Emodin attenuates TNF- α -induced apoptosis and autophagy in mouse C2C12 myoblasts through the phosphorylation of Akt

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Abstract: Emodin, [a](#) major component of *Rheum palmatum*, has been reported to significantly protect neural tissue against apoptosis and autophagy. However, the effects and underlying mechanisms of [action of](#) emodin in muscle atrophy are still poorly defined. In this study, we investigated the protective effects and the underlying mechanisms [by which](#) emodin [acts](#) on tumor necrosis factor alpha (TNF- α)-induced apoptosis and autophagy in mouse C2C12 myoblasts. Emodin, at various concentrations, decreased [TNF- \$\alpha\$ -induced](#) apoptosis in C2C12 myoblasts, which were analyzed by Hoechst 33342 staining and [annexin V/PI](#) analysis. Emodin also inhibited [the](#) collapse of [the](#) mitochondrial membrane potential and [the](#) generation of reactive oxygen species in TNF- α -[stimulated](#) C2C12 myoblasts. Consistent [with these results](#), the expression of Bcl-2 was increased, [whereas](#) the expression of Bax, cleaved-caspase 3 and cleaved-PARP [was](#) decreased after emodin treatment. These data demonstrate that emodin attenuated apoptosis in TNF- α -[stimulated](#) C2C12 myoblasts through mitochondrial [signaling](#) pathways. In addition, emodin inhibited autophagy in TNF- α -[stimulated](#) C2C12 myoblasts by suppressing the expression of LC3-II, Beclin-1 and Atg7. Emodin also resulted in the upregulation of the phosphorylated [ed](#) forms of Akt. Taken together, these results suggest that emodin inhibited apoptosis and autophagy in TNF- α -induced C2C12 myoblasts, [possibly](#) through the activation of phosphorylated Akt. Our findings suggest that emodin could be a potential therapeutic agent in [the treatment of](#) muscle atrophy.

Keywords: C2C12 myoblasts; Apoptosis; Autophagy; Tumor necrosis factor alpha; Emodin

1. Introduction

Skeletal muscle atrophy is defined as the deficiency of both muscle mass and muscle function, which is caused by [an](#) imbalance between protein degradation and synthesis. Muscle atrophy appears in [response to](#) various [types](#) of physiological and pathological stimuli, including aging, chronic kidney disease (CKD), cancer, diabetes, muscle disuse and malnutrition [1, 2]. Pro-inflammatory cytokines, [such as tumor necrosis factor alpha \(TNF- \$\alpha\$ \), are](#) involved in this process. The level of TNF- α plays a critical role in muscle atrophy through [its effects on](#) cell growth and myogenic differentiation inhibition, and apoptosis [and through the induction of](#) autophagy [3-7].

Muscle fibers are post-mitotic syncytia formed by the fusion of myogenic progenitors during pre- and post-natal development [8]. The bulk of aggregated myonuclei develop into fibers during post-natal growth, leading to a dramatic increase in muscle mass [9]. Recent research has demonstrated that apoptosis eliminates [superfluous](#) tissues during normal embryonic development [10]. In addition, apoptosis is important for the preservation of tissue homeostasis in adults [10]. [The](#) progressive elimination of myonuclei via apoptosis may drive the onset and progression of muscle atrophy [11-14]. In vertebrates, apoptosis is induced by two major pathways, mitochondrion-mediated signaling and death receptor-mediated apoptotic signaling. In apoptotic cells, caspase-9 is activated [15], and [this](#) leads to the activation and cleavage of executioner caspases-3 and -7 [16, 17]. The critical downstream protein PARP is cleaved [in this process](#) [18]. These [are](#) the typical [characteristics](#) of mitochondrion-mediated apoptosis. [Additionally](#), studies [on death receptor pathways](#) [have](#) indicated that death receptors, such as Fas, TNFR1, [and](#) TRAIL, contribute to the activation of caspase-8 [and](#) caspase-3 as well as the cleaving of PARP [19, 20]. As

a result, caspase-3 and PARP are potential [links](#) between [the](#) mitochondrion-mediated pathway and [the](#) death receptor-mediated apoptotic pathway.

Research [shows](#) that [the](#) autophagic system also contributes to muscle atrophy. Clinical [studies have](#) reported that [autophagy is a significant characteristic of myopathic](#) conditions [21, 22]. Some studies [have](#) shown that [the](#) autophagy-related genes LC3, Beclin1 and Atg12 are upregulated in different models of muscle atrophy [23, 24]. Indeed, we have reported in previous research that TNF- α was expressed [by](#) [C2C12](#) myoblasts [during](#) muscle atrophy and [that](#) TNF- α alone or in combination with other cytokines stimulated [the](#) apoptosis and autophagy of C2C12 myoblasts [25-27].

Emodin (1,3,8-trihydroxy-6-methylantraquinone), a key anthraquinone derivative of *Rheum palmatum*, exhibits anti-inflammatory, anti-fibrotic, anti-tumor, [and](#) anti-atherosclerotic effects [18, 28-33]. However, the role of emodin in TNF- α -induced apoptosis and autophagy in C2C12 myoblasts [is unknown](#). This study [presents the first demonstration](#) that emodin can ~~inhibit~~[inhibit](#) apoptosis and autophagy in TNF- α -[stimulated](#) C2C12 myoblasts. Our findings shed light on the potential of [emodin to](#) modulate apoptosis and autophagy as [a](#) novel therapeutic strategy for the treatment of muscle atrophy, and [they further](#) promote [our understanding of](#) this field.

2. Materials and methods

2.1. Chemicals and reagents

5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) probes [were](#) purchased from Cayman (Ann Arbor, MI, [USA](#)). The [annexin V-FITC](#) apoptosis detection kit and RIPA lysis buffer were [purchased](#) from Keygen (Nanjing, China). [The](#) BCA protein assay kit was purchased from Thermo Fisher (Pittsburgh, PA). Antibodies were purchased from Cell Signaling Technologies (Danvers, MA).

Recombinant rat TNF- α was [purchased](#) from PeproTech (Rocky Hill, NJ). All other chemicals were purchased from Sigma (Saint-Louis, MO).

2.2. Cell culture

C2C12 myoblasts were purchased from type culture collection of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM/F-12 medium (HyClone) supplemented with 10% (v/v) fetal bovine serum (FBS, HyClone), 100 U/ml penicillin, and 100 μ g/ml streptomycin (Invitrogen) in a humidified incubator containing 5% CO₂ at 37 °C.

2.3. Cytotoxicity assay

C2C12 myoblasts were seeded at a density of 1×10^4 /ml in 96-well plates and cultured overnight. Cells were treated with different concentrations of emodin (Fig. 1A, [purity \(HPLC\)](#) $\geq$ 98%, [Sigma](#)) for 24 h. [Then](#), 20 μ l of MTT (5 mg/ml, Sigma) was added and incubated for another 4 h. [After this](#), the supernatants in each well were removed and 150 μ l of DMSO was added into each well followed by incubation at 37 °C for 10 min with gentle shaking. The optical density was detected at 490 nm [using](#) a microplate reader (Spectra Max M5, USA).

2.4. Hoechst 33342 staining

Hoechst 33342 dye was used to evaluate the [occurrence](#) of apoptosis [based on](#) the aggregation of chromatin and the fragmentation of [the](#) nuclei in [the](#) cells. C2C12 myoblasts were plated in 6-well plates and treated with emodin in the presence or absence of TNF- α (20 ng/ml) for 24 h. [Then](#), cells were incubated with 10 μ g/ml Hoechst 33342 dye for 20 min at 37 °C and rinsed with cold phosphate buffered saline (PBS). [The cells were immediately observed with a fluorescence microscope \(Eclipse-Ti, Nikon, Japan\) using filters for blue fluorescence. To quantify the extent of apoptosis, the frequency of apoptotic cells \(%\) was calculated from five images of randomly selected microscopic fields of view from each treatment. The percent of](#)

apoptotic cells was calculated as the total number of nuclei that were apoptotic relative to the overall number of cells observed. Apoptotic nuclei were identifiable because they appeared bright and condensed compared to normal nuclei.

2.5. Apoptosis assay

Apoptosis was assessed using the annexin V-FITC apoptosis detection kit according to the manufacturer's instruction. C2C12 myoblasts were seeded in 6-well plates and incubated with emodin in the presence or absence of TNF- α (20 ng/ml) for 24 h. Cells were harvested, washed twice with cold PBS, and resuspended in binding buffer at 1×10^5 cells/ml. A 250 μ l aliquot of the suspension was incubated with 5 μ l of annexin V-EGFP and 5 μ l of propidium iodide (PI, 20 μ g/ml) in the dark at room temperature for 15 min, and the samples were analyzed with a flow cytometer (BD Biosciences, USA). Cells that were positive for annexin V but negative for PI were considered to be undergoing apoptosis. The third quadrant cells were regarded as control cells, whereas the first and the fourth quadrant cells were considered as apoptosis, the percent of apoptotic cells was calculated as the first and the fourth quadrant cells relative to the overall number of cells.

2.6. Mitochondrial membrane potential ($\Delta\Psi_m$) measurement

The mitochondrial membrane potential was analyzed using a JC-1 $\Delta\Psi_m$ assay kit according to the manufacturer's protocol. In brief, C2C12 myoblasts were seeded in 6-well plates and incubated with emodin in the presence or absence of TNF- α (20 ng/ml) for 24 h. Then, the cells were collected and resuspended in a JC-1 working solution and incubated at 37 °C in 5% CO₂ for 15 min. Next, the cells were collected and washed in incubation buffer twice. Then, the stained cells were assayed using a flow cytometer (BD Biosciences, USA).

2.7. Autophagosome and ROS observations

After emodin treatment with or without TNF- α (20 ng/ml), the cells were fixed with 4% paraformaldehyde for 30 min at room temperature, permeabilized with 0.2% Triton X-100 for 5 min and blocked with 1% FBS containing 0.25% Tween-20 for 30 min at room temperature. Then, cells were incubated overnight with anti-LC3B antibody (1:200 dilution). After washing with PBS, the cells were incubated [with the](#) appropriate secondary antibody for 1 h at room temperature. Cells were then rinsed and counterstained with DAPI for 15 min. Images were [obtained using](#) confocal microscopy (Carl Zeiss LSM710, Jena, Germany).

[To](#) determine the intracellular generation of ROS, C2C12 myoblasts were stained with [a](#) 2'-7'-dichlorofluorescein diacetate (H₂DCF-DA) fluorescent probe ([Sigma](#)). Cells were incubated with H₂DCF-DA (10 μ M) for 20 min at 37 °C and washed with PBS [and](#) then counterstained with DAPI for 15 min. Images were [obtained using](#) confocal microscopy (Carl Zeiss LSM710, Jena, Germany).

2.8. Western blotting analysis

C2C12 myoblasts were collected and washed twice with ice-cold PBS and then lysed for 30 min at 4 °C with RIPA buffer containing a cocktail of protease inhibitors. Samples of [the](#) cell lysates were separated by 10% SDS-PAGE [and](#) subsequently transferred onto a polyvinylidene difluoride (PVDF) membrane. The membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) on the shaker at room temperature for 1 h and then incubated with primary antibodies at 4 °C overnight. Subsequently, the membranes [es were](#) washed with TBS-T followed by [an](#) incubation with the HRP-conjugated secondary antibodies at room temperature for 1 h. Images were [obtained](#) and analyzed with a CCD system (Image Station 2000 [MM](#), Kodak).

2.9. Statistical analysis

Statistical analyses were performed with SPSS_13.0 (SPSS, Chicago, IL). All data were presented [as the means](#) $\pm$ SD from at least 3 independent experiments. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test for multiple comparisons. A value [of](#) $P < 0.05$ was considered statistically significant.

3. Results

3.1. Emodin shows no obvious cytotoxicity in C2C12 myoblasts below 20 μ M

In the present study, we first investigated whether emodin [exhibited any](#) potential cytotoxic [effects](#) on C2C12 myoblasts [using an](#) MTT assay. After incubation with emodin for 24 h, low concentrations (1 ~ 20 μ M) of emodin had no obvious influence on cell viability. However, [a](#) high concentration (30 μ M) of emodin significantly decreased the viability of C2C12 myoblasts (Fig. 1B). Based on the results above, 5, 10 and 20 μ M emodin were used in our next experiments.

Fig. 1. Chemical structure of emodin and [its effect on the](#) viability of C2C12 myoblasts. (A) Chemical structure of emodin. (B) The [effects](#) of emodin [on the viability](#) C2C12 myoblasts. C2C12 myoblasts were cultured with different concentrations of emodin for 24 h. [Cell](#) viability was determined by MTT assay. The values are presented as the means $\pm$ SD of three independent experiments. * $P < 0.05$ compared with [the](#) TNF- α group, # $P < 0.05$ compared with [the](#) control cells.

3.2. Emodin suppresses TNF- α -induced apoptosis in C2C12 myoblasts through the mitochondrial apoptosis pathway.

Our previous studies have shown that pathologic levels of TNF- α can induce apoptosis in C2C12 myoblasts [25]. Therefore, we further investigated whether emodin could protect [these cells](#) against TNF- α -induced apoptosis.

As shown in Fig. 2A, C2C12 myoblasts were incubated with emodin in the presence or absence of TNF- α (20 ng/ml) for 24 h and then stained with Hoechst 33342 dye. The TNF- α -treated cells exhibited apoptotic morphological characteristics with nuclear condensation and DNA fragmentation. In contrast, TNF- α -induced apoptosis was efficiently abrogated by treatment with emodin. [In particular, treatment with](#) 10 μ M emodin showed better anti-apoptotic effects [than did](#) the other treatments.

We subsequently examined [the](#) apoptotic ratio using flow cytometry with [annexin V/PI](#) staining (Fig. 2B). We found that the percentage of cells undergoing apoptosis (but not necrosis) increased after treatment with TNF- α [but that](#) emodin treatment [significantly down-regulated](#) TNF- α -induced apoptosis in C2C12 myoblasts. These results were consistent with that of Hoechst staining.

Mitochondrial potential ($\Delta\Psi_m$) has been reported to be associated with apoptosis through [the](#) exposure of cytochrome c to the inter-membrane space [34, 35]. To confirm whether emodin affects the cellular $\Delta\Psi_m$, [fluorescent JC-1 probes](#) were used. Our data suggest that [the](#) $\Delta\Psi_m$ in response to treatment with TNF- α [was](#) significantly [decreased](#) compared to [that of](#) the control cells. Emodin treatment blocked the TNF- α -induced decrease in $\Delta\Psi_m$, especially at 10 μ M ([Fig. 2 C](#)).

Emodin has been shown to be a potent ROS scavenger [36]. Therefore, we speculated that [ability of](#) emodin [to](#) reduce TNF- α -induced apoptosis might be [attributable](#) to the same property. To confirm this hypothesis, we measured ROS

production [using](#) CM-H2DCF-DA staining. [Our results show that](#) TNF- α treatment led to strong H2DCF-DA staining, whereas emodin treatment decreased the staining intensity. These results indicate that emodin has a protective effect against TNF- α -induced ROS production ([Fig. 2 D](#)).

Western blotting analysis results show that the Bcl-2/Bax ratio [was reduced and that the](#) cleaved-caspase 3 and cleaved-PARP expression [levels](#) were increased in the TNF- α group. However, emodin treatment led to [an increase in the](#) Bcl-2/Bax ratio and the decrease of both cleaved-caspase 3 and cleaved-PARP ([Fig. 2 E & F](#)). Collectively, these results suggest that emodin inhibits C2C12 myoblast apoptosis induced by TNF- α through the mitochondrial pathway.

Fig. 2. Emodin ameliorates TNF- α -induced-apoptosis in C2C12 myoblasts through [the](#) mitochondrial pathway. C2C12 myoblasts were plated in 6-well plates and treated with emodin in the presence or absence of TNF- α (20 ng/ml) for 24 h. Cells incubated with 10 μ g/ml Hoechst 33342 dye were observed under a fluorescence microscope (A) or analyzed [to determine](#) the apoptotic rate (B) and mitochondrial membrane potential (C) by flow cytometry. (D) Emodin decreases the generation of ROS in C2C12 myoblasts induced by TNF- α . C2C12 myoblasts were plated in 6-well plates and treated with emodin in the presence or absence of TNF- α (20 ng/ml) for 24 h. C2C12

myoblasts were loaded with H₂DCF-DA and DAPI and observed under a fluorescence microscope (400×). (E) Emodin regulates the expression levels of apoptosis-related proteins. C2C12 myoblasts were incubated with emodin in the presence or absence of TNF- α (20 ng/ml) for 24 h. After treatment, the expression of PARP, cleaved-caspase 3, Bcl-2 and Bax was determined by western blotting. GAPDH were used as a loading control. (F) The quantification of the expression levels of apoptosis-related proteins. Data derived from three separate experiments are presented as the means $\pm$ SD. * P < 0.05 compared with the TNF- α group, # P < 0.05 compared with control cells.

3.3. Emodin inhibits TNF- α -induced autophagy in C2C12 myoblasts

TNF- α -induced upregulation of autophagy had been demonstrated previously [27, 37, 38]. We further investigated the effects of emodin on TNF- α -induced autophagy in C2C12 myoblasts. LC3 is a marker for the autophagic process and an upregulation of LC3 is required to replenish the LC3 protein pool and allow for the progression of autophagy [2]. To determine the cellular expression levels of the autophagosome marker LC3B, we used immunofluorescent staining in C2C12 myoblasts in response to emodin treatment. The previous experimental results showed that treatment with 10 μ M emodin exhibited the best protective effects against TNF- α . Therefore, in this experiment, emodin at a concentration of 10 μ M was used with the treatment group. The results from fluorescence microscopy revealed that the expression of LC3B (red) was abundant in the TNF- α group (Fig. 3A), but those effects were reduced after emodin treatment.

Next, we determined whether emodin could protect against the autophagy induced by TNF- α in C2C12 myoblasts. It was observed that TNF- α induced an increase in the expression of LC3-II (autophagosomal membrane-localized form). However, emodin treatment limited the increase in LC3-II that was induced by TNF- α (Fig. 3 B & C).

Beclin-1 and Atg7 are [considered to be](#) important genes in the canonical autophagy pathway and are required for autophagosome formation [39]. In our findings, the expression [patterns](#) of Beclin-1 and Atg7 were consistent with [those of](#) LC3-II in [the](#) emodin-treated group ([Fig. 3](#) B & C). Consequently, these results indicate that emodin can repress TNF- α -induced autophagy in C2C12 myoblasts.

Fig. 3. Emodin inhibits TNF- α -induced autophagy in C2C12 myoblasts. (A) Immunofluorescence analysis of the autophagosome marker LC3B in C2C12 myoblasts. The cells were stained with an antibody against LC3B (red) and [with](#) the nuclear dye DAPI (blue) (400 $\times$). (B) Emodin regulates the expression levels of autophagy-related proteins. C2C12 myoblasts were incubated with emodin in the presence or absence of TNF- α (20 ng/ml) for 24 h. After treatment, the expression of Atg-7, Beclin-1 and LC3B [was](#) determined by western blotting. GAPDH [was](#) used as a loading control. (C) The quantification of the expression levels of autophagy-related proteins. Data are expressed as the means $\pm$ SD of three independent experiments. * P < 0.05 compared with [the](#) TNF- α group, # P < 0.05 compared with [the](#) control cells.

3.4. Emodin attenuates [TNF- \$\alpha\$ -induced apoptosis and autophagy in C2C12 myoblasts through the activation of the phosphorylation of Akt](#)

Akt is a critical factor in cellular survival, differentiation, and energy metabolism, [and](#) [the](#) phosphorylation of Akt can regulate apoptosis and autophagy [39-41]. The results presented in Fig. 4A and B show that, in the presence of TNF- α , [the](#) expression of p-Akt (Ser-473) and p-Akt (Thr-308) [was](#) reduced, whereas emodin treatment resulted in the upregulation of the phosphorylated [ed](#) forms of Akt, and the group treated with 20 μ M emodin showed the highest [st](#) level of phosphorylated Akt. Overall, these results [suggest](#) that the TNF- α -induced [apoptosis and autophagy of C2C12 cells](#) may be attenuated by emodin through the activation of the phosphorylation of Akt.

Fig. 4. Emodin increases the levels of phosphorylated Akt. C2C12 myoblasts were incubated with emodin in the presence or absence of TNF- α (20 ng/ml) for 24 h. (A) After treatment, the proteins from the total cell lysates were separated by SDS-PAGE gel electrophoresis and immunoblotted with antibodies against total Akt and phosphorylated Akt (Ser-473 and Thr-308). (B) The quantification of the expression levels of total Akt and phosphorylated Akt (Ser-473 and Thr-308). Data are expressed as the means $\pm$ SD from at least three independent experiments. * $P < 0.05$ compared with the TNF- α group, [#] $P < 0.05$ compared with the control cells.

4. Discussion

[Apoptosis plays a significant role](#) in age-related muscle atrophy [14, 42, 43]. Previous studies have shown that pathological [levels](#) of the pro-inflammatory cytokine TNF- α can inhibit myogenic differentiation and induce apoptosis [4, 7, 44]. However, apoptosis induced by TNF- α and its corresponding receptor-mediated signaling often [overlaps](#) with mitochondrion-mediated signaling. Both of these [pathways](#) can lead to cellular destruction by caspases [45]. In the mitochondrial apoptosis signaling pathway, pro-apoptotic (Bax and Bad) and anti-apoptotic (Bcl-2 and Bcl-xl) members of [the](#) Bcl-2 family play important roles [46, 47]. Bax assists in the release of cytochrome c from [the](#) mitochondrial intermembrane into [the](#) cytosol and the [in](#) activation of caspase [48]. In contrast, Bcl-2 acts to sustain mitochondrial

integrity, suppressing [the](#) release of cytochrome c and subsequently inhibiting apoptosis [49]. Overexpression of Bcl-2 in cells has been reported to prevent the loss of mitochondrial potential, [the](#) release of cytochrome c, and [the](#) activation of caspases, whereas Bax reduces these changes [48]. Therefore, striking a balance between the levels of expression of pro-apoptotic and anti-apoptotic proteins [is](#) of paramount importance for [regulating the](#) survival or death of cells [50]. Thus, the Bcl-2/Bax ratio is often used as an indicator of [a cell's](#) apoptotic potential; a high ratio protects against apoptosis and a low ratio favors apoptosis. [The](#) activation of caspases is a biochemical feature of apoptosis. [Caspases](#) are responsible for [the](#) proteolytic cleavage of a broad spectrum of cellular targets [10]. The initiator caspases (caspase-8, -9) [are](#) cleaved [by stimulators of apoptosis](#) and thereby activate executioner caspases (caspase-3), which results [in](#) small and large subunits [generated by the](#) cleaving. A caspase cascade occurs [that](#) leads [to](#) PARP ([a](#) DNA repair enzyme and a known caspase substrate) cleavage and DNA degradation [49]. In our study, we demonstrated that emodin could decrease pro-apoptotic Bax, cleaved-caspase 3 and cleaved-PARP expression [and](#) elevate anti-apoptotic Bcl-2 in C2C12 myoblasts. It is well known that ROS mediates the intracellular signaling of apoptotic proteolytic cascades [51]. Chronic and acute overproduction of ROS can induce [a](#) loss of $\Delta\Psi_m$, which is accompanied [by](#) the loss of cellular function and apoptosis [52]. Furthermore, it has been shown that enhanced production of ROS may induce a pro-apoptotic shift [in](#) the expression pattern of Bcl-2 proteins as evidenced by a decreased Bcl-2/Bax ratio [53, 54]. Increased ROS production stimulates the pro-apoptotic factor Bax, resulting in the activation of the mitochondrial apoptotic pathway [55]. Our results show that emodin treatment [resulted](#) an increase in $\Delta\Psi_m$ and a decrease in ROS. [Taken together,](#)

these findings [suggest](#) that emodin protects against TNF- α -induced apoptosis through [effects on the](#) mitochondrial apoptotic pathway.

Autophagy is a complex catabolic [process](#) for lysosomal degradation of proteins [and](#) is essential for [cellular](#) survival, differentiation, development, and homeostasis [39]. It leads to [the](#) recycling of organelles and other cytoplasmic substances to provide metabolic precursors. Autophagy principally serves an adaptive role to protect organisms against diverse pathologies [39]. However, excessive stimulation of autophagy is deleterious and may lead to caspase-independent cell death [21]. It [has been](#) found that the excessive activation of autophagy aggravates muscle wasting by removing portions of [the](#) cytoplasm, proteins, and organelles [56]. TNF- α plays an important role in autophagy [57]. Therefore, tight control of TNF- α -induced gene expression is needed to avoid [the](#) unbalanced [induction of](#) autophagy and muscle loss. To further clarify the protective [effects](#) of emodin [against](#) TNF- α -induced autophagy in C2C12 myoblasts, we measured the changes [in](#) LC3, Beclin-1 and Atg7 expression and found that the expression of LC3-II, Beclin-1 and Atg7 [was](#) decreased with emodin treatment, indicating that emodin treatment suppresses TNF- α -induced autophagy in C2C12 myoblasts.

Increasing evidence [indicates](#) that the cross talk between autophagy and apoptosis is made especially complicated by the fact that they share many common regulatory factors, such as [the](#) PI3K/Akt/mTOR signaling pathway [15, 46, 58-60]. The activation of this pathway allows cells to inhibit apoptosis and autophagy, which might be responsible for malignant transformation and proliferation [39, 41]. On the other hand, inhibition of [the](#) PI3K/Akt/mTOR signaling pathway causes cell death associated with apoptosis and/or autophagy [15]. Emodin has been reported to

modulate the PI3K/Akt signaling pathway [29, 33]. In the present study, emodin increased the level of phosphorylated Akt. [These data support](#) the suggestion that [the](#) PI3K/Akt [pathway](#) might be involved [in](#) the anti-apoptotic and anti-autophagic actions of emodin.

In summary, [the](#) present study [is the](#) first [to](#) suggest that emodin protects against apoptosis and autophagy in TNF- α -[stimulated](#) C2C12 myoblasts [and that this protection](#) is associated with phosphorylated Akt. These findings demonstrate that emodin may be [a](#) potential therapeutic agent to improve muscle atrophy. MuRF1 and MAFbx have been demonstrated to be upregulated significantly in multiple models of skeletal muscle atrophy in rodents and humans [29, 33]. In addition, studies have shown that myotubes could be induced to [undergo](#) hypertrophy by the IGF/Akt pathway, which induces [the](#) protein synthesis required for muscle hypertrophy and blocks the up-regulation of MAFbx and MuRF1 [that occurs](#) during muscle atrophy [29, 33]. Therefore, [in future work, we will focus](#) on studying atrophy-related signaling pathways and genes to further [confirm the](#) function of emodin in this process.

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Figure-1

Figure-2

Figure-3

Figure-4

Figure-5