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Cytoplasmic receptor-interacting protein 140 (RIP140) interacts with perilipin to regulate lipolysis

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ABSTRACT

Receptor-interacting protein 140 (RIP140) is abundantly expressed in mature adipocyte and modulates gene expression involved in lipid and glucose metabolism. Protein kinase C epsilon and protein arginine methyltransferase 1 can sequentially stimulate RIP140 phosphorylation and then methylation, thereby promoting its export to the cytoplasm. Here we report a lipid signal triggering cytoplasmic accumulation of RIP140, and a new functional role for cytoplasmic RIP140 in adipocyte to regulate lipolysis. Increased lipid content, particularly an elevation in diacylglycerol levels, promotes RIP140 cytoplasmic accumulation and increased association with lipid droplets (LDs) by its direct interaction with perilipin. By interacting with RIP140, perilipin more efficiently recruits hormone-sensitive lipase (HSL) to LDs and enhances adipose triglyceride lipase (ATGL) forming complex with CGI-58, an activator of ATGL. Consequentially, HSL can more readily access its substrates, and ATGL is activated, ultimately enhancing lipolysis. In adipocytes, blocking cytoplasmic RIP140 accumulation reduces basal and isoproterenol-stimulated lipolysis and the pro-inflammatory potential of their conditioned media (i.e. activating NF-κB and inflammatory genes in macrophages). These results show that in adipocytes with high lipid contents, RIP140 increasingly accumulates in the cytoplasm and enhances triglyceride catabolism by directly interacting with perilipin. The study suggests that reducing nuclear export of RIP140 might be a useful means of controlling adipocyte lipolysis.

1. Introduction

Adipocytes store energy as triglyceride (TG) in lipid droplets (LDs) under a nutrient-excess condition, and triglyceride can be used as the energy source through lipolysis in a nutrient-deprived state [1]. In healthy individuals, adipocytes alter their lipid storage, lipolysis, and glucose uptake according to the nutritional status and hormonal fluctuations. Defects in homeostasis of the adipose tissue (e.g., glucose uptake, proper lipid storage, adiponectin secretion) can contribute to the initiation and progression of metabolic disorders, including type 2 diabetes mellitus (T2DM) [2–4]. Increased lipolysis following a high fat diet (HFD) can also contribute to T2DM as a result of lipotoxicity, which can manifest in the liver, muscle and heart and cause insulin resistance and cardiomyopathy [2,5–8]. Free fatty acids (FFAs) released by increased lipolysis can also enhance low-grade chronic inflammation by activating adipocyte tissue macrophages (ATMs), which further suppresses insulin sensitivity and adipocyte function [6,9]. However, the exact mechanism contributing to the dysregulation of lipolysis remains elusive [2,10].

During fasting, or in time of energy demand, TG is hydrolyzed into fatty acids and glycerol to provide a source of energy [2]. Lipolysis is a tightly regulated process, modulated by catecholamine, insulin and natriuretic peptides [1,11]. Perilipin, a structural protein associated with LDs and involved in their formation, is the major regulator of lipolysis and can modulate the basal and stimulated lipolytic rates. In resting adipocytes, perilipin reduces lipolysis and increases lipid storage, partly by sequestering comparative gene identification-58 (CGI-58), an activator of adipose triglyceride lipase (ATGL). Normally, the cytoplasmic hormone-sensitive lipase (HSL) cannot access its substrates within the LDs. In catecholamine-stimulated adipocytes, activated protein kinase A (PKA) phosphorylates both perilipin and HSL. Phosphorylation of perilipin frees CGI-58 to stimulate ATGL activity, whereas phosphorylation of HSL increases its association with the phosphorylated perilipin on LDs, thereby enhancing its access to substrate [12,13]. Both mechanisms are promoted by perilipin phosphorylation, but it is less clear if the actions of perilipin can be modulated by other mechanisms.

Receptor-interacting protein 140 (RIP140) is well known as a co-regulator for numerous transcription factors and nuclear receptors. It is abundantly expressed in various tissues including ovary, uterus, and testis, as well as in metabolic tissues/organs such as adipose tissue,

Abbreviations: RIP140, receptor-interacting protein 140; PKCε, protein kinase C epsilon; LD, lipid droplet; HSL, hormone-sensitive lipase; ATGL, adipose triglyceride lipase; CGI-58, comparative gene identification-58; DAG, diacylglyceride.

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liver and muscle [14,15]. Studies of RIP140 knockout mice demonstrated that RIP140 plays roles in numerous biological processes such as ovulation and metabolism [14,16,17]. In the nucleus, it recruits additional cofactors such as histone deacetylases (HDACs) and C-terminal binding protein (CtBP) for transcriptional regulation [15,18]. Depleting RIP140 from adipocytes leads to decreased TG accumulation and a higher rate of fatty acid oxidation [19,20]. Conversely, RIP140 expression is elevated during adipogenesis. Following a series of post-translational modifications (PTMs), RIP140 is increasingly exported to the cytoplasm [15,21,22]. In animals, HFD can induce cytoplasmic accumulation of RIP140 in adipocytes, but the signal triggering cytoplasmic accumulation of RIP140 is unclear [23]. Mechanistically, the export of RIP140 to the cytoplasm is stimulated by nuclear protein kinase C epsilon (PKCε)-elicited serine phosphorylation, followed by protein arginine methyltransferase 1 (PRMT1)-stimulated arginine methylation of RIP140 [22]. Predictably, cytoplasmic RIP140 performs functions different from those involved in gene regulation [19,20,22]. For example, cytoplasmic RIP140 can negatively regulate glucose transporter type 4 (GLUT4) trafficking by interacting with the 160-KDa Akt substrate (AS160), thereby reducing glucose uptake [23]. Questions remain to be answered concern the possible roles of cytoplasmic RIP140 in lipid-loaded adipocytes, especially with regards to their lipid metabolism, and the identity of specific mediators that might transmit lipid signals to promote aberrant accumulation of RIP140 in the cytoplasm of fully differentiated, lipid-loaded adipocytes after a HFD feeding.

This study shows that the adipocyte fat content can trigger cytoplasmic accumulation of RIP140, and demonstrates a new functional role for cytoplasmic RIP140 in adipocyte; specifically, it positively modulates lipolysis through its direct interaction with perilipin. The physiological relevance of this pathway is validated by examining the pro-inflammatory potential of conditioned media collected from adipocyte cultures with altered cytoplasmic RIP140 accumulation. Our findings provide new insights into the roles of cytoplasmic RIP140 in HFD-induced adipocyte dysfunction and support the notion that targeting cytoplasmic RIP140 could be a therapeutic strategy in managing T2DM or other metabolic syndromes.

2. Materials and methods

2.1. Cell culture and treatment

3T3-L1 cells were maintained and differentiated as described [24]. For DAGK inhibitor, mature 3T3-L1 adipocytes were treated with R59022 for 24 h and cell lysates were collected. For lipolysis, mature 3T3-L1 adipocytes were starved in serum-free medium for 3 h and then incubated with serum-free medium with or without 10 μM isoproterenol for another 2 h.

2.2. Reagents and transfection

Antibodies for actin, lamin, CGI-58 and PKCε were from Santa Cruz Biotechnology. Anti-flag, anti-alpha tubulin, anti-calnexin and anti-perilipin A were from Sigma Aldrich. Anti-Oxophos complex IV antibody was from Upstate. Anti-HSL, anti-ATGL and anti-Phospho-PKA substrate antibodies were from Cell signaling. Anti-perilipin A, anti-giantin and anti-calreticulin antibodies were from Abcam. Anti-RIP140 (ab42126) was from Abcam and its specificities in immunofluoresence and immunoblotting were determined in previous report [23]. Anti-cyclophilin A antibody was from calbiochem. siRNAs were from Qiagen. Insulin was from Sigma Aldrich. Isoproterenol was from Cayman. BODIPY 493/503 was from Molecular Probes (Invitrogen). siRNA transfect ion was conducted by DeliverX Plus siRNA transfection kit (Panomics) as manufacturer's instruction. Plasmid of full length and different fragments of Flag-RIP140 constructs were as described [23]. Plasmid of full length and different fragment of perilipin were cloned from cDNA of 3T3-L1 adipocyte and then cloned into pCMV-PL vector-containing 3xFlag tags.

2.3. Western blotting, immunoprecipitation and immunofluoresence

Western blotting was conducted as described previously [24]. For immunoprecipitation, 500 μg whole cell lysates were incubated with 5 μg indicated antibodies for 2–3 h in 500 μl Co-IP buffer (50 mM Tris–HCl pH 8.0, 10% glycerol, 100 mM NaCl, 1 mM EDTA and 0.1% NP-40) and then incubated with protein G beads (Upstate) overnight. After centrifugation, beads were washed using a Co-IP buffer three times, and the precipitates were subjected into SDS-PAGE for western blotting. Immunofluorescence assay was conducted as a previous report [23]. For co-staining with lipid droplet, BODIPY 493/503 and fluorescence-conjugated secondary antibodies were co-incubated with cells for 1–3 h. Images were acquired by Olympus FluoView1000 IX2 inverted confocal microscope. Colocalization analysis was performed by Manders Coefficients in Image J as previous described [25].

2.4. Cell fractionation

Nuclear and cytoplasmic fractionation, cells were collected and fractions were collected as in a previous report [23]. Forty 10-cm plates with mature 3T3-L1 adipocytes were collected for isolating organelles as reported (31). 80 μg proteins from indicated organelles were subjected into western blotting. Organelles isolation was performed as previous report [26].

2.5. TG content measurement and lipolysis assay

TG content was assayed as described previously [24]. For lipolysis assay, cells were starved in serum-free medium and then stimulated with or without isoproterenol for 2 h. Media were collected and glycerol levels within the media were determined by adipolysis assay kit (Cayman) as manufacture's instruction. The glycerol levels were normalized to the protein amounts of the cell lysates.

2.6. In vitro GST pull-down assay

GST-RIP140 and GST-perilipin were produced by BL-21 strain. Expression was induced by IPTG at 20 °C overnight. Bacteria pellets were lysed in PBS. For GST-perilipin, lysate was collected and incubated with GST beads to purify GST-perilipin. For GST-RIP140, after centrifugation, insoluble pellet was lysed by Inclusion Body Solubilization Reagent (Thermo Scientific) and lysate was dialyzed as instruction. Dialyzed lysate was incubated with GST beads to purify refolded GST-RIP140. For pull-down assay, it was conducted as a previous report with modifications [23]. In GST-RIP140 set, the washing condition was 50 mM Tris–HCl pH 8.0, 10% glycerol, 100 mM NaCl, 1 mM EDTA and 2% NP-40. Peptide fragments were synthesized *in vitro* by TNT assay kit as previous report [23].

2.7. Mice

Male mice (C56BL/6J) (Jackson Laboratory) were housed in a temperature-controlled environment with 12-hr light/dark photocycle and fed with a normal diet (5% fat) (#2018, Harlan Teklad) or a high-fat diet (HFD) (60% fat) (#F3282, Bio-Serv). Animal experiments were conducted in procedures approved by University of Minnesota Institutional Animal Care and Use Committee.

2.8. Tissue collection and immunohistochemical staining

Epididymal adipose tissues were fixed, embedded and sectioned by the Histology & Microscopy Core Facility (University of Minnesota). Immunohistochemical staining of RIP140 and perilipin was performed