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AMP-activated kinase α2 deficiency protects mice from denervation-induced skeletal muscle atrophy

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ABSTRACT

AMP-activated protein kinase (AMPK) is a master regulator of skeletal muscle metabolic pathways. Recently, AMPK activation by AICAR has been shown to increase myofibrillar protein degradation in C2C12 myotubes via stimulating autophagy and ubiquitin proteasome system. However, the impact of AMPKα on denervation induced muscle atrophy has not been tested. In this study, we performed sciatic denervation on hind limb muscles in both wild type (WT) and AMPKα2$^{-/-}$ mice. We found that AMPKα was phosphorylated in atrophic muscles following denervation. In addition, deletion of AMPKα2 significantly attenuated denervation induced skeletal muscle wasting and protein degradation, as evidenced by preserved muscle mass and myofiber area, as well as lower levels of ubiquitinated protein, Atrogin-1 and MuRF-1 expression, and LC3-II/I ratio in tibial anterior (TA) muscles. Interestingly, the phosphorylated FoxO3a at Ser253 was significantly decreased in atrophic TA muscles, which was preserved in AMPKα2$^{-/-}$ mice. Collectively, our data support the notion that the activation of AMPKα2 contributes to the atrophic effects of denervation.

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1. Introduction

Muscle atrophy is characterized by a decrease in muscle fiber diameter, protein content, force production, and fatigue resistance [1]. The disruption of the balance between catabolic and anabolic processes in muscle atrophy could result from a plethora of causes including fasting, denervation, aging and neuromuscular diseases [2]. Much evidence suggests that the protein degradation in muscle atrophy is regulated by the ubiquitin-proteasome system (UPS) and autophagy-lysosome system, which work coordinately to remove the myofibrillar proteins and myocyte organelles [1,3–8]. Two E3 ligase genes, Atrogin-1/MAFbx and MuRF-1, as well as several autophagy genes, including LC3, Bnip-3, have been described to be essential for muscle atrophy [5,6,9].

The mammalian AMP-activated protein kinase (AMPK) is a cellular energy status sensor that acts as a master regulator of skeletal muscle metabolic pathways. AMPK has been thought to be a negative regulator of protein synthesis in muscle by inhibiting the mammalian target of rapamycin complex 1 (mTORC1) [10]. Moreover, activation of AMPK has also been found to promote protein degradation through UPS and autophagy in muscle atrophy [11–13]. AMPK is a heterotrimeric enzyme complex with a catalytic subunit (α) and regulatory subunits (β and γ). Both α and β subunit have two different isoforms (α1 and α2 or β1 and β2), while the γ subunit has three different isoforms (γ1, γ2 and γ3) [14]. In muscles, AMPKα2 is the major catalytic subunit isoform [15]. Using specific siRNA for AMPK α1/2, a recent study demonstrated that AMPK α2 isoform is necessary for MuRF1 up-regulation and myofiber size reduction in high CO2 induced muscle atrophy [16]. However, the role of AMPK α2 in other types of muscle atrophy is still not clear.

Abbreviations: AICAR, aminoimidazole carboxamide ribonucleotide; AMPK, AMP-activated protein kinase; CSA, cross-sectional area; EDL, extensor digitorum longus; GAS, gastrocnemius; mTORC1, mammalian target of rapamycin complex 1; Sol, soleus; TA, tibial anterior; UPS, ubiquitin-proteasome system; WGA, wheat germ agglutinin; WT, wild type.

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In this study, we investigated the impact of the AMPK α2 during development of muscle atrophy, by inducing sciatic denervation on hind limb muscles in mice with genetic disruption of the AMPK α2 (AMPK $\alpha2^{-/-}$) and compared the results with wild type (WT) mice. We found that deletion of AMPK α2 significantly attenuated denervation-induced muscle atrophy, as evidenced by the preserved muscle mass and myofiber area. Of interest, AMPK$\alpha2^{-/-}$ attenuated the expression of atrogin1 and MuRF-1. Taken together, these data support the role of inhibition of AMPK α2 activity in attenuating neurogenic skeletal muscle atrophy.

2. Methods

2.1. Mice and denervation-induced muscle atrophy

Male C57BL/6J and AMPK $\alpha2^{-/-}$ mice (C57BL/6J background), 8–10 weeks of age, were purchased from Beijing HFK Bioscience Co., LTD. This study was approved by the Animal Care and Use Committee of the University of Chinese Academy of Science. Mice were anesthetized and then the sciatic nerve of left hind limb was exposed by blunt dissection and a 3 mm piece was excised. An identical incision into the right hind limb was made but the muscles remained innervated to serve as control. The denervation procedure was performed on WT ($n = 15$) and AMPK $\alpha2^{-/-}$ mice ($n = 17$) as previously described [3,4]. Muscle samples were harvested at 7 and 14 days after the denervation surgery.

2.2. Western blotting

Mice were anesthetized and muscles were quickly isolated, and 10–20 mg pieces of the muscle tissues were homogenized in buffer (50 mM Tris-Cl, 150 mM NaCl, 100 μg/ml phenylmethylsulfonyl fluoride, protease and phosphatase inhibitor cocktail from Roche and 1%Triton X-100) on ice. After centrifugation at 12,000*g* for 20 min at 4 °C, the supernatant was used for western blot analysis as previous described [17]. Primary antibodies used in this study were as follows: phospho-AMPKα^{Thr172}, phospho-FOXO3a^{Ser253}, phospho-AKT^{ser473}, phospho-4E-BP1$^{Thr37/46}$, AMPKα, FOXO3a, AKT, 4E-BP1, LC-3b and Ubiquitin were from Cell Signaling Technology (Danvers, MA, USA); AMPKα1 and AMPKα2 were from GeneTex (Irvine, CA, USA); Atrogin-1, MuRF-1 and β-actin were from Abcam (Cambridge, UK).

2.3. Cross-sectional area assessment

Muscles were freshly isolated and mounted in a plastic bowl containing OCT (Thermo Scientific, Rockfield, IL, USA) as previous reported [4]. The mounted muscle tissues were frozen in isopentane pre-chilled at −159 °C for 30–40 s and stored at −80 °C. Transverse sectioning of the muscle tissues was performed using a Leica CM3050S cryostat (Wetzlar, Germany). Serial sections with 8 μm thickness were used for wheat germ agglutinin (WGA) staining. After staining, the cross-sectional areas (CSA) were quantified using NIH Image J software (Bethesda, Maryland, USA) and results were expressed as mean CSA S.E. and as percentage of fibers distributed.

2.4. Data and statistical analysis

All values are expressed as mean ± standard error. Statistical significance was defined as $p < 0.05$. One-way or two-way analysis of variance (ANOVA) was used to test each variable for differences among the treatment groups with StatView (SAS Institute Inc). If ANOVA demonstrated a significant effect, pair wise post hoc comparisons were made with Fisher's least significant difference test.

3. Results

3.1. AMPKα is phosphorylated in some skeletal muscles during muscle atrophy

In order to elucidate the function of AMPKα during the pathological remodeling process of adult skeletal muscles, we initially examined the expression of total and phosphorylated AMPKα in different kinds of skeletal muscle, including tibial anterior (TA), gastrocnemius (GAS), soleus (Sol) and extensor digitorum longus (EDL) muscles. As shown in Fig. 1, after denervation for 7 days, the expression of p-AMPKα was significantly increased in TA, Gas and Sol, suggesting AMPKα was activated in those muscles in response to denervation.

3.2. AMPKα2 deficient TA skeletal muscles are resistant to denervation induced atrophy

The increase in AMPKα activity levels in muscles during denervation induced atrophy suggests that AMPKα may contribute to the muscle wasting process. Since α2 is the more abundant isoform found in skeletal muscle, we examined the pathological process of atrophy process in adult WT and AMPKα2 KO mice by measuring muscle mass and myofiber size. Seven and fourteen days after denervation, WT TA muscle mass decreased to 74.9% ± 1.7% and 59.9 ± 0.8% of contralateral level, respectively; while AMPKα2 deficient TA muscle mass remained at 86.5 ± 2.5% and 73.6 ± 1.4% of its contralateral level (Fig. 2A). Myofiber area quantification using WGA staining on TA muscle cryosections also revealed better preservation of myofiber size in AMPKα$2^{-/-}$ TA muscles in response to denervation (Fig. 2B). Seven days after denervation, the averaged myofiber cross-sectional area of denervated TA muscle decreased to 63% ± 1.7% of the contralateral level in WT mice and 84.2% ± 3.4% in AMPKα$2^{-/-}$ mice (Fig. 2C). In addition, myofiber size distribution calculated from AMPKα$2^{-/-}$ TA muscles showed a reduced leftward shift from its contralateral conditions (Fig. 2D, E). As compared with WT mice, the atrophy in GAS, Sol and EDL muscles tended to be lower in the AMPKα$2^{-/-}$ mice after denervation for 7 days. However, this difference was not significant (Supplemental Fig. 1). Together, our data suggested that AMPKα2 deficiency protected TA muscles against denervation induced atrophy and the activation of AMPKα after denervation potentially represents a pro-atrophy mechanism.

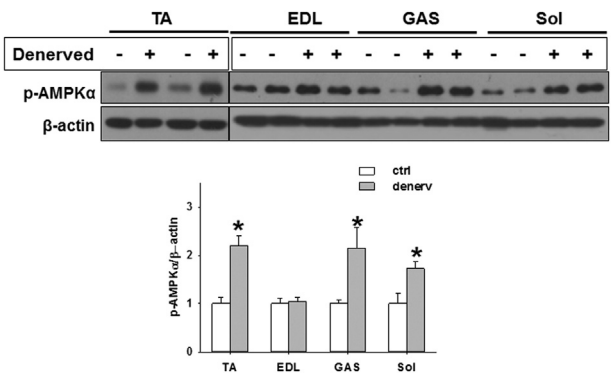

Fig. 1. AMPKα phosphorylation is increased in multiple skeletal muscles after denervation. 7 days after denervation, phosphorylated and total AMPKα were determined by western blot in tibial anterior (TA), extensor digitorum longus (EDL), gastrocnemius (GAS) and soleus (Sol) muscles. Results were collected from six independent experiments and values were normalized to control. *$p < 0.05$ compared to control (Two-way ANOVA).

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