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Resveratrol protects muscle cells against palmitate-induced cellular senescence and insulin resistance through ameliorating autophagic flux

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ABSTRACT

Skeletal muscle, a highly metabolic tissue, is particularly vulnerable to increased levels of saturated free fatty acids (FFAs). The role of autophagy in saturated FFAs-induced cellular senescence and insulin resistance in skeletal muscle remains unclear. Therefore, the present study was aimed to explore autophagic flux in cellular senescence and insulin resistance induced by palmitate in muscle cells, and whether resveratrol limited these responses. Our results showed that palmitate induced cellular senescence in both myoblasts and myotubes. In addition, palmitate delayed differentiation in myoblasts and inhibited expression of insulin-stimulated p-AKTSer473 in myotubes. The accumulations of autophagosome assessed by tandem fluorescent-tagged LC3 demonstrated that autophagic flux was impaired in both palmitate-treated myoblasts and myotubes. Resveratrol protected muscle cells from palmitate-induced cellular senescence, apoptosis during differentiation, and insulin resistance via ameliorating autophagic flux. The direct influence of autophagic flux on development of cellular senescence and insulin resistance was confirmed by blockage of autophagic flux with chloroquine. In conclusion, impairment of autophagic flux is crucial for palmitate-induced cellular senescence and insulin resistance in muscle cells. Restoring autophagic flux by resveratrol could be a promising approach to prevent cellular senescence and ameliorate insulin resistance in muscle.

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1. Introduction

Skeletal muscle comprises 40–60% of total body mass and is responsible for almost 80% of whole body insulin-stimulated glucose disposal [1]. The long-term exposure of cells to high levels of free fatty acids (FFAs), particularly saturated FFAs such as palmitate, results in cellular senescence via promoting inflammation [2] and leads to deficiency of insulin-stimulated glucose uptake via PI3K-Akt signaling pathway [3]. Acceleration of cellular senescence and impairment of insulin pathway by palmitate eventually lead to a series of diseases such as obesity, type 2 diabetes, metabolic syndrome and cancer. However, the mechanisms underlying palmitate-induced cellular senescence and insulin resistance in muscle remain unclear.

Autophagy is a conserved cellular housekeeping process for the clearance of dysfunctional organelles and denatured proteins in living cells [4]. The autophagy-lysosome system is a degradation pathway that contributes to muscle protein degradation especially during muscle wasting [5]. Conversely, growing evidences indicate that autophagy is required to maintain muscle mass and regenerative function of muscle stem-cells [6,7]. These studies display a dual role of autophagy in muscle homeostasis. Several signaling pathways involved in cellular senescence and insulin resistance, including the mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK), are reported to regulate autophagy [8,9]. The mTOR-dependent autophagy activators exhibit a potent anti-senescent effect through the restoration of autophagy [8]. Inactivation of the AKT/mTOR signaling impairs insulin signaling pathway and induces abnormal autophagosome formation [10,11]. These evidences indicate that impairment of autophagy may contribute to cellular senescence and insulin resistance.

Resveratrol, a natural polyphenol found in grapes and berries, exhibits a variety of biochemical and physiological effects including reversion of senescence process and amelioration insulin resistance [12]. It has been shown that resveratrol protects cellular senescence by reducing the production of ROS through the SIRT1/NADPH pathway [13] and improves glucose uptake and insulin sensitivity through SIRT1/AMPK pathway [14]. Recently, many studies have indicated that resveratrol is an autophagy regulator that exerts cardiovascular protection, neuroprotection, anti-inflammatory and anti-cancer effect by regulating autophagy [12,15–18]. Nonetheless, evidence linking resveratrol to underlying mechanisms of skeletal muscle regulated by autophagy is still lacking.

The objective of this study was to investigate the role of autophagy in palmitate-induced cellular senescence and insulin resistance in skeletal muscle. The efficacy of resveratrol in ameliorating cellular senescence and insulin resistance induced by palmitate via modulation of autophagy in C2C12 myoblasts and myotubes was further studied.

2. Methods

2.1. Materials

Resveratrol (RSV), palmitate (PA), chloroquine (CLQ) and other chemicals were from Sigma–Aldrich Chemical Co (St. Louis, MO, USA). For Western blotting, primary antibodies: p16, p21, p62 (Santa Cruz, CA, USA), LC3, AKT, phospho-AKT (Ser473), mTOR, phospho-mTOR (Ser2448), AMPKα, phospho-AMPKα (Thr172) (Cell signaling, Danvers, MA, USA), α-actin, GAPDH (GeneTex Inc., Hsinchu City, Taiwan), and caspase-9 (Millipore, Billerica, MA, USA) were commercially available. Goat anti-rabbit and sheep anti-mouse horseradish peroxidase (HRP) conjugated secondary antibodies were purchased from Bio-Rad (Hercules, CA, USA) and GE Healthcare Life Sciences (Pittsburgh, PA, USA).

2.2. Cell culture and treatment

C2C12 myoblasts were passaged in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA) under an atmosphere of 10% CO_2 in air at 37 °C. Twenty-four hours after seeding, myoblasts were pretreated with or without 100 μM resveratrol for 16 h, and then incubated with 250 μM palmitate for 24 h (Fig. 3A).

When the myoblasts reached approximately 80% confluency, they were differentiated into myotubes in DMEM containing 2% horse serum (Invitrogen, Carlsbad, CA, USA), with medium changes every two days. After five days differentiation, differentiated myotubes were pretreated with or without 100 μM resveratrol for 16 h, and then incubated with 250 μM palmitate for 24 h (Fig. 4A).

There was no evidence for toxicity of resveratrol to myoblasts and myotubes at the concentrations employed in this study based on cell viability assay by MTT (data not show).

2.3. β-galactosidase staining

β-galactosidase activity was determined using the Senescence Detection Kit (BioVision, Milpitas, CA, USA) according to a standard protocol. Images were observed under the phase contrast microscopy and captured with a digital camera (Nikon Corporation, Tokyo, Japan). Four random fields were counted to determine the percentage of β-galactosidase-positive cells in the total cell population.

2.4. MTT assay

MTT (3-[4, 5-dimethylthiazol-2, 5-diphenyl tetrazolium bromide) assay was carried out for assessment of the growth curves. MTT dissolved in PBS at 5 mg/ml was added to the culture medium at a dilution 1:10. The cell in the number of 5000, 10,000, 20,000, 40,000, 80,000 and 16,0000 were incubated for depicting standard curve, and 10,000 cells were incubated for assessing. After 4 h incubation period with MTT, medium