Integrin αV Mediates the Effects of Irisin on Human Mature Adipocytes

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Keywords
Integrin αV · Irisin · Obesity · Adipocyte browning

Abstract
Introduction and Aims: The myokine irisin is critical to modulating adipocytes thermogenesis and influence whole-body metabolism. However, whether there is difference in the effects of irisin on adipocytes derived from different depots remains unknown, and the receptor of irisin on adipocytes is still unclear. In this study, we determine the browning effect of irisin on adipocytes of subcutaneous and visceral human adipose tissue and explore the possibility that integrin αV was the receptor of irisin on human adipocytes. Methods: Human adipose-derived stem cells were isolated from human subcutaneous and visceral white adipose tissues and induced to differentiate into mature adipocytes, and the expression of UCP1 and thermogenic genes in mature adipocytes were examined with or without irisin treatment and compared between groups of different adiposity and anatomic position. Results: Irisin treatment could increase the expression level of beige adipocyte marker protein UCP1 and specific thermogenic genes in mature adipocytes derived from subcutaneous white adipose tissue but not in visceral adipose tissue. The results of immunoprecipitation showed that irisin could be attached to integrin αV on mature adipocytes, and there was no significant difference in the gene and protein expression of integrin αV in adipocytes, either derived from subcutaneous and visceral adipose tissue, or derived from obese and normal-weight individuals. Conclusion: The results of the present study indicated that irisin contributed to the transformation of mature white adipocytes to beige adipocytes in human subcutaneous adipose tissue but not in visceral adipose tissue. Integrin αV may mediate the browning effects of irisin on human mature adipocytes, which could provide the potential therapeutic targets for obesity and metabolic syndrome by promoting human brown adipose tissue activity.

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Introduction

The prevalence of obesity has increased to a pandemic level in the past decades [1]. Obesity represents a major health challenge because it is an independent risk factor for diabetes, hypertension, hyperlipidemia, ischemic heart disease, and some cancers, thereby contributing to a decline in both quality of life and life expectancy [2, 3]. Obesity is defined as the excessive fat accumulation that may impair health, and adipose tissue function is critical to the development and treatment of obesity. There are two types of adipose tissue in humans, white adipose tissue (WAT) that mainly stores energy and brown adipose tissue (BAT) which contains uncoupling protein 1 (UCP1) and burns energy to generate heat [4]. Following the identification of functional BAT in human adults [5], there has been a great deal of interest in finding out how it is induced and the mechanisms by which it regulates thermogenesis, hoping to find new therapeutic target for obesity and metabolic disease.

There are several ways that could promote human white adipose tissue browning including cold [6, 7], calorie-restricted diets [8], and exercise [9, 10]. Exercise improves quality of life and reduces the incidence of several disorders including obesity and the endocrine function of skeletal muscle involved in the mechanisms of this protective effect [11]. Myokines are released from skeletal muscle in response to contraction and can influence metabolism in other tissues and organs, and it is acknowledged that some optimal effects on metabolism are mediated by the transcriptional coactivator PPARγ coactivator 1α [12]. The expression of PPARγ coactivator 1α in muscle stimulates the increased expression of the fibronectin type III domain 5, which divides and secretes irisin for release into the blood circulation [13].

Irisin has been reported to promote UCP1 protein expression in 3T3-L1 cell line [14] and human white adipose tissue [15], but the receptor of irisin in adipose tissue has not been confirmed yet. A recent study reported that integrin αV may mediate the effect of irisin on rat bone and adipose tissue [16], colon cancer cells [17], and microvascular endothelial cells [18], and it is reasonable to speculate that integrin αV may also act as a irisin receptor on human mature adipocytes. To confirm this hypothesis, we used immunoprecipitation analysis to detect the interaction between integrin αV and irisin on human mature adipocytes; the integrin αV expression and irisin browning ability on human mature adipocytes were also compared between groups with different adiposity and different anatomic position.

Materials and Methods

Participants

Adipose tissue samples (2–3 g) from the abdominal subcutaneous and the peripheral portion of the greater omentum were collected from 71 patients who were undergoing elective abdominal surgery in Jinan Central Hospital. Entry criteria were patients aged 30–60 years old and body mass index (BMI) below 24 or over 28 kg/m². No patient had intra-abdominal inflammatory disease, advanced cancer, or diabetes. This study is approved by the Ethics Committee of Jinan Central Hospital. Written, informed consent was obtained from all the participants.

Participants were divided into normal-weight group (NW group BMI ≤24 kg/m²) and obese group (OB group BMI ≥28 kg/m²) according to BMI, and the two groups were further divided into subgroups according to the adipose tissue location: normal-weight-subcutaneous group (NW-sub group), normal-weight-visceral group (NW-vis group), obese-subcutaneous group (OB-sub group), and obese-visceral group (OB-vis group). The four groups were matched for age and sex when compared. Height, weight, systolic blood pressure, and diastolic blood pressure were measured before surgery. BMI was calculated as weight (kilograms) divided by height (meters) squared.

Isolation of Human Mature Adipocytes and Stromal Vascular Cells

Adipose tissues were minced and digested with 1 mg/mL collagenase in 37°C shaking H2O bath for 30–40 min. Then the digested tissues were filtered through a 250-micron mesh and left to settle for 10 min. The floating mature adipocytes were collected from the top layer and used for immunoprecipitation analysis. The remaining cell suspension was spun down by centrifugation at 2,000 rpm (10 min), and human adipose-derived stem cells (hADSCs) were obtained in the pellet and cultured with basic medium (DMEM/f12 medium, 10% fetal bovine serum, 100 U/mL penicillin-streptomycin).

Primary Adipocyte Culture and Adipogenic Differentiation

Human subcutaneous and visceral ADSCs were cultured in 6-well plates. Cell differentiation was induced for 14 days by adding a differentiation cocktail: DMEM medium supplemented with 10% fetal bovine serum ( Gibco Life Technologies), 0.5 mM 3-isobutyl-1-methylxanthine, 10 μg/mL insulin, 0.1 μM dexamethasone, and 200 μM indomethacin; medium was refreshed every third day. The differentiated mature adipocytes were treated with or without irisin (50 nm) for 4 days. Adipogenic differentiation was confirmed by Oil Red O staining [19].

Co-Immunoprecipitation

The mature adipocytes isolated from adipose tissue were treated with irisin (100 nM) for 20 min, lysed by IP lysis buffer (P0013, Beyotime Biotechnology, China), and centrifuged at 12,000 rpm at 4°C for 15 min to get the protein solution, and protein concentration was determined by the BCA method. Approximately 300 μg proteins were incubated with the rabbit anti-integrin αv antibody (1:100, 4711S, CST, USA) and anti-IgG antibody (1 μg, 2729S, CST, USA) overnight, respectively, and then incubated with 20 μL Protein A/G PLUS-Agarose (sc-2003, Santa, CA, USA) at 4°C on a rocker platform for 3 h. Immunoprecipitates were collected by centrifugation at 2,500 rpm for 5 min at 4°C, and the pellet was washed by PBS.
(including PMSF) and resuspended in loading buffer. The immunoprecipitated solution (20 μL) was analyzed by Western blot analysis using the rabbit anti-irisin antibody (1:1,000, ab174833, Abcam) and rabbit anti-integrin αv antibody (4711s, CST, USA, 1:1,000).

Western Blot Analysis
Mature adipocytes isolated from adipose tissue were lysed by RIPA lysis buffer plus protease inhibitor (vol/vol 100/1) and then centrifuged and obtained the protein solution. Protein concentrations were determined by the BCA Protein Assay kit (Beyotime Biotechnology). Approximately 20 μg protein of each sample was separated by 10% SDS-PAGE and transferred onto PVDF membranes (0.45-μm pore size, Merck Millipore, Burlington, MA, USA). The membranes were incubated overnight at 4°C with specific primary antibodies consisting of the anti-integrin αv antibody (1:500, 4711S, CST, USA), anti-UCP1 antibody (1:1,000, 14670, CST, USA), and anti-β actin antibody (1:1,000, 3700S, CST, USA), which was followed by incubation with appropriate secondary anti-rabbit/mouse IgG conjugated with horseradish peroxidase (HRP) (Beijing Zhongshan Gold Bridge Biotechnology, Beijing, China) for 1 h at room temperature. The protein bands were detected using the Chemiluminescent HRP Substrate present in the FluorChem E system (Cell Biosciences, Santa Clara, CA, USA) and analyzed with Alphaimager 2200.

Quantitative Real-Time PCR
Total RNA was extracted from mature adipocytes using the TRIzol reagent (AG21102, Accurate Biology, China) following the manufacturer’s recommendations. RNA was dissolved in nuclease-free water and quantified using a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (1 μg) was reverse-transcribed using the Reverse Transcription Reaction Kit (AG11705, Accurate Biology, China), and quantitative real-time PCR was performed on the LightCycler 480 System (Roche, Basel, Switzerland). The relative mRNA expression levels were calculated using the 2^(-ΔΔCt) method after standardization to β-actin mRNA levels. The quantitative real-time PCR reactions were performed using primers as described in online supplementary material (see www.karger.com/doi/10.1159/000523871 for all online suppl. material).

Immunohistochemistry
Fresh adipose tissue samples were fixed, embedded in paraffin, and cut into slices and then deparaffinized and the antigen retrieved at 95°C. Tissue sections were incubated with anti-integrin αV antibody (1:100) overnight at 4°C, then with the HRP-conjugated antirabbit secondary antibody for 1 h at room temperature, stained with DAB to detect the images and take photos by positive fluorescence microscope (Olympus).

Statistical Analysis
The analysis was performed using SPSS (version 23.0, IBM Corporation, Chicago, IL, USA). Normal distribution data were analyzed statistically using the nonpaired Student’s t test. The expression levels of integrin αV, UCP1, and thermogenic genes between different groups were compared using the nonpaired Student’s t test. Data were expressed as mean ± SD, and the statistically significant difference were defined as p < 0.05.

Results
Subject Characterization
Adipose tissue samples from 71 participants (28 subcutaneous and 43 visceral adipose tissue samples) were used in this study. Unfortunately, we cannot get all the data needed from each sample because of the limited adipose tissue volume that could be provided by surgery. As showed in Table 1, 35 and 32 visceral adipose tissue samples and 20 and 16 subcutaneous adipose tissue samples
were used for adipocytes integrin αV mRNA and protein expression analysis, respectively. hADSCs were isolated from 6 adipose tissue samples and were induced to differentiate into mature adipocytes, which were used for irisin treatment and UCP1 and thermogenic genes expression analysis. Human mature adipocytes were isolated from 3 subcutaneous adipose tissues. The groups were matched for age and gender when compared.
**Irisin Treatment Could Improve Human Mature Adipocytes Beige Transformation**

hADSCs were isolated from subcutaneous (n = 3) and visceral (n = 3) adipose tissue and were induced to differentiate into mature adipocytes. On day 3 of the induction, a few lipid droplets were visible, and after 14 days of induction, most cells differentiated into mature adipocytes which were identified by Oil Red O staining (Fig. 1a). Irisin was reported to have the ability of improving subcutaneous adipose tissue browning [13, 14, 20] in the previous study, which was assumed to due to the promotion of precursor beige adipocyte de novo differentiation. To examine if the irisin treatment can increase the transformation of mature adipocytes into beige adipocytes, the differentiated mature adipocytes were treated with irisin (50 nM) for 4 days. The results showed that with the treatment of irisin, the gene expression of UCP1, PRDM16, and CDIEA was significantly increased in adipocytes derived from subcutaneous adipose tissue but not from visceral adipose tissue. The gene expression of PPARγ did not change with irisin treatment in adipocytes derived from both visceral and subcutaneous adipose tissues (Fig. 1b). Similar results were also found in UCP1 protein expression analysis, which indicated that the adipocytes UCP1 protein expression also increased significantly with irisin treatment in subcutaneous adipose tissue group but not in the visceral adipose tissue group (Fig. 1c).

**Irisin Conjugates with Integrin αV in Human Mature Adipocytes**

Mature adipocytes were isolated from human adipose tissue (Fig. 2a), which were verified by Nile red and Hoechst33342 staining (Fig. 2b). Immunoprecipitate was used to explore the interaction between irisin and integrin αv on mature adipocytes and white adipose tissue. Immunoprecipitate was analyzed by Western blot analysis. The results showed that irisin bands (24 kD) were visible with addition of anti-integrin αv in the integrin group, while no irisin expression was found with IgG addition in the control group (Fig. 2c), which indicated that irisin could bind to the integrin αv receptor on mature adipocytes and white adipose tissue.

**Mature Adipocytes Integrin αv Expression Is Independent of Adiposity and Anatomic Location**

Thirty-five visceral adipose tissue samples (17 normal-weight individuals and 18 obese patients) and 20 subcutaneous adipose tissue samples (10 normal-weight indi-
Fig. 3. Adipocyte integrin αV mRNA and protein expression was independent of adiposity and anatomic location. **a** Comparison of adipocyte integrin αV gene expression levels, which were examined by qPCR, between subcutaneous adipose tissue and visceral adipose tissue groups, NW and OB groups. **b** Adipocyte integrin αV protein expression levels were detected by Western blot in subcutaneous adipose tissue and visceral adipose tissue groups. **c** Integrin αV protein expression in white adipose tissue was detected by immunohistochemistry analysis. NW-sub, normal-weight-subcutaneous group; NW-vis, normal-weight-visceral group; OB-sub, obese-subcutaneous group; NW-vis, obese-visceral group.
viduals and 10 obese patients) were used to isolate mature adipocytes. The NW group and OB group were matched for age and gender, and the clinical data of the participants were showed in Table 1.

The results of qPCR analysis showed that there were no significant difference in adipocytes integrin αv gene expression between NW and OB groups, neither in visceral adipose tissue and subcutaneous adipose tissue group (Fig. 3a). The results of Western blot analysis was also similar to qPCR, which showed that the adipocyte integrin αv protein expression level in subcutaneous adipose tissue has no significant difference compared to that in the visceral adipose tissue and so did in adipocytes derived from normal-weight and obese individuals (Fig. 3b). The results of integrin αV immunohistochemical analysis in adipose tissue were also in accordance with the above results (Fig. 3c).

Discussion

The myokine irisin is reported to be critical to modulating adipocytes thermogenesis and influence whole-body metabolism [21, 22], and it is considered as a hope in understanding and managing obesity and metabolic syndrome. However, the diverse browning effects of irisin on adipocytes derived from different anatomic locations and the receptor of irisin on human adipocytes are still unclear. In this study, we determine that irisin treatment could improve the transformation of mature white adipocytes into beige adipocytes derived from human subcutaneous adipose tissue but not from visceral adipose tissue. The browning effects of irisin on human subcutaneous mature adipocytes may be mediated by integrin αV, but the expression level of integrin αV on adipocytes was independent of adiposopathy and the adipose tissue anatomic location.

Because beige adipocyte development is highly inducible, a special emphasis has been given to this cell type as an appealing cellular target of new obesity therapeutics. It was reported that irisin treatment could switch on brown-fat like program in white fat in mice [13, 20], resulting in ameliorated metabolism and reduced insulin resistance [23, 24], and could promote beige gene and UCP1 protein expression in human subcutaneous white adipocytes [14, 15], which was assumed to promote white adipocyte browning in humans [25]. Another debated topic is whether beige adipocytes induced by irisin treatment arise from preexisting mature adipocytes or via the de novo differentiation of resident precursor cells, which was not illuminated in the previous studies. The present study indicated that irisin treatment could induce UCP1/thermogenic characteristics in human mature adipocytes. These adipocytes may appear morphologically white but have a beige identity and thus the capacity to quickly reinitiate the thermogenic program in response to thermogenic inducers [26]. This study also found that irisin had diverse effects on the browning of human subcutaneous and visceral adipose tissue. Irisin increased the expression of UCP1 as well as beige adipocytes specific thermogenic genes in adipocytes established from subcutaneous adipose tissue but not from visceral adipose tissue, which was in accordance with the previous studies [15, 27].

A growing number of studies on the association of irisin with different subunits of integrin have drawn attention to the possibility that irisin may act as a new ligand for the integrin [16, 28]. Integrins represent a large family of transmembrane cell adhesion molecules that consist of noncovalently associated α/β heterodimers [29]. Eighteen types of α chain and eight types of β chain associate with each other to form 24 different heterodimers.

Irisin was revealed to bind to several integrins in adipocytes and osteocytes, with integrin αV classes, including αVβ5 and αVβ1, exhibiting the strongest binding affinity [30]. The integrin αV was also involved in irisin-mediated FAK signaling in CD81 + adipocyte progenitor cells [31]. Moreover, irisin was proven to bind to the integrin αVβ5 receptor on gut epithelial cells both in vitro and in vivo [17], and irisin exerted ameliorating effects on endothelial damage [18] and promotes osteoclast generation via integrin-triggered signaling [32]. The co-immunoprecipitation results of the present study revealed that irisin could bond to integrin αV on human mature adipocytes, which indicated that integrin αV maybe the proposed receptor of irisin and mediated its browning effect on human mature adipocytes.

Since the discovery of irisin might exert potentially beneficial effects on metabolic diseases [13, 27], many studies have tried to determine the association between serum irisin levels and obesity. Our previous study has proved that obese subjects had significantly higher serum irisin levels compared to nonobese subjects in nondiabetes population [33], which was in accordance with the result of Sahin-Efe et al. [34, 35]. Muscle and adipose tissue were the main source of human circulating irisin. Most of studies showed a positive correlation between circulating levels of irisin and whole-body mass [36, 37], which may possibly maximize energy usage and glucose homeostasis to achieve metabolic balance [21, 23]. Elevated levels of irisin in obese subjects may also be due to irisin resistance developed during the course of obesity. As
the proposed receptor of irisin on mature adipocytes, we also want to determine if the integrin αV expression in mature adipocytes was also associated with personal adiposity or adipose tissue anatomic location. But, the present results showed that there was no significant difference in integrin αV expression between mature adipocytes derived from individuals with different adiposity and from a different anatomic location. We still cannot rule out the possibility that there is a functional connection between irisin and other receptors on adipocytes that may contribute to the regulation of white adipose tissue browning.

In conclusion, the results of present study indicated that irisin may contribute to the transformation of mature white adipocytes to beige adipocytes in human subcutaneous adipose tissue but not in visceral adipose tissue. Integrin αV may mediate the browning effects of irisin on human mature adipocytes, but the expression level of integrin αV on adipocytes was independent of adiposity and adipose tissue anatomic location. However, the molecular mechanisms that underlie these effects and benefits are not well understood, partly due to the lack of knowledge regarding the irisin receptor. Future work is needed to elucidate further details of interaction between irisin and integrin αV and the underlying post-receptor signaling pathway, which may lead to the exploitation of advanced treatments to obesity and metabolic syndrome by promoting adipocytes browning in human white adipose tissue.

Acknowledgments

We are thankful to Lulu Zhang and Han Li for technical assistance.

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