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Irisin inhibition of growth hormone secretion in cultured tilapia pituitary cells

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ABSTRACT

Irisin, the product of fibronectin type III domain-containing protein 5 (FNDC5) gene, is well-documented to be a regulator of energy metabolism. At present, not much is known about its biological function in non-mammalian species. In this study, a full-length tilapia FDNC5 was cloned and its tissue expression pattern has been confirmed. Based on the sequence obtained, we produced and purified recombinant irisin which could induce uncoupling protein 1 (UCP1) gene expression in tilapia hepatocytes. Further, the rabbit polyclonal irisin antiserum was produced and its specificity was confirmed by antiserum preabsorption. In tilapia pituitary cells, irisin inhibited growth hormone (GH) gene expression and secretion and triggered rapid phosphorylation of Akt, Erk1/2, and p38 MAPK. Furthermore, irisin-inhibited GH mRNA expression could be prevented by inhibiting PI3K/Akt, MEK1/2, and p38 MAPK, respectively. Apparently, fish irisin can act directly at the pituitary level to inhibit GH transcript expression via multiple signaling pathways.

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1. Introduction

Irisin is a novel peptide hormone released upon cleavage of the plasma membrane protein fibronectin type III domain-containing protein 5 (FNDC5). In mammalian species, irisin is highly conserved across species with 100% identity between human and mice. The secreted irisin, binding to yet-to-be-discovered receptor, has the potential to increase energy expenditure and improve glucose homeostasis (Bostrom et al., 2012). The circulating irisin induces the browning of subcutaneous white adipocytes and stimulates thermogenic genes including uncoupling protein 1 (UCP1) both in stromal vascular fraction (SVF) cells and in mouse models resulting in increased thermogenesis and subsequently energy expenditure (Bostrom et al., 2012). The distributions of FDNC5 mRNA and irisin are widespread, suggesting that this peptide is a multi-functional endocrine factor in mammalian species (Polyzos et al., 2015). Abnormal circulating irisin levels were linked with several pathophysiological conditions, including type 2 diabetes (Erol et al., 2016; Garcia-Fontana et al., 2016; Zhang et al., 2016), the risk of non-alcoholic fatty liver disease (Choi et al., 2014), chronic kidney disease (Wen et al., 2013; Yang et al., 2015), systolic heart failure (Lecker et al., 2012), oxidative stress (Huh and Mantzoros, 2015) and inflammation (Dulian et al., 2015). At present, the studies on biological actions of irisin are restricted to mammals and no information is available in fish species.

Although the mouse (Bostrom et al., 2012) and human (Huh et al., 2012) skeletal muscle represents a major source of irisin in circulation, FDNC5 mRNA is also detected at relatively high expression levels in chicken (Li et al., 2015) and rat (Varela-Rodriguez et al., 2016) pituitary. More recent data suggest a relationship between the GH/IGF1 axis and irisin (Srinivasa et al., 2016). In human, the GH treatment results in the increase of irisin concentration in plasma (Wikiera et al., 2015). Whether irisin generated within the pituitary and/or from peripheral tissues can also exert a feedback control on GH release by effects acting on at the pituitary level remains an interesting topic for research. GH is primarily produced in the pituitary gland. The biological actions of GH are pleiotropic, including growth promotion, energy mobilization, gonadal development, appetite, and social behavior (Canosa et al., 2007). The neuroendocrine regulation of GH secretion has been recognized as a balance between stimulatory and inhibitory factors acting on the somatotrope. However, no functional data are available on irisin contributions to GH secretion.

Abbreviations: GH, Growth hormone; UCP1, uncoupling protein 1; PI3K, Phosphoinositide-3-kinase; Akt, Akt/PKB Serine/threonine Kinase; MAPK, Mitogen-activated Protein Kinase; Erk, Extracellularly Regulated Kinase; MEK, MAPK/Erk Kinase.

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The primary objective of this study was to examine molecular characteristics, tissue expression, and biological actions of irisin on GH secretion in talipia. Tilapia is an economically important species for fish farming in China and is favored for its meat texture, flavor, and high nutritive value (Chiu et al., 2013). It is inevitable that efforts will be directed towards controling growth and metabolism of finfish to enhance production. As a first step, tilapia FNDC5 was cloned by 5'/3' RACE and identification of tissue expression profiles was characterized by real-time PCR. Moreover, the bioactive recombinant protein and specific antiserum for tilapia irisin were produced. Using primary cultures of tilapia pituitary cells as a model, the pituitary actions of irisin for regulation of GH gene expression and secretion were investigated and signal transduction pathways regulating irisin-inhibited GH mRNA expression were also elucidated.

2. Materials and methods

2.1. Animals

Male tilapia (*oreochromis niloticus*) (standard length: 11 ± 0.5 cm, body weight: 50 ± 5.0 g) were maintained in freshwater aquaria at 28 °C under 10 h dark/14 h light photoperiod for at least 7 days prior to tissue sampling. The fish were fed commercial diet (40% protein, 12% fat, 2% fiber, 8.5% moisture, 8% ash, Tongwei, China) to satiety twice a day at 10:00 and 16:00. During the process of tissue sampling, the fish were sacrificed by spinosectomy after anesthesia with 0.05% MS222 (Sigma, St Louis, MO) according to the procedures approved by the Animal Ethics Committee of Sichuan University.

2.2. Test substances

Human recombinant irisin was purchased from Enzo Life Sciences (Farmingdale, USA), dissolved in double-distilled deionized water, and stored frozen at −80 °C as 0.1 mM stocks in small aliquots. The inhibitors for various signaling pathways, including wortmannin, LY294002, 1L-6-Hydroxymethyl-chiro-inositol-2-R-2-O-methyl-3-O-octadecylcarbonate (HIMOC), PD98059, U0126, SB203580 and PD169316 were obtained from Calbiochem (San Diego, CA). Frozen stocks of these pharmacological tools were prepared in a similar manner as in the case of irisin, except that dimethyl sulfoxide (DMSO) was used as the solvent. On the day of experiments, stock solutions of test substances were diluted with prewarmed culture medium to appropriate concentrations 15 min prior to drug administration. The final dilutions of DMSO were always less than 0.1% and had no effects on GH mRNA expression in tilapia pituitary cells.

2.3. Molecular cloning of tilapia FNDC5

Total RNA was extracted from tilapia brain using RNAzol (Molecular Research Center) and reversely transcribed using M-MLV (TaKaRa, China). Based on the Ensembl predicted cDNA sequence of tilapia FNDC5, gene-specific primers were designed and 5'/3'-RACE was conducted using a GeneRacer Kit (Invitrogen) and sequenced by ABI3100 Genetic Analyzer (BGI, Shanghai, China). After that, phylogenetic analysis of tilapia FNDC5 was conducted using the neighbor-joining method with MEGA 6.0 programs (http://www.megasoftware.net/). The amino acid sequence of tilapia irisin was aligned with that of other species by using the ClustalW program and MacVector V.9.5.2 programs (Acclelrys, San Diego, CA). Motifs were predicted using the Motif Scan program (http://myhits.isb-sib.ch/cgi-bin/motif_scan).

2.4. Tissue distribution of tilapia FNDC5

The tissue distribution of FNDC5 was examined by using quantitative real-time PCR. After the RNA extraction and reverse transcription, real-time PCR assays were performed on the CFX96 real-time PCR Detection System (Bio-Rad) by using a SYBR Select Master Mix kit (Invitrogen). For the measurement of FNDC5 mRNA, real-time PCR was conducted using the following primers [forward primer: 5′ ACCCAACAGAAGAAGGATGTA 3′ and reverse primer: 5′ GCTTCCTCCCATGCTAATACT 3']. The PCR reactions were conducted with the following thermal cycling parameters: 94 °C for 3 min, followed by 35 cycles of amplification with denaturation at 94 °C for 5 s, annealing at 56 °C for 30 s, extension at 72 °C for 10 s and then fluorescent signal collection at 80 °C for 1 s. Serial dilutions of plasmid DNA carrying the ORF of irisin was used as the standards for data calibration. As an internal control, real-time PCR for β-actin was conducted using the primers specific for β-actin [forward primer: 5′ GTGATGGTGGGTATGGGT 3′ and reverse primer: 5′ GGCAACTCTCAGCTCGTT 3']. In these experiments, the quantitative results were normalized as a ratio of the target gene/β-actin expression level.

2.5. Recombinant protein for tilapia irisin

The tilapia irisin peptide region was PCR-isolated using an Expand High Fidelity system using primers specific for tilapia irisin [forward primer: 5′ GGAATTCGACACCCTGCTGTCAGCA 3' (*EcoR*I site underlined) and reverse primer: 5′ CCAAGCTTCTCCTCCATCGTCACCTC 3' (*HindIII* site underlined)]. The restriction sites introduced into the respective primers were used for subsequent cloning of PCR products into the prokaryotic expression vector pET28a His-tag vector (Novagen, Madison, WI). PCR amplification of irisin was initiated by denaturation at 94 °C for 1 min followed by primer annealing at 55 °C for 1 min and extension at 72 °C for 2 min for a total of 30 cycles. After size fractionation by 1% agarose gel electrophoresis, PCR products for irisin was gel-purified using a QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) and subcloned into the prokaryotic expression vector pET28a His-tag vector. Sequence identity of the irisin inserts were confirmed by DNA sequencing using ABI3100 Genetic Analyzer (BGI, Shanghai, China). The expression vector generated, namely "pET28a-irisin" was introduced into *E. coli* BL21 (DE3) competent cells and single colonies of transformed bacteria were used for recombinant protein production in 250 ml Luria-Bertani (LB) medium with 50 μg/ml antibiotics ampicillin at 18 °C with constant shaking (220 rpm). IPTG induction (0.5 mM, Sigma-Aldrich, St. Louis, MO) was added when the OD260 reading of bacteria culture reached the level of 0.4–0.5. After incubation for appropriate duration, the bacteria with irisin protein expression were harvested by centrifugation at 3000 g for 20 min at 4 °C and resuspended in 1× PBS (pH 8.0) for protein extraction. In this case, the cell suspension obtained was then subjected to 3 cycles of repeated freezing and thawing, followed by a 5 min sonication using a Branson Sonifier 250 (VWR Scientific, San Diego, CA). Recombinant proteins are readily produced at high yield in a soluble fraction. Soluble protein fractions of the cell lysates were isolated by high-speed centrifugation at 12,000 g for 20 min at 4 °C. After that, tilapia irisin expressed as His-tagged recombinant protein were purified from soluble fraction by immobilized metal ion affinity chromatography using Ni-NTA Superflow ™ columns (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Fractions containing the eluted protein are pooled and subjected to PD-10 column (GE Healthcare) in order to remove salt. The recombinant proteins produced were quantified using a BCA protein assay kit (Pierce, Rockford, IL).

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