EBioMedicine 6 (2016) 139–148

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Irisin Inhibits Hepatic Cholesterol Synthesis via AMPK-SREBP2 Signaling

Hong Tang [a], Ruili Yu [a], Shiying Liu [a], Bahetiyaer Huwatibieke [a], Ziru Li [b], Weizhen Zhang [a,b,*]

[a] Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing 100191, China
[b] Department of Surgery, University of Michigan Medical Center, Ann Arbor, MI 48109-0346, USA

ARTICLE INFO

Article history:
Received 5 November 2015
Received in revised form 23 February 2016
Accepted 25 February 2016
Available online 27 February 2016

Keywords:
Irisin
AMPK
SREBP2
Cholesterol
Liver

ABSTRACT

Irisin, a myokine released during exercise, promotes browning of subcutaneous adipose tissue and regulates energy homeostasis. Although exercise constantly reduces blood cholesterol, whether irisin is involved in the regulation of cholesterol remains largely unknown. In the present study, subcutaneous infusion of irisin for 2 weeks induced a reduction in plasma and hepatic cholesterol in high fat diet-induced obese (DIO) mice. These alterations were associated with an activation of 5′ AMP-activated protein kinase (AMPK) and inhibition of sterol regulatory element-binding transcription factor 2 (SREBP2) transcription and nuclear translocation. In primary hepatocytes from either lean or DIO mice, irisin significantly decreased cholesterol content via sequential activation of AMPK and inhibition of SREBP2. Suppression of AMPK by compound C or AMPKα1 siRNA blocked irisin-induced alterations in cholesterol contents and SREBP2. In conclusion, irisin could suppress hepatic cholesterol production via a mechanism dependent of AMPK and SREBP2 signaling. These findings suggest that irisin is a promising therapeutic target for treatment of hypercholesterolemia.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Irisin was recently identified as a myokine proteolytically cleaved from fibronectin type β domain containing 5 (FNDC5) (Bostrom et al., 2012). Exercise could induce irisin expression and secretion into circulation to exert its effects on browning of subcutaneous adipose tissue (Bostrom et al., 2012; Wu et al., 2014; Shan et al., 2013; Rodriguez et al., 2015; Lee et al., 2014; Wu et al., 2012), as well as subsequent improvement of obesity (Huh et al., 2014; Miyamoto-Mikami et al., 2015) and its related disorders such as type 2 diabetes (Xiong et al., 2015; Bostrom et al., 2012; Vaughan et al., 2014). These observations suggest that irisin may contribute to the regulation of energy homeostasis and thus is the potential target for therapy of metabolic dysfunction associated with obesity. This concept is further supported by clinical observations. Circulating irisin was found to be reduced in obese human (Yan et al., 2014; Moreno-Navarrete et al., 2013; Duran et al., 2015; Hou et al., 2015) and rodents (Bilski et al., 2015) and in patients with diabetes (Espes et al., 2015; Li et al., 2015a; Choi et al., 2013; Xiang et al., 2014; Liu et al., 2013; Duran et al., 2015; Moreno-Navarrete et al., 2013; Kurdiova et al., 2014), or chronic kidney disease (Wen et al., 2013; Ebert et al., 2014). Conversely, it was positively associated with endothelium-dependent vasodialation (Xiang et al., 2014; Hou et al., 2015; Zhu et al., 2015) and myocardiac infraction in type 2 diabetes (Aronis et al., 2015; Hou et al., 2015).

The relationship between irisin and lipid metabolism has been controversial. Evidence suggesting a negative relation between irisin and lipid dysregulation has been emerging. Circulating levels of irisin were negatively correlated with total cholesterol, LDL cholesterol and triglyceride (Huh et al., 2012; Zhang et al., 2013; Ebert et al., 2015; Duran et al., 2015) and intrahepatic triglyceride contents in obese adults (Zhang et al., 2013), while positively correlated with HDL cholesterol. In obese human, diet intervention-induced reduction in glucose and triglyceride was greater in those with higher baseline irisin levels (Lopez-Legarrea et al., 2014). Lentivirus-mediated overexpression of FNDC5 or subcutaneous perfusion of irisin reduced blood triglyceride, cholesterol, free fatty acid and glucose in obese mice (Xiong et al., 2015). Further studies in vitro showed an inhibitory effect of irisin on palmitic acid (PA)-induced lipid accumulation and lipogenic markers via inhibition of protein arginine methyltransferase-3 in AML12 cells and mouse primary hepatocytes (Park et al., 2015). Other studies have suggested a positive relation between irisin and lipid dysfunction. Circulating irisin levels were positively associated with total cholesterol, LDL cholesterol and fasting fatty acids in a Chinese population independent of BMI (Tang et al., 2015) and in women with polycystic ovary syndrome (Li et al., 2015b). Energy restriction induced depletion of irisin was associated with decrease in total cholesterol, total cholesterol/HDL-cholesterol

Abbreviations: DIO, high fat diet-induced obese; AMPK, 5′ AMP-activated protein kinase; SREBP2, sterol regulatory element-binding transcription factor 2; FNDC5, fibronectin type β domain containing 5; OA, oleic acid; NCD, normal chow diet; HFD, high-fat diet; BW, body weight; WAT, white adipose tissue; CC, compound C; siRNA, small interfering RNA.

* Corresponding author at: Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing 100191, China.
E-mail addresses: tangyunlian626@126.com (H. Tang), yyyrrrlll@126.com (R. Yu), from_jessica@163.com (S. Liu), tafcalgen@163.com (B. Huwatibieke), liziru@umich.edu (Z. Li), weizhenz@umich.edu (W. Zhang).

http://dx.doi.org/10.1016/j.ebiom.2016.02.041
2352-3964/© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ratio, LDL-cholesterol and apolipoprotein B, independent of changes in body weight (de la Iglesia et al., 2014). In adults at higher cardiovascular risk, irisin was negatively associated with HDL cholesterol and large HDL particles (Panagiotou et al., 2014). In addition, serum irisin was significantly higher in patients with nonalcoholic fatty liver disease (Choi et al., 2014) and in patients with portal inflammation (Polyzos et al., 2014). Moreover, plasma irisin was positively related to total cholesterol in adults with Prader–Willi Syndrome (Hirsch et al., 2015). Nevertheless, other studies have found no significant association of irisin with raised triglyceride and reduced HDL in obese adults with metabolic syndrome (Yan et al., 2014) or with NAFLD (Polyzos et al., 2015). In addition, irisin has been demonstrated to exercise no effect on either lipolysis in 3T3-L1 adipocytes or fatty acid metabolism in HepG2 hepatocytes (Wang et al., 2015). Therefore, further investigation is necessary to define the role of irisin in lipid metabolism.

Here we reported that irisin suppressed cholesterol synthesis in hepatocytes through the activation of 5′ AMP-activated protein kinase (AMPK) and subsequent inhibition of transcription and nuclear translocation of sterol regulatory element-binding transcription factor 2 (SREBP2).

2. Materials and Methods

2.1. Materials

Antibodies used in the study were: pAMPKα Thr172 (2535, CST, Beverly, MA, USA) and AMPKα (2532, CST), SREBP2 (ab30682, Abcam, Cambridge, MA, USA) and β-actin (AT 0001, Milwaukee, WI). Donkey-anti-rabbit Alexa Fluor® 488-IgG (711-545-152) was from Jackson ImmunoResearch (West Grove, PA, USA). IRDye-conjugated affinity purified anti-rabbit and anti-mouse IgGs were purchased from Rockland (Gilbertsville, PA, USA). Irisin (067-16) was from Pheonix (Burlingame, CA, USA). Recombinant irisin-Fc and Fc control were expressed in HEK293 cells (Abgent, Nanjing, China) and purified by high-performance liquid chromatography. Oleic acid (OA), collagenase IV and compound C were purchased from Sigma Aldrich (St. Louis, MO, USA). Alzet microosmotic pumps (1002) were from DURECT Corporation (Cupertino, CA, USA). Aprotinin was purchased from Amersham Biosciences (Pittsburgh, PA, USA). Triglyceride and cholesterol Colorimetric Assay Kits were from Cayman Chemical Company (Ann Arbor, MI, USA). BCA protein quantitative assay kit was from Applied Gene (Beijing, China).

2.2. Animals

Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). All experimental protocols were approved by the Animal Care and Use Committee of Peking University. Mice were housed in standard plastic rodent cages and maintained in a regulated environment (24 °C, 12-h light and 12-h dark cycle with lights on at 7 AM and off at 7 PM). Four-week-old male C57BL/6J mice were fed a normal chow diet (NCD) or a high-fat diet (HFD) (60% fat, D12492; Research Diets, New Brunswick, NJ, USA) ad libitum for 14 weeks followed by 2-week subcutaneous perfusion of irisin-Fc or Fc control at a dose of 12 nmol/d·kg body weight (BW). Food intake was measured and averaged for 2 mice in one cage. Mice were sacrificed without fasting after anesthesia by intraperitoneal injection of pentobarbital sodium at 70 mg/kg BW. Liver was frozen in liquid nitrogen and stored in −80 °C freezer for future experiments. Blood samples were transcardially collected and immediately transferred to chilled polypropyrene tubes containing EDTA-Na_2 (12.5 mg/mL) and aprotinin (1000 units/mL) and centrifuged at 4 °C. The plasma was separated and stored at −80 °C before use.

2.3. Implantation of Osmotic Minipumps

The minipumps were placed in a Petri dish with sterile 0.9% saline at 37 °C for at least 4 h, then filled with the test agent before implantation. Mice were anesthetized with pentobarbital sodium and a 1 cm incision was made in the back skin through which an Alzet osmotic minipump (model 1002) filled with irisin-Fc or Fc control was implanted subcutaneously.

2.4. Isolation and Culture of Primary Hepatocytes

Twelve-week old lean or diet-induced-obese (DIO) C57BL/6J mice were anesthetized with pentobarbital sodium at 60 mg/kg BW and injected intraperitoneally with 1000 IU heparin. After laparotomy, the portal vein was cannulated. The liver was perfused with 20 mL of pre-warmed 37 °C Hanks buffer, followed by 20 mL of 0.02% collagenase IV at a flow rate of 2 mL/min. After perfusion, liver tissues were removed and washed with warm Hanks buffer. Liver capsule was removed, and hepatic tissues dispersed and incubated in 20 mL of 0.01% collagenase IV in a shaking water bath at 37 °C for 20 min. Cell suspension was then filtered through 80-μm nylon mesh twice, centrifuged at 500 rpm [SorvallRT7 Benchtop Centrifuge with RTH 250 rotor (Ramsey, MN)] and washed twice with DMEM to remove tissue dissociation enzymes, damaged cells, and nonparenchymal cells. Dispersed hepatocytes were counted and seeded at a concentration of 1–2 × 10^5 cells per well in a 6-well plate containing 2 mL of high glucose DMEM supplemented with 10% (vol/vol) FBS. Cells were cultured at 37 °C in a humidified atmosphere of 5% (vol/vol) CO_2. Culture medium was changed to high glucose DMEM supplemented with 2% (vol/vol) FBS 24 h later. Hepatocytes were then treated with 10 nM irisin for 20 min or 3, 6, 12 and 24 h as indicated in the absence or presence of 125 μM OA. Where indicated, compound C was added 1 h prior to the addition of irisin and OA.

2.5. Cell Culture and siRNA Transfection

HepG2 cells were cultured with high glucose DMEM supplemented with 10% (vol/vol) FBS at 37 °C in a humidified atmosphere of 5% (vol/vol) CO_2. Cells were seeded in a 12-well plate at 30–50% confluency. siRNA was transfected using siRNA-Mate (G04002, GenePharma, Shanghai, China) following the manufacturer's instructions. Culture medium was changed to high glucose DMEM supplemented with 2% (vol/vol) FBS 48 h later. Cells were stimulated with 10 nM irisin for 12 h or 24 h as indicated. siRNA sequences were for Scrambled sense: 5′-UUCUCCGAACGUGUCACGUTT-3′, antisense: 5′-ACGUGACACGUUCG GAGAATT-3′; for siAMPKα1 sense: 5′-CGGGAUCAGUUAGCAACUATT-3′, antisense: 5′-UAGUUGCUAACUGAUCCCGTT-3′.

2.6. Measurements of Triglyceride and Cholesterol Content

Twenty milligram liver tissues were homogenized in 1 mL of 2:1 chloroform/methanol mix on ice and placed at 4 °C for 18 h. Two hundred microliters of distilled water was added to the homogenates. The mixture was vortexed, then centrifuged for 10 min at 3000 rpm, 4 °C. The lower phase was collected, lyophilized and resolved in 5% Triton X-100 in PBS for measurements of lipids. Primary hepatocytes were homogenated according to manufacturer's instructions, and the supernatant was used for lipid detection. Plasma, hepatic and primary hepatocytes triglyceride and cholesterol were measured according to the manufacturer's instructions. Values were normalized to protein concentration.

2.7. Western Blot Analysis

Liver tissues and primary hepatocytes were homogenized in RIPA lysis buffer. Proteins were subjected to SDS/PAGE separation

Download English Version:

https://daneshyari.com/en/article/2120672

Download Persian Version:

https://daneshyari.com/article/2120672

Daneshyari.com