Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Excessive irisin increases oxidative stress and apoptosis in murine heart

Ming-Yun Ho [1], Ming-Shien Wen[*, 1], Jih-Kai Yeh, I-Chang Hsieh, Chun-Chi Chen, Ming-Jer Hsieh, Ming-Lung Tsai, Chia-Hung Yang, Victor Chien-Chia Wu, Kuo-Chun Hung, Chun-Chieh Wang, Chao-Yung Wang[**]

Department of Cardiology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taiwan

ARTICLE INFO

Article history:
Received 26 June 2018
Accepted 2 July 2018
Available online xxx

Keywords:
Irisin
Reactive oxygen species
Mitochondria
Apoptosis

ABSTRACT

Irisin is an exercise-related myokine. The abundance of irisin is associated with many diseases, such as myocardial infarction, chronic kidney disease, metabolic syndrome, obesity, and diabetes mellitus. In cardiomyocytes, irisin modulates the mitochondrial thermogenesis, regulates ischemic responses, and affects calcium signaling. Previous studies suggested that irisin increases cardiomyoblast mitochondrial functions and protects ischemic and reperfusion injury in *ex vivo* murine heart. In human, clinical studies have shown that acute myocardial infarction patients with more elevated serum irisin abundances are associated with increased major adverse cardiovascular events. However, the mechanisms responsible for this discrepancy between in myocardial infarction patients and *ex vivo* murine heart is unclear. Based on the clinical observations, we hypothesized that excessive irisin might lead to mitochondrial dysfunctions and cardiomyocyte damages. Our data showed that overexpression of irisin in mice with the adenovirus resulted in enhanced mitochondrial respiration with a higher oxygen consumption rate. Enhanced irisin expression in heart and irisin treatment in cardiomyocytes increased reactive oxygen species production. Furthermore, irisin treatment in cardiomyocytes enhanced the apoptosis and the cleaved caspase 9 levels in hypoxic condition. Pathway analysis in the murine heart with the overexpression of irisin showed that angiopoietin-Tie2, IL-8, IL-13, TGF-β, and thrombopoietin signaling were affected by irisin. Collectively, these results supported that excessive irisin causes mitochondrial overdrive with a higher reactive oxygen species production, which results in increased apoptosis of cardiomyocytes in a hypoxic environment.

1. Introduction

Irisin is a hormone secreted by the muscle and adipose tissue [1]. The cell membrane prohormone FNDC5 is cleaved at its ectodomain to generate irisin. Human irisin circulates at a serum level of 3.6 ng/ml in sedentary individuals and increases to 4.3 ng/ml in individuals after aerobic training [2]. Circulating irisin abundances have essential functions in many disease processes [3–5]. Patients with diabetes mellitus and renal disease have lower irisin level [6]. Decreased irisin correlates with increased carotid atherosclerosis in the patients receiving hemodialysis [7]. Irisin can prevent mice hepatic steatosis through protein arginine methyltransferase-3 signaling [8]. Decreased irisin secretion is associated with altered insulin signaling and contributes to insulin resistance in high-fat diet mice [9]. Irisin treatment in mice also prevents bone loss and muscle atrophy [10,11].

Irisin is highly expressed in the myocardium. Treatment of irisin in cardiomyoblasts H9C2 cells significantly inhibits cell proliferation activates intracellular calcium signaling and increases cellular oxygen consumption [12]. In the *ex vivo* Langendorff perfused heart, irisin produces protective effects from ischemic and reperfusion injury [13]. In the rat, serum irisin levels decreased after myocardial infarction [14]. However, a recent clinical study showed that acute myocardial infarction patients who have higher irisin serum levels had worse cardiovascular outcomes, including higher mortality, stroke, and heart failure [15]. The exact mechanism

* Corresponding author. 5 Fu-Hsing Street, Taoyuan, 333, Taiwan
** Corresponding author. 5 Fu-Hsing Street, Taoyuan, 333, Taiwan
E-mail addresses: wenms123@gmail.com (M.-S. Wen), cwang@ocean.ag (C.-Y. Wang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.07.005
0006-291X/

leading to these different observations between *ex vivo* mice and myocardial infarction patients are unclear.

Irisin is a critical regulator for mitochondrial homeostasis. Intravenous irisin enters alveolar lung cells and targets mitochondria [16,17]. The mitochondria play essential roles in oxygen stress, apoptosis, and cellular survival under hypoxia condition [18–20]. Homeostasis of mitochondria in myocardial infarction plays critical roles in myocardial survival [21–24]. Circulating irisin is positively correlated with the levels of exercise and multiple exercise-related physiological functions, including hippocampal brain-derived neurotrophic factor secretion and the browning of beige fat [1,2,25]. Based on the beneficial effects of exercise on the cardiovascular systems after myocardial infarction, we assumed that elevated irisin level is associated with reduced adverse cardiovascular events [26]. Because of this discrepancy, we decided to investigate the direct function of irisin in cardiomyocytes and mice. In this study, we evaluated the direct effect of irisin in the mice and found that irisin can overdrive mitochondria and increases reactive oxygen species.

2. Materials and methods

2.1. Mice and adenovirus injection

The adenoviruses were generated using adenovirus type-5 vector kits with pDC516 (Microbix, Toronto, Canada). pCR-blunt-TOPO-FNDC5 was a gift from Bruce Spiegelman (Addgene plasmid # 35970). FNDC5 cDNA was blunt-end cloned into pDC516. Adeno-FNDC5 and control adenovirus adeno-516 titers were measured using adenovirus titration kits (Clontech, California, USA). Tail vein injections were performed with 1.5×10^{10} viral particles in C57BL6/J mice. The mice were analyzed 10–14 d after adenovirus injection.

2.2. Mitochondrial function and lucigenin chemiluminescent assays

The XF96 Extracellular Flux Analyzer (Seahorse Bioscience, MA, USA) was used to measure the oxygen consumption rate in mitochondria freshly isolated from mice. Oligomycin (1 μM), FCCP (Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone, 0.75 μM), and rotenone/antimycin A (1 μM) were used to determine the maximal respiration, proton leak, ATP production, and spare respiratory capacity. For lucigenin chemiluminescent assays, mouse whole-heart homogenates were suspended in 200 μL of the assay buffer (100 mmol/L potassium phosphate (pH 7.0), 10 μmol/L flavin adenine dinucleotide (FAD), 1 mmol/L NaN3, and 1 mmol/L EGTA). After preincubation with 5 μM lucigenin, NADH or NADPH was added to a final concentration of 500 μM. The chemiluminescence was continuously monitored with a luminometer.

2.3. Cell culture, RNA, and protein analysis

Rat cardiomyocytes were prepared from 1~2-d-old neonatal rats. The atria were excised, and the ventricles were minced and digested in collagenase II and pancreatin enzymatic solutions[53]. The cardiomyocytes were then purified with percoll gradients and cultured. For hypoxia experiments, cardiomyocytes were placed into serum-free hypoxia buffer (125 mM NaCl, 8 mM KCl, 1.2 mM KH_2PO_4, 1.25 mM $MgSO_4$, 1.2 mM $CaCl_2$, 6.25 mM $NaHCO_3$, 20 mM 2-deoxyglucose, 5 mM sodium lactate, and 20 mM HEPES, pH 6.6) and transferred to a hypoxia chamber (5% CO_2 AND 95% N_2) for the indicated time. A TUNEL assay was performed according to the ApopTag Plus Fluorescein In Situ Kit (Millipore, Darmstadt, Germany). Total RNA was extracted using TRI reagent (Ambion, Massachusetts, USA) according to the manufacturer's instructions. One microgram of total RNA was reverse-transcribed and analyzed using the Applied Biosystems Real-time PCR system. The relative gene expression method was used for analysis, and the expression of the target genes was normalized to that of 18 S rRNA. The assay was independently repeated at least three times. Protein was isolated from homogenized frozen kidneys or cells with cell lysis buffer (Cell Signaling Technology, Massachusetts, USA). The lysates were separated by electrophoresis, transferred to polyvinylidene fluoride membranes, and probed with specific cleaved caspase 9 antibody (Cell Signaling Technology, Massachusetts, USA). The results were normalized to the GAPDH band and calculated with Image J (NIH, Maryland, USA). The activity of irisin (Enzo, New York, USA) was verified in well-differentiated 3T3-L1 preadipocytes that uncoupling protein-1 was significantly elevated after 20 nM or 100 nM irisin treatment. We choose 20 nM for the experiments in cardiomyocytes because we noticed the effects of 20 nM and 100 nM irisin in adipocytes were similar in inducing uncoupling protein-1.

2.4. Microarray analysis

Microarray experiments were performed at the Genomic Medicine Research Core Laboratory (GMRCL) of Chang Gung Memorial Hospital. The RNA samples were hybridized using Affymetrix Mouse Genome 430 A 2.0 Oligonucleotide Microarrays. All analysis was performed in duplicate, and dye swap experiments were used. The signals that were differentially expressed >2 or <0.75 were considered significant and further analyzed. Network and pathway analyses were performed with MetaCore (GeneCo).

2.5. Statistical analysis

For mouse or cell experiments with smaller sample sizes or non-normally distributed data, two-sample Mann-Whitney *U*-tests were used.

3. Results

Increased irisin level in mice is associated with increased cardiac mitochondrial respiration and elevated reactive oxygen species.

To study the *in vivo* function of irisin in mice, we injected *FNDC5* (*irisin*)-overexpressing and control adenovirus through the tail veins of C57BL6/J mice. After injecting for over a 10-d period, analysis of the *irisin* mRNA confirmed the overexpression in the liver and heart (Fig. 1). A significant increase in *UCP*-1 mRNA abundances in the heart after the injection of irisin adenoviruses compared to control adenoviruses was noted (Fig. 1). In line with the results of previous reports, adenoviral injections through tail veins in mice result in high expression in the liver but not in the white fat of mice [27,28]. Analysis of mitochondrial respiration via oxygen consumption rate in hearts with an XF24 mitochondrial flux analyzer (Fig. 2A). It showed that the injection of irisin adenovirus increased the maximal mitochondrial respiration and ATP production rates more than the control adenovirus (Fig. 2B).

Increased energy expenditure and mitochondrial respiration are beneficial for body weight control and energy metabolism [28]. However, elevated mitochondrial respiration can result in increased generation of reactive oxygen species, which lead to cardiovascular diseases [29]. We tested superoxide production with lucigenin-enhanced chemiluminescence [30]. Left ventricular tissues isolated from mice injected with irisin adenovirus exhibited a significantly higher level of reactive oxygen species production compared to that seen with control adenovirus (Fig. 2C). The increased level of reactive oxygen species by irisin treatment was significantly inhibited by the mitochondrial specific antioxidant, Mito-TEMPO, in rat cardiomyocytes (Fig. 2C).

Please cite this article in press as: M.-Y. Ho, et al., Excessive irisin increases oxidative stress and apoptosis in murine heart, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.005