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A novel insulin receptor-signaling platform and its link to insulin resistance and type 2 diabetes

Farah Alghamdi [1], Merry Guo [2], Samar Abdulkhalek [3], Nicola Crawford [4], Schammim Ray Amith [5], Myron R. Szewczuk *

Department of Biomedical & Molecular Sciences, Queen's University, Kingston, Ontario K7L 3N6, Canada

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ABSTRACT

Insulin-induced insulin receptor (IR) tyrosine kinase activation and insulin cell survival responses have been reported to be under the regulation of a membrane associated mammalian neuraminidase-1 (Neu1). The molecular mechanism(s) behind this process is unknown. Here, we uncover a novel Neu1 and matrix metalloproteinase-9 (MMP-9) cross-talk in alliance with neuromedin B G-protein coupled receptor (GPCR), which is essential for insulin-induced IR activation and cellular signaling. Neu1, MMP-9 and neuromedin B GPCR form a complex with IRβ subunit on the cell surface. Oseltamivir phosphate (Tamiflu®), anti-Neu1 antibodies, broad range MMP inhibitors piperazine and galardin (GM6001), MMP-9 specific inhibitor (MMP-9i), and GPCR neuromedin B specific antagonist BIM-23127 dose-dependently inhibited Neu1 activity associated with insulin stimulated rat hepatoma cells (HTCs) that overly express human IRs (HTC-IR). Tamiflu, anti-Neu1 antibodies and MMP-9i attenuated phosphorylation of IRβ and insulin receptor substrate-1 (IRS1) associated with insulin-stimulated cells. Olanzapine, an antipsychotic agent associated with insulin resistance, induced Neu3 sialidase activity in WG544 or 1140F01 human sialidosis fibroblast cells genetically defective in Neu1. Neu3 antagonist 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA) and anti-Neu3 antibodies inhibited sialidase activity associated with olanzapine treated murine Neu4 knockout macrophage cells. Olanzapine attenuated phosphorylation of IGF-R and IRS1 associated with insulin-stimulated human wild-type fibroblast cells. Our findings identify a novel insulin receptor-signaling platform that is critically essential for insulin-induced IRβ tyrosine kinase activation and cellular signaling. Olanzapine-induced Neu3 sialidase activity attenuated insulin-induced IGF-R and IRS1 phosphorylation contributing to insulin resistance.

1. Introduction

The insulin receptor (IR) is a transmembrane tyrosine kinase receptor that is activated by insulin and insulin growth factors-I and II. Metabolically, insulin-induced IR tyrosine kinase activation plays a key role in the regulation of glucose homeostasis. A dysfunctional process of insulin induced IR activation may result in a range of clinical manifestations including insulin resistance [1], type 2 diabetes (T2DM), obesity, cancer, hypertension, and cardiovascular disorders [2–5]. In particular, insulin resistance occurs when the insulin-sensitive tissues, such as skeletal muscle, adipose tissue and liver, do not have the ability to respond to insulin [6,7], and consequently develop several of these chronic diseases [8–10]. The precise mechanism(s) involved in insulin resistance is not well understood [1]. However, several contributing factors have been proposed to contribute to insulin resistance, and they include increased plasma-free fatty acid levels [11], elastin-derived peptides [12], subclinical chronic inflammation [1], oxidative and nitrative stress, altered gene expression, and mitochondrial dysfunction [1,11].

Abbreviations: IR, insulin receptor; IRS1, insulin receptor substrate-1; IGF-R, insulin growth factor receptor; EGF, epidermal growth factor; MMP, matrix metalloproteinase; RTK, receptor tyrosine kinase; NGF, nerve growth factor; Neu1, mammalian neuraminidase-1; 4-MUNANA, [2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid]; DANA, 2-deoxy-2,3-didehydro-N-acetylneuraminic acid; IC_{50}, the concentration of a drug that is required for 50% inhibition in vitro; TLR, Toll-like receptor; GPCR, G-protein-coupled receptor; MAPK, mitogen-activated protein kinase; IGF-1, insulin like growth factor-1; GRK, G-protein-coupled receptor kinase; PDGF, platelet-derived growth factor; PDGFβR, PDGF β receptor; PI3K, phosphoinositide 3-kinase; EGF, epidermal growth factor; S1P, sphingosine 1-phosphate; Gi, inhibitory G-protein; Gαi, α subunit of Gi; Grb, growth factor receptor binding protein.

* Corresponding author. Tel.: +1 613 533 2457; fax: +1 613 533 6796.
E-mail addresses: farah_gg@hotmail.com (F. Alghamdi), merry.guo11@gmail.com (M. Guo), samarma@hotmail.com (S. Abdulkhalek), nicola.crawford@medportal.ca (N. Crawford), amith@ualberta.ca (S.R. Amith), szewczuk@queensu.ca (M.R. Szewczuk).
[1] Present address: The King Fahd Armed Forces Hospital, Serology, Jeddah, Saudi Arabia.
[2] Present address: Faculty of Medicine, University of Ottawa, Ottawa, ON K1N 6N5, Canada.
[3] Present address: Department of Molecular Genetics, Cleveland Clinic Foundation Molecular Genetics, Lerner Research Institute, Cleveland, Ohio, U.S.A.
[4] Present address: Department of Family Medicine, McMaster University, Hamilton, Ontario, Canada.
[5] Present address: Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada.

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Several studies have separately demonstrated a connection between insulin resistance and cell membrane sialic acid content. Cytidine 5′-monophospho-N-acetylneuraminic acid (CMP-NANA; substrate used for the sialyltransferase sialylation of glycans) was reported to enhance insulin responsiveness by 39% providing support for a role for cell surface sialic acid residues in hepatic insulin action with a concomitant contribution to insulin resistance of diabetes mellitus [13]. Structurally, IR is a heavily glycosylated receptor with 18 glycosylation sites [14,15]. Fourteen of the glycosylation sites are localized on the IRα subunits, while the other sites are on the IRβ subunits [16]. Specifically, the IRα subunits contain only the N-linked carbohydrate, while IRβ subunits contain both O- and N-linked carbohydrates [17].

The importance of glycosylation in IR biosynthesis, insulin binding and activation has been studied in detail using site-directed mutagenesis analyses [16,18]. Leconte et al. reported on the potential contribution of N-linked oligosaccharides of the IRβ subunit in the processing, structure and function of the IR [18]. They specifically targeted and mutated IRβ subunits (IRβ-N1234) which were mutagenized on four potential N-glycosylation sites (ASn-X-Ser/Thr). They compared the mutated IR-N1234 with the wild type receptor. There were no differences on the molecular weight of IRα subunits between both receptors. However, a reduction in the molecular weight from 95 kDa to 80 kDa of the IRβ subunit was depicted in the mutated IR form. The data indicated that the mutation of the four glycosylation sites in IRβ subunit had no effect on the α-subunit, but caused a reduction in the β-subunit molecular weight. This IRβ-N1234 mutation had no effect on IR cell surface expression and on insulin binding, but attenuated IRβ tyrosine kinase activation by insulin [18]. These early findings provided supporting evidence for a critical role of oligosaccharide side chains of the IRβ subunit in the molecular events responsible for the IR enzymatic activation and signal transduction. Indeed, the role of membrane glycosylation in the function of insulin receptors and glucose transporters is well known for two decades. A neuraminidase was found to release sialic acid from isolated rat adipocytes [19]. The report disclosed that pretreatment of adipocytes with a neuraminidase resulted in an increase in basal glucose transport, the process of which suggested sialic acids playing multiple roles in the control of glucose-transport activity.

Recently, the treatment of rat skeletal L6 myoblast cells with either mouse-derived mammalian Neu1 sialidase or *Clostridium perfringens* neuraminidase resulted in a desialylation of IR, which coincided with a significant increase of L6 myoblast cell proliferative responses to low concentrations of insulin [20]. In addition, the inhibition of endogenous Neu1 abolished this increase in proliferation of L6 cells induced by 1 and 10 nM of insulin, but amplified the proliferative effect of 100 nM insulin. For IGF receptors, the opposite results were observed where the desialylation process of this receptor coincided with an elimination of the heightened proliferative response of L6 myoblasts to 100 nM insulin. To explain this dichotomy, it was proposed that Neu1 enhanced the mitogenic response of L6 myoblasts to low dose of insulin, but the cytosolic Neu2 sialidase [21] and the cell membrane-bound Neu3 sialidase induced myoblast differentiation, but not proliferation, through the direct modulation of the GM3 ganglioside content in myoblasts [22,23]. In support of this hypothesis, it has been reported that a transient upregulation of Neu3 expression in L6 myocytes caused a significant decrease in IR signaling, the mechanism of which was proposed to be in direct modulation of plasma membrane gangliosides by Neu3 activity and the interaction with the growth factor receptor-bound protein 2 (Grb2) [24].

Another possibility to explain this dichotomy of insulin-induced mitogenic responses may involve GPCR-receptor tyrosine kinase (RTK) partners forming novel signaling platforms. Using human embryonic kidney 293 cells, Alderton et al. have clearly demonstrated that the platelet-derived growth factor β receptor (PDGFβR) forms a complex with Myc-tagged endothelial differentiation gene-1, a GPCR whose agonist is sphingosine 1-phosphate, in cells co-transfected with these receptors [25]. PDGF was shown to stimulate tyrosine phosphorylation of the inhibitory Giα subunit to increase p42/p44 mitogen activated protein kinase (MAPK) activation in the regulation of cell proliferation. Furthermore, both GPCR kinase 2 and β-arrestin-1 were found to associate with the PDGFβR, which play critical roles in the regulation of GPCR signal complexes endocytosis, a requirement for the activation of p42/p44 MAPK. The premise is that PDGFβR signaling is initiated by GPCR kinase 2/β-arrestin-1 complexes that have been recruited to the PDGFβR via tethering to a GPCR(s). These results provide a novel platform for the integrative signaling by these receptors that may account for the co-mitogenic effect of certain GPCR agonists with PDGF on cell proliferation. It is noteworthy that the basally tyrosine-phosphorylated Giα subunits do not induce activation of p42/p44 MAPK on their own [25]. The potentiating effects of Giα signaling on the PDGF-stimulated p42/p44 MAPK activation may require the PDGF-induced recruitment to tyrosine-phosphorylated Giα subunits of other intermediates. Indeed, several reports have shown that insulin receptors can interact with Giα subunit [26–28]. The integration of GPCR-RTK partners forming unique signaling platforms in which protein components specific for each receptor are shared to produce a response upon engagement of ligands is eloquently reviewed by Pyne and colleagues [29–32] and Abdulkhalek et al. [33].

Recently, insulin binding to its receptor was reported to rapidly induce the interaction of the insulin receptor with Neu1 sialidase, the activity of which hydrolyzes sialic acid residues of IR and, consequently, induces receptor activation [34]. The report also disclosed that Neu1 deficient mice with ~10% of the normal Neu1 activity exposed to a high-fat diet developed hyperglycemia and insulin resistance twice as fast as the wild-type cohort. They also proposed that endogenous Neu1 sialidase activity is involved in the insulin receptor glycosylation modification. Indeed, Blaise et al. provided additional confirmation to support that Neu1 interacts with IRβ and desialylates the receptor [12]. Other reports have suggested that receptor glycosylation modification may in fact be the connecting link between ligand-binding, receptor dimerization and activation for several other receptors [35–39].

This present report describes a novel organizational IR signaling platform describing the intermediates linked to the insulin-induced receptor activation process in live IR-expressing cells. Here, insulin binding to its receptor induces a Neu1 and matrix metalloproteinase-9 (MMP-9) cross-talk in activating IRs. Central to this process is that Neu1 and MMP-9 cross-talk in alliance with neuromedin B GPCR forms a complex tethered at the ectodomain of IRβ subunits on the cell surface. This signaling paradigm proposes that insulin binding to its receptor on the cell surface induces a conformational change of the receptor to initiate GPCR Giα-signaling and MMP-9 activation to induce Neu1. Activated Neu1 hydrolyzes α-2,3-sialyl residues linked to β-galactosides, which are distant from the insulin binding sites. These findings are consistent with another report [34] that predicts a prerequisite desialylation process by activated Neu1 enabling the removal of steric hindrance to IRβ subunit association and the activation of tyrosine kinases. This Neu1-MMP-9 crosstalk in alliance with neuromedin B GPCR at the ectodomain of IRβ subunits forms the essential signaling platform on the cell surface that is critical for insulin-induced receptor activation.

Olanzapine, an antipsychotic agent associated with insulin resistance [40–42], induced Neu3 sialidase activity in Neu1-deficient human WG544 or 1140F01 sialidosis fibroblast cells and Neu4 knockout primary murine macrophage cells. Olanzapine-induced Neu3 activity attenuated IRβ and IRS1 phosphorylation associated with insulin-stimulated human fibroblast cells. This study provides confirmation additional evidence that Neu1 sialidase is involved in the regulation of insulin signaling and that a down-regulation Neu1 by Neu3 activity may lead to an impaired insulin receptor signaling and subsequently contributing to insulin-resistant diabetes.