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CD36 mediates lipid accumulation in pancreatic beta cells under the duress of glucolipotoxic conditions: Novel roles of lysine deacetylases

Sabbir Khan, Anjaneyulu Kowluru*

β-Cell Biochemistry Laboratory, John D. Dingell VA Medical Center, and Department of Pharmaceutical Sciences, Wayne State University, Detroit, MI, 48201, USA

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ABSTRACT

The cluster of differentiation 36 (CD36) is implicated in the intake of long-chain fatty acids and fat storage in various cell types including the pancreatic beta cell, thus contributing to the pathogenesis of metabolic stress and diabetes. Recent evidence indicates that CD36 undergoes post-translational modifications such as acetylation-deacetylation. However, putative roles of such modifications in its functional activation and onset of beta cell dysregulation under the duress of glucolipotoxicity (GLT) remain largely unknown. Using pharmacological approaches, we validated, herein, the hypothesis that acetylation-deacetylation signaling steps are involved in CD36-mediated lipid accumulation and downstream apoptotic signaling in pancreatic beta (INS-1832/13) cells under GLT. Exposure of these cells to GLT resulted in significant lipid accumulation without affecting the CD36 expression. Sulfo-*n*-succinimidyl oleate (SSO), an irreversible inhibitor of CD36, significantly attenuated lipid accumulation under GLT conditions, thus implicating CD36 in this metabolic step. Furthermore, trichostatin A (TSA) or valproic acid (VPA), known inhibitors of lysine deacetylases, markedly suppressed GLT-associated lipid accumulation with no discernible effects on CD36 expression. Lastly, SSO or TSA prevented caspase 3 activation in INS-1832/13 cells exposed to GLT conditions. Based on these findings, we conclude that an acetylation-deacetylation signaling step might regulate CD36 functional activity and subsequent lipid accumulation and caspase 3 activation in pancreatic beta cells exposed to GLT conditions. Identification of specific lysine deacetylases that control CD36 function should provide novel clues for the prevention of beta-cell dysfunction under GLT.

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1. Introduction

Type 2 diabetes (T2D) is a chronic metabolic disorder, which is manifested by abnormal glucose and lipid homeostasis. Insulin resistance and pancreatic beta-cell dysfunction are the two key contributors for the pathogenesis of T2D. Diabetic patients frequently exhibit hyperlipidemia in addition to hyperglycemia [1]. Chronic exposure of cells to elevated glucose and unsaturated fatty acids (often referred to as glucolipotoxicity; GLT), is a major contributor to the beta-cell dysfunction and progression of T2D [2,3]. GLT causes excess fat accumulation and functional impairment in several metabolic pathways in adipose tissue as well as pancreas, liver, heart and muscle [2,4]. Increased circulating lipids and the metabolic alterations in fatty acid utilization and downstream intracellular signaling, have also been implicated in beta-cell dysfunction [1,3]. Lastly, long-term exposure of primary islets to elevated fatty acid has been shown to cause insulin secretory defects, loss of beta cell mass and apoptosis [5,6]. Numerous mechanisms have been proposed for GLT-induced beta-cell dysregulation, and these include alterations in lipid metabolism, endoplasmic reticulum stress and mitochondrial dysfunction [7–9]. Interestingly, increased intracellular generation of reactive oxygen species and associated oxidative stress mediated by phagocyte-like NADPH oxidase (Nox2) have also been implicated in metabolic dysfunction and demise of the pancreatic beta cell [10–12].

Cluster of differentiation 36 (CD36), also known as fatty acid translocase (FAT), is a membrane glycoprotein present on many cell types including pancreatic beta-cells. CD36 is a multifunctional protein that facilitates lipid/fat intake, fat taste perception, fat absorption, immunity, and angiogenesis [13]. CD36 is also a receptor for fatty acids and is involved in lipid utilization and storage, thus

* Corresponding author. B-4237 Research Service, John D. Dingell VA Medical Center, 4646 John R, Detroit, MI, 48201, USA.
E-mail address: akowluru@med.wayne.edu (A. Kowluru).

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contributing in the pathogenesis of metabolic disorders including obesity and diabetes [14]. It is noteworthy that CD36 undergoes a variety of post-translational modifications, such as ubiquitination, glycosylation, palmitoylation, acetylation and phosphorylation [15]. Such modifications are likely to exert regulatory effects on subcellular localization and function of CD36. However, little is known with regard to regulation of CD36 function in the pancreatic beta cell.

It has been shown recently that sulfosuccinimidyl oleate (SSO), an irreversible inhibitor of CD36, prevents CD36-mediated fatty acid intake in a variety of cell types. SSO inhibits CD36 function *via* formation of *n*-hydroxysuccinimidyl esters with the lysine (Lys) 164 of CD36 in the fatty acid binding pocket leading to a conformational change in CD36 thereby preventing fatty acid intake and/or fatty acid-induced signaling [16,17]. It should also be noted that the binding pocket of CD36 contains another lysine (Lys-166), which does not bind SSO. Thus, the acetylation-deacetylation of Lys-166 and/or other Lys-52, -231, and -403 residues might dictate access of fatty acids to the binding pocket, thus contributing to regulation of CD36 and it s downstream signaling events [14]. Along these lines published evidence also suggests that acetylation-deacetylation steps control function of a number of proteins involved in glucose and fatty acid metabolism potentially resulting in the beta cell dysfunction and the onset of diabetes [18–20]. In this context, published evidence suggests that acetylation-deacetylation of histone and non-histone proteins can directly or indirectly control signaling steps involved in glucose and lipid metabolism [21,22]. Indeed, recent findings by Daneshpajooh et al., demonstrated that HDAC7 is overexpressed in human diabetic islets and contributes to impaired insulin secretion [23]. Based on the findings reviewed above, we undertook the current investigation to validate the hypothesis that acetylation-deacetylation signaling steps underlie functional activation of CD36 leading to lipid accumulation and caspase 3 activation in pancreatic beta cells under the duress of GLT conditions.

2. Materials and methods

2.1. Materials

Sulfosuccinimidyl oleate (SSO; CAS No. 135661-44-8; purity ≥95%), valproic acid (VPA; CAS No. 1069-66-5; purity ≥95%) and trichostatin A (TSA; CAS No. 58880-19-6; purity ≥98%) were from Cayman Chemical (Ann Arbor, MI, USA). Anti-CD36 was from Santa Cruz Biotechnology (CA, USA). Antisera directed against cleaved (active) caspase 3 and mouse HRP-conjugated secondary antibodies were obtained from Cell Signaling (Danvers, MA, USA). Antibody for β-actin and all other reagents employed in the current studies were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Cell culture and experimental conditions

INS-1832/13 cells (kindly provided by Prof. Chris Newgard, Duke University Medical Center) were cultured in RPMI-1640 medium containing 10% heat inactivated FBS supplemented with 100 IU/ml penicillin and 100 IU/ml streptomycin, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol and 10 mM HEPES (pH 7.4). The cultured cells were sub-cloned twice weekly following trypsinization and passages 55–61 were used for the experiments. To assess the roles of CD36 in lipid accumulation and caspase-3 activation, INS-1832/13 cells were exposed to glucotoxic (20 mM), lipotoxic (0.5 mM palmitate) and GLT (20 mM glucose plus 0.5 mM palmitate) conditions in the absence or presence of SSO (200 μM), VPA (2.5–5.0 mM) or TSA (0.25–0.5 μM), as indicated.

2.3. Western blotting

Following incubations (above), the cells were harvested and lysed in RIPA buffer containing 1 mg/ml protease inhibitor cocktail, 1 mM NaF, 1 mM PMSF and 1 mM Na_3VO_4. Cellular lysate proteins (30–50 μg) were separated by SDS-PAGE and electro-transferred onto the nitrocellulose membrane. The membranes were then blocked with 1% casein in 0.2X PBS for 1 h at room temperature. Blots were then incubated overnight at 4 °C with appropriate primary antibody in 0.2X PBS-T containing 0.1% casein. The membranes were washed three times for 15 min each with PBS-T and probed with appropriate HRP-conjugated secondary antibody in 0.1% casein in PBS-T at room temperature for 1 h. After washing, the immune complexes comprised of the target proteins, were detected on X-ray film using Pierce-ECL western blotting substrate Thermo Fisher Scientific (Waltham, MA, USA). The intensity of bands was quantified using Carestream® Molecular Imaging Software.

2.4. Quantification of lipid accumulation

INS-1832/13 cells were cultured in chamber slides and incubated in a medium containing the low glucose (Con) and GLT (20 mM glucose plus 0.5 mM palmitate) in the absence and presence of SSO (200 μM), VAP (2.5–5.0 mM) or TSA (0.25–0.5 μM) for 24 h. At the end of the incubation, media was discarded and cells were washed twice with ice-cold 1x PBS. The cells were then fixed with 10% buffer formalin at room temperature for 1 h. Then lipid (Oil Red O) staining was performed using a staining kit (MAK194-1KT, Sigma) as per the manufacturer's instructions. Slides were mounted with aqueous media using glass cover-slip and stored at 4 °C until observed under microscope (Olympus, XI71) at 40x and 100x objectives. Images were captured for each treatment condition. For quantitative analysis 12-15 images were process and % ORO stained area (lipid accumulation) was measured by Image J software (https://imagej.nih.gov/ij/).

2.5. Statistical analysis

All the data were presented as mean ± standard error of mean (SEM) of three independent experimental conditions unless otherwise mentioned. The statistical significance of the differences between the experimental conditions was determined by ANOVA. The p values < 0.05 was considered as statistically significant.

3. Results

3.1. Lipotoxic or glucolipotoxic conditions promote lipid accumulation in INS-1832/13 cells without affecting CD36 expression

At the outset we assessed the effects of gluco-, lipo-, or glucolipotoxic conditions on lipid accumulation in insulin-secreting INS-1832/13 cells. Data depicted in Fig. 1A and Fig. S1 indicate that lipotoxic, but not glucotoxic conditions significantly augmented lipid accumulation within 24 h of exposure. It is noteworthy that the lipid accumulation was much more significant in the combined presence of both glucose and palmitate (glucolipotoxicity). We next assessed if increased lipid accumulation is, in part, due to increased expression of CD36, which is known to mediate the uptake and storage of lipids in various cell types, including the islet β-cell. Data in Fig. 1B and Fig. S2 indicate no significant effects of GLT conditions on CD36 expression, especially under conditions of increased lipid accumulation as shown in Fig. 1.

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