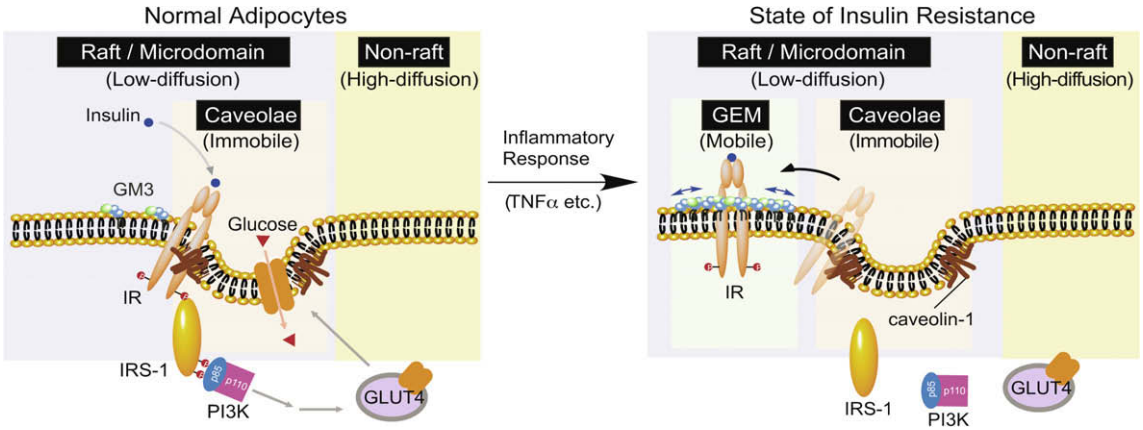

Fig. 5. Proposed mechanism behind the shift of insulin receptors from the caveolae to the glycosphingolipid-enriched microdomains (GEM) in adipocytes during a state of insulin resistance. A schematic representation of raft/microdomains comprising caveolae and non-caveolae rafts such as GEM. Caveolae 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 (Table 1). 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 [2]. The present 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 [4].

increases the expression of GM3 in adipocytes and the TNFα-induced insulin resistance is prevented by treatment with a glucosylceramide synthase inhibitor, D-PDMP, and decreases GM3 contents [1]. Recently, an improved PDMP analog [37,38] and another type of glucosylceramide synthase inhibitor [39], were proven to have therapeutic value by oral administration in diabetic rodent models.; (ii) GM3 contents increase in the adipose tissue of Zucker *fa/fa* rats and *ob/ob* mice, which are typical rodent models of obesity [1] and diet induced obesity (unpublished observation); (iii) insulin sensitivity is enhanced in GM3 synthase knockout mice [3]; (iv) the accumulation of GM3 in insulin resistance results in dissociation of the insulin receptor from caveolae [2]; (v) dissociation of the insulin receptor from caveolae is caused by electrostatic interaction between GM3 and the lysine residue (Lys-944) located just above the transmembrane of the insulin receptor [4]; (vi) treatment of glucosylceramide synthase inhibitors significantly im-

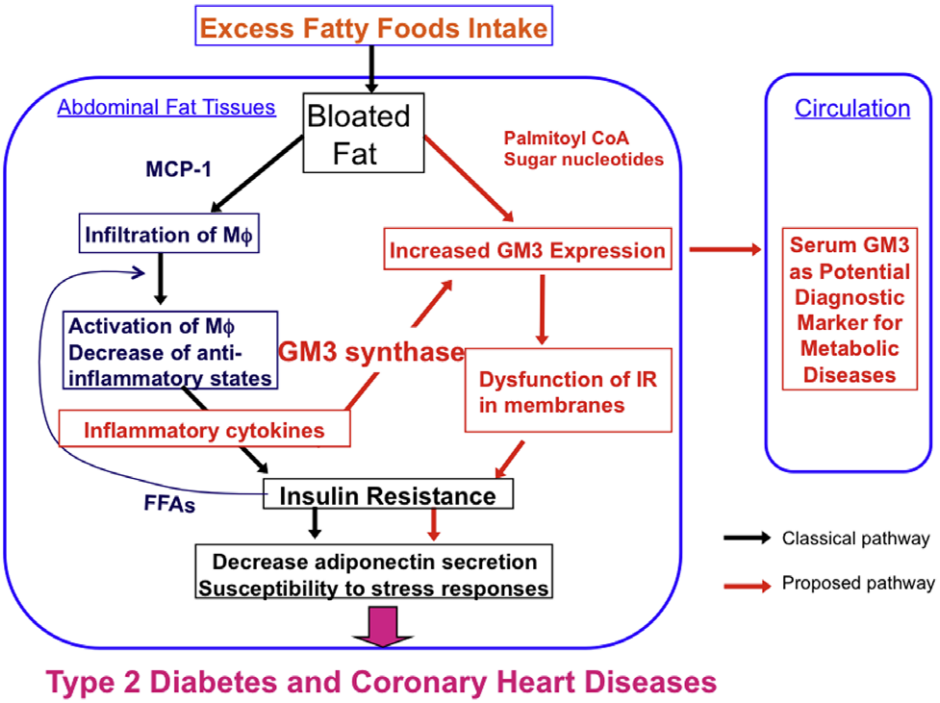

Fig. 6. Involvement of ganglioside GM3 in insulin resistance and metabolic disorder. Here we propose a pathway of insulin resistance caused by the elevated levels of GM3 under a chronic and low grade inflammation in bloated abdominal fat tissues. The bloated fat tissue in the state of insulin resistance is no more able to store triglycerides and release FFAs including palimitic acid. Palmitic acid is converted to palmitoyl CoA, which is a substrate for sphingosine biosynthesis. At the same time, the levels of sugar nucleotides including UDP-glucose and UDP-galactose increase due to the insulin resistance. These events accelerate the biosynthesis of GM3 in the state of insulin resistance which accompanied with the activation of GM3 synthase gene transcription by inflammatory cytokines including TNFα. Consequently, the elevated levels of GM3 in plasma membranes especially in lipid raft resulted in the dissociation of IR–caveiolin-1 complex, leading to the attenuation of insulin signaling as summarized in Fig. 5. MCP-1: monocyte chemoattractant protein-1.

proved insulin sensitivity and glucose homeostasis in rodent models of obesity [37,40]. Taken together, a new therapeutic intervention by inhibiting GM3 biosynthesis can be proposed for the treatment of metabolic syndrome including type 2 diabetes.

7. Concluding remarks

A growing body of evidence implicates glycosphingolipids including gangliosides in the pathogenesis of insulin resistance. We demonstrated that in 3T3-L1 adipocytes in a state of TNF-induced insulin resistance, the inhibition of insulin metabolic signaling was associated with an accumulation of the ganglioside GM3 [1], and, moreover, the pharmacological inhibition of GM3 biosynthesis by the glucosylceramide synthase inhibitors proved to be effective to ameliorate the state of insulin resistance induced in vitro and obese rodent models, suggesting a new target for therapy against insulin resistance and type 2 diabetes [1,37–39]. In addition, our data substantiate a rationale for designing novel therapies against type 2 diabetes and related diseases based on inhibition of ganglioside biosynthesis [4]. The extensive reduction of all gangliosides by inhibiting GM3 biosynthesis would carry to important physical and chemical modifications of the all cellular plasma membrane and in particular of lipid microdomains. However, we expect that such extensive depletion of gangliosides will not be necessarily for the treatment of metabolic disorders. When we demonstrated the effectiveness of D-PDMP on the impaired insulin signaling in TNFα-treated 3T3-L1 adipocytes, the normalization of elevated levels of GM3 was enough to ameliorate the state of insulin resistance [1]. Thus, a new therapeutic intervention by inhibiting GM3 biosynthesis for the treatment of metabolic syndrome including type 2 diabetes will be proved for clinical usefulness. Here I have summarized the distinct pathway of metabolic disorder including the aberrant expression of GM3 (Fig. 6).

Acknowledgements

The author thanks all the collaborators concerned with the studies described in this review article, who are listed as the co-authors of the paper. This work was supported by grants from Core Research for Evolutional Science and Technology (CREST) of the Japan Science and Technology Agency, and the Ministry of Education, Culture, Sports, Science and Technology to promote multidisciplinary research projects.

References

[1] Tagami, S., Inokuchi Ji, J., Kabayama, K., Yoshimura, H., Kitamura, F., Uemura, S., Ogawa, C., Ishii, A., Saito, M., Ohtsuka, Y., Sakaue, S. and Igarashi, Y. (2002) Ganglioside GM3 participates in the pathological conditions of insulin resistance. J. Biol. Chem. 277, 3085–3092.

[2] Kabayama, K., Sato, T., Kitamura, F., Uemura, S., Kang, B.W., Igarashi, Y. and Inokuchi, J. (2005) TNFalpha-induced insulin resistance in adipocytes as a membrane microdomain disorder: involvement of ganglioside GM3. Glycobiology 15, 21–29.

[3] Yamashita, T., Hashiramoto, A., Haluzik, M., Mizukami, H., Beck, S., Norton, A., Kono, M., Tsuji, S., Daniotti, J.L., Werth, N., Sandhoff, R., Sandhoff, K. and Proia, R.L. (2003) Enhanced insulin sensitivity in mice lacking ganglioside GM3. Proc. Natl. Acad. Sci. USA 100, 3445–3449.

[4] Kabayama, K., Sato, T., Saito, K., Loberto, N., Prinetti, A., Sonnino, S., Kinjo, M., Igarashi, Y. and Inokuchi, J. (2007) Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proc. Natl. Acad. Sci. USA 104, 13678–13683.

[5] Guo, D. and Donner, D.B. (1996) Tumor necrosis factor promotes phosphorylation and binding of insulin receptor substrate 1 to phosphatidylinositol 3-kinase in 3T3–L1 adipocytes. J. Biol. Chem. 271, 615–618.

[6] Inokuchi, J. and Radin, N. (1987) Preparation of the active isomer of 1-phenyl-2-decanoylamino-3-morpholino-1-propanol, inhibitor of murine glucocerebroside synthetase. J. Lipid Res. 28, 565–571.

[7] Hotamisligil, G.S., Shargill, N.S. and Spiegelman, B.M. (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259, 87–91.

[8] Hotamisligil, G.S. and Spiegelman, B.M. (1994) Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes 43, 1271–1278.

[9] Parpal, S., Karlsson, M., Thorn, H. and Stralfors, P. (2001) Cholesterol depletion disrupts caveolae and insulin receptor signaling for metabolic control via insulin receptor substrate-1, but not for mitogen-activated protein kinase control. J. Biol. Chem. 276, 9670–9678.

[10] Fan, J.Y., Carpentier, J.L., van Obberghen, E., Grunfeld, C., Gorden, P. and Orci, L. (1983) Morphological changes of the 3T3–L1 fibroblast plasma membrane upon differentiation to the adipocyte form. J. Cell Sci. 61, 219–230.

[11] Bickel, P.E. (2002) Lipid rafts and insulin signaling. Am. J. Physiol. Endocrinol. Metab. 282, E1–E10.

[12] Cohen, A.W., Combs, T.P., Scherer, P.E. and Lisanti, M.P. (2003) Role of caveolin and caveolae in insulin signaling and diabetes. Am. J. Physiol. Endocrinol. Metab. 285, E1151–E1160.

[13] Virkamaki, A., Ueki, K. and Kahn, C.R. (1999) Protein–protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J. Clin. Invest. 103, 931–943.

[14] Couet, J., Li, S., Okamoto, T., Ikezu, T. and Lisanti, M.P. (1997) Identification of peptide and protein ligands for the caveolin-scaffolding domain. Implications for the interaction of caveolin with caveolae-associated proteins. J. Biol. Chem. 272, 6525–6533.

[15] Nystrom, F.H., Chen, H., Cong, L.N., Li, Y. and Quon, M.J. (1999) Caveolin-1 interacts with the insulin receptor and can differentially modulate insulin signaling in transfected Cos-7 cells and rat adipose cells. Mol. Endocrinol. 13, 2013–2024.

[16] Imamura, T., Takata, Y., Sasaoka, T., Takada, Y., Morioka, H., Haruta, T., Sawa, T., Iwanishi, M., Hu, Y.G., Suzuki, Y., et al. (1994) Two naturally occurring mutations in the kinase domain of insulin receptor accelerate degradation of the insulin receptor and impair the kinase activity. J. Biol. Chem. 269, 31019–31027.

[17] Cohen, A.W., Razani, B., Wang, X.B., Combs, T.P., Williams, T.M., Scherer, P.E. and Lisanti, M.P. (2003) Caveolin-1-deficient mice show insulin resistance and defective insulin receptor protein expression in adipose tissue. Am. J. Physiol. Cell Physiol. 285, C222–C235.

[18] Gustavsson, J., Parpal, S., Karlsson, M., Ramsing, C., Thorn, H., Borg, M., Lindroth, M., Peterson, K.H., Magnusson, K.E. and Stralfors, P. (1999) Localization of the insulin receptor in caveolae of adipocyte plasma membrane. FASEB J. 13, 1961–1971.

[19] Iwanishi, M., Haruta, T., Takata, Y., Ishibashi, O., Sasaoka, T., Egawa, K., Imamura, T., Naitou, K., Itazu, T. and Kobayashi, M. (1993) A mutation (Trp1193 → Leu1193) in the tyrosine kinase domain of the insulin receptor associated with type A syndrome of insulin resistance. Diabetologia 36, 414–422.

[20] Kimura, A., Mora, S., Shigematsu, S., Pessin, J.E. and Saltiel, A.R. (2002) The insulin receptor catalyzes the tyrosine phosphorylation of caveolin-1. J. Biol. Chem. 277, 30153–30158.

[21] Mastick, C.C. and Saltiel, A.R. (1997) Insulin-stimulated tyrosine phosphorylation of caveolin is specific for the differentiated adipocyte phenotype in 3T3–L1 cells. J. Biol. Chem. 272, 20706–20714.

[22] Muller, G., Jung, C., Wied, S., Welte, S., Jordan, H. and Frick, W. (2001) Redistribution of glycolipid raft domain components induces insulin-mimetic signaling in rat adipocytes. Mol. Cell Biol. 21, 4553–4567.

[23] Mastick, C.C., Brady, M.J. and Saltiel, A.R. (1995) Insulin stimulates the tyrosine phosphorylation of caveolin. J. Cell Biol. 129, 1523–1531.

[24] Schuck, S., Honsho, M., Ekroos, K., Shevchenko, A. and Simons, K. (2003) Resistance of cell membranes to different detergents. Proc. Natl. Acad. Sci. USA 100, 5795–5800.

[25] Ohashi, M. (1979) A comparison of the ganglioside distributions of fat tissues in various animals by two-dimensional thin layer chromatography. Lipids 14, 52–57.

[26] Hakomori Si, S.I. (2002) Inaugural article: the glycosynapse. Proc. Natl. Acad. Sci. USA 99, 225–232 (Epub 2002 January 2).

[27] Sonnino, S., Mauri, L., Chigorno, V. and Prinetti, A. (2007) Gangliosides as components of lipid membrane domains. Glycobiology 17, 1R–13R.

[28] Uemura, S., Kabayama, K., Noguchi, M., Igarashi, Y. and Inokuchi, J. (2003) Sialylation and sulfation of lactosylceramide distinctly regulate anchorage-independent growth, apoptosis, and gene expression in 3LL Lewis lung carcinoma cells. Glycobiology 13, 207–216.

[29] Iwabuchi, K., Handa, K. and Hakomori, S. (1998) Separation of "glycosphingolipid signaling domain" from caveolin-containing membrane fraction in mouse melanoma B16 cells and its role in cell adhesion coupled with signaling. J. Biol. Chem. 273, 33766–33773.

[30] Senn, H.J., Orth, M., Fitzke, E., Wieland, H. and Gerok, W. (1989) Gangliosides in normal human serum. Concentration, pattern and transport by lipoproteins. Eur. J. Biochem. 181, 657–662.

[31] Sato, T., Nihei, Y., Nagafuku, M., Tagami, S., Chin, R., Kawamura, M., Miyazaki, S., Suzuki, M., Sugawara, S., Takahashi, Y., Saito, A., Igarashi, Y. and Inokuchi, J. (2008) Circulating levels of ganglioside GM3 in metabolic syndrome: a pilot study. Obes. Res, Clin. Pract. 2, 231–238.

[32] Austin, M.A., Breslow, J.L., Hennekens, C.H., Buring, J.E., Willett, W.C. and Krauss, R.M. (1988) Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 260, 1917–1921.

[33] de Graaf, J., Hak-Lemmers, H.L., Hectors, M.P., Demacker, P.N., Hendriks, J.C. and Stalenhoef, A.F. (1991) Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arterioscler. Thromb. 11, 298–306.

[34] Tribble, D.L., Krauss, R.M., Lansberg, M.G., Thiel, P.M. and van den Berg, J.J. (1995) Greater oxidative susceptibility of the surface monolayer in small dense LDL may contribute to differences in copper-induced oxidation among LDL density subfractions. J. Lipid Res. 36, 662–671.
[35] Bobryshev, Y.V., Lord, R.S., Golovanova, N.K., Gracheva, E.V., Zvezdina, N.D., Sadovskaya, V.L. and Prokazova, N.V. (1997) Incorporation and localisation of ganglioside GM3 in human intimal atherosclerotic lesions. Biochim. Biophys. Acta 1361, 287–294.
[36] Bobryshev, Y.V., Lord, R.S., Golovanova, N.K., Gracheva, E.V., Zvezdina, N.D. and Prokazova, N.V. (2001) Phenotype determination of anti-GM3 positive cells in atherosclerotic lesions of the human aorta. Hypothetical role of ganglioside GM3 in foam cell formation. Biochim. Biophys. Acta 1535, 87–99.
[37] Zhao, H., Przybylska, M., Wu, I.H., Zhang, J., Siegel, C., Komarnitsky, S., Yew, N.S. and Cheng, S.H. (2007) Inhibiting glycosphingolipid synthesis improves glycemic control and insulin sensitivity in animal models of type 2 diabetes. Diabetes 56, 1210–1218.
[38] van Eijk, M., Aten, J., Bijl, N., Ottenhoff, R., van Roomen, C.P., Dubbelhuis, P.F., Seeman, I., Ghauharali-van der Vlugt, K., Overkleeft, H.S., Arbeeny, C., Groen, A.K. and Aerts, J.M. (2009) Reducing glycosphingolipid content in adipose tissue of obese mice restores insulin sensitivity, adipogenesis and reduces inflammation. PLoS One 4, e4723 (Epub 2009 March 23).
[39] Aerts, J.M., Ottenhoff, R., Powlson, A.S., Grefhorst, A., van Eijk, M., Dubbelhuis, P.F., Aten, J., Kuipers, F., Serlie, M.J., Wennekes, T., Sethi, J.K., O'Rahilly, S. and Overkleeft, H.S. (2007) Pharmacological inhibition of glucosylceramide synthase enhances insulin sensitivity. Diabetes 56, 1341–1349 (Epub 2007 February 7).
[40] Karlsson, M., Thorn, H., Danielsson, A., Stenkula, K.G., Ost, A., Gustavsson, J., Nystrom, F.H. and Stralfors, P. (2004) Colocalization of insulin receptor and insulin receptor substrate-1 to caveolae in primary human adipocytes. Cholesterol depletion blocks insulin signalling for metabolic and mitogenic control. Eur. J. Biochem. 271, 2471–2479.
[41] Foti, M., Porcheron, G., Fournier, M., Maeder, C. and Carpentier, J.L. (2007) The neck of caveolae is a distinct plasma membrane subdomain that concentrates insulin receptors in 3T3–L1 adipocytes. Proc. Natl. Acad. Sci. USA 104, 1242–1247.