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Effect of diosgenin on metabolic dysfunction: Role of ERβ in the regulation of PPARγ

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ABSTRACT

The present study was designed to investigate the effect of diosgenin (DSG) on metabolic dysfunction and to elucidate the possible molecular mechanisms. High fat (HF) diet-fed mice and 3T3-L1 preadipocytes was used to evaluate the effect of DSG. We showed that DSG attenuated metabolic dysfunction in HF diet-fed mice, as evidenced by reduction of blood glucose level and improvement of glucose and insulin intolerance. DSG ameliorated oxidative stress, reduced body weight, fat pads, and systematic lipid profiles and attenuated lipid accumulation. DSG inhibited 3T3-L1 adipocyte differentiation and reduced adipocyte size through regulating key factors. DSG inhibited PPARγ and its target gene expression both in differentiated 3T3-L1 adipocytes and fat tissues in HF diet-fed mice. Overexpression of PPARγ suppressed DSG-inhibited adipocyte differentiation. DSG significantly increased nuclear expression of ERβ. Inhibition of ERβ significantly suppressed DSG-exerted suppression of adipocyte differentiation and PPARγ expression. In response to DSG stimulation, ERβ bound with RXRα and dissociated RXRα from PPARγ, leading to the reduction of transcriptional activity of PPARγ. These data provide new insight into the mechanisms underlying the inhibitory effect of DSG on adipocyte differentiation and demonstrate that ERβ-exerted regulation of PPARγ expression and activity is critical for DSG-inhibited adipocyte differentiation.

1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM) and related metabolic disorders is dramatically serious and reaches epidemic proportions worldwide (Wild et al., 2004). It is estimated that 350–400 million people will suffer from T2DM in the next two decades (Wild et al., 2004). In parallel with the burst of T2DM, obesity has become a worldwide public health problem which is closely associated with the development of T2DM (Kopelman, 2000).

In general, fat tissue is generally controlled by adipocyte proliferation, differentiation and lipolysis. Adipocyte differentiation, also called adipogenesis, is a complex process which is regulated by a battery of molecular pathways triggered by an adipogenic stimulus. The transcription factor, peroxisome proliferator activated receptor γ (PPARγ) (Tontonoz et al., 1995), plays a pivotal role in the sequential regulation of adipogenic signals and terminal maturation of fat cell. The important role of PPARγ in metabolic regulation was demonstrated by the observation that antidiabetic drugs thiazolidinediones were high affinity ligands of PPARγ (Lehmann et al., 1995). Animals deficient with PPARγ are unable to form adipose tissue, indicating the essential role of PPARγ in fat formation (Barak et al., 1999).

In the last decades, much attention has been paid to dietary agents and natural products that can be used for treating metabolic diseases. Diosgenin (DSG) is an important natural source of steroidal hormones in the pharmaceutical industry. As an aglycone of the steroidal saponin, DSG has been shown to have favorable effects in the improvement of diabetes and regulation of lipid metabolism (Roman et al., 1995; Chiang et al., 2007). Estrogen receptors (ER) α and ERβ belong to the nuclear receptor family of transcription factors (Bjornstrom and Sjoberg, 2005; Hewitt et al., 2005), which have been reported to mediate the effects of DSG (Wu et al., 2015). However, whether ER is involved in DSG-exhibited effect on metabolic function and adipocyte differentiation is still unclear. The present study was designed to investigate the effect of DSG on high fat (HF) diet-induced metabolic dysfunction and adipocyte differentiation, and to elucidate the possible role of ER.

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2. Materials and methods

2.1. Materials

β-Actin, PPARγ, RXRα and ERβ antibodies and IBMX were purchased from Santa Cruz Biotechnology. Hoechst and DHE were purchased from Beyotime Institute of Biotechnology. MitoSOX were purchased from Invitrogen. DSG, Oil Red O, insulin, the inhibitors and most of the chemicals and reagents used in this study were procured from Sigma.

2.2. Animal treatment

All experiments were performed according to the procedures approved by Fourth Military Medical University Animal Care and Use Committee. 30 male C57 mice were purchased from Animal Centre of Fourth Military Medical University. The mice were housed under temperature (23 ± 2 °C) and humidity (55 ± 5%) condition with a standard light (12 h light/dark) cycle. Mice were randomly divided into three groups: Control, high fat (HF) diet (Supplemental Table 1) group, and HF diet + DSG group. Mice in HF diet group were given HF diet for 4 months. Mice in HF diet + DSG group were administrated with DSG (in drinking water, 5 mg/L) for the last 1 month. Water containing DSG was changed every 2 days. After the treatment, fasted blood glucose level (12 h) and refed blood glucose level (4 h) were measured. At the end, the mice were anesthetized with sodium pentobarbital, perirenal and epididymal adipose tissues were weighed and then tissue and blood samples were harvested for the assays.

2.3. IPGTT and IPITT

To evaluate glucose and insulin tolerance, intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were performed as we previously described (Wang et al., 2012). Mice were fasted for 12 h and 4 h, respectively, and the blood samples dropped from their tails for baseline detection of glucose level. After that, each mouse was intraperitoneally injected with glucose (Sigma, 1 g/kg body weight), or insulin (Novolin R, 0.75 U/kg body weight). Blood samples dropped from tails at 30, 60, and 120 min after the injection and were analyzed immediately for glucose concentration.

2.4. ROS determination

ROS was detected by specific probe DHE and MitoSOX as we previously described (Wang et al., 2013). Briefly, frozen sections of livers and fat tissues were stained with 10 μM DHE or 1 μM MitoSOX (kept in the dark, 37 °C) for 30 min. Inflorescence intensity was observed under a laser scanning confocal microscope (Olympus).

2.5. Biochemical analysis

At the end of the animal treatment, blood samples were harvested and serum was separated for estimation of triglyceride (TG), free fatty acids (FFA), and total-cholesterol (TC) using commercial kits (Nanjing Jiancheng Company, China). Assays were conducted according to the manufacture's instruction.

2.6. Cell culture and treatment

3T3-L1 cells were cultured as previously described (Tormos et al., 2011). Briefly, cells were cultured in DMEM containing 10% bovine calf serum until the cells became confluent. To induce differentiation, 2 days post confluence (Day 0), 3T3-L1 preadipocytes were stimulated with 0.5 mM isobutylmethylxanthine (IBMX), 1 μM dexamethasone, and 167 nM insulin in DMEM containing 10% FBS (DMI differentiation medium) for 2 days (Day 2). Cells were then maintained in a 10% FBS/DMEM medium with 167 nM insulin for another 2 days (D4) and then cultured in 10% FBS/DMEM medium for an additional 4 days (D8), at which time more than 90% of cells became mature adipocytes with lipid-filled droplets. All media contained penicillin (100 U/mL), streptomycin (100 μg/mL), and glutamine (292 μg/L). The cells were maintained at 37 °C in a humidified 5% CO_2 atmosphere. In some experiments, DSG was added in DMSO along with DMI and the same concentration of DSG was maintained when the medium was changed.

2.7. Determination of lipid accumulation

For the determination of adipocyte differentiation, cells were fixed with 4% paraformaldehyde for 30 min, rinsed with water. Frozen liver sections and fix cells were stained with Oil Red O solution (6 parts of saturated Oil Red O dye in isopropanol + 4 parts of water) for 30 min. Cells were washed by water to remove excessive dye. Lipid accumulation in cells was observed under a microscope (Olympus BX51 with DP70 camera).

2.8. Real-time PCR

Following treatment, RNA was extracted from cells and was reverse transcribed using commercial kits (TIANGEN) according to the manufacturer's protocol. Real-time PCR was performed using a BIORAD PCR instrument in 20 μL reaction mixtures. The relative amount of RNA was quantified using the comparative cycle threshold (C_T) ($2^{-\Delta\Delta}CT$) method.

2.9. Western blot

Protein extractions of livers and cells were separated by using SDS-PAGE on 8–12% polyacrylamide gels, and transferred to nitrocellulose membranes. Protein bands were detected using chemiluminescent reagents according to the manufacturer's protocol and observed using an image analyzer Quantity One System (Bio-Rad). The protein quantifications were adjusted for the corresponding Histone level.

2.10. Chromatin immunoprecipitations

Chromatin immunoprecipitations (CHIP) was conducted using a Thermo Scientific Pierce Agarose ChIP Kit according to the manufacture's instruction. After the treatment, cells were cross-linked using formaldehyde (1%). Then, cells were harvested, and cross-linked chromatin was sheared by sonication. Sonicated cell lysate was immunoprecipitated using PPARγ antibody. DNA present in the immunoprecipitated chromatin was isolated after reversed cross-link, and PCR was performed to examine the presence of aP2 gene promoter.

2.11. Co-immunoprecipitations

Co-immunoprecipitations (Co-IP) was conducted using a Thermo Scientific Pierce Co-Immunoprecipitation Kit. Antibodies for bait proteins (RXRα or ERβ) were immobilized covalently using amino-link columns according to manufacturer's protocol. Lysates were obtained, cleared on agarose resin, and immunoprecipitated according to the protocol. Western blot detection of RXRα, ERβ or PPARγ was conducted on IP eluates as described above. IP column flow through with no bait antibody was run as an input control.

2.12. Statistical analysis

Results were expressed as the means ± SD. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the Newman Keuls multiple-comparison post hoc test using Graph-Pad