HIF-1α Negatively Regulates Irisin Expression Which Involves in Muscle Atrophy Induced by Hypoxia

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Research

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Abstract

Background: Exposure to high altitude environment leads to skeletal muscle atrophy. As a hormone secreted by skeletal muscles after exercise, irisin contributes to promoting muscle regeneration and ameliorating skeletal muscle atrophy, but its role in hypoxia-induced skeletal muscle atrophy is still unclear. Methods & Results: Our results showed that 4 w of hypoxia exposure significantly reduced body weight and gastrocnemius muscle mass of mice, as well as grip strength and the duration time of treadmill exercise. Hypoxia treatment increased HIF-1α expression and decreased both the circulation level of irisin and its precursor protein FNDC5 expression in skeletal muscle. In vitro, CoCl₂-induced chemical hypoxia and 1% O₂ ambient hypoxia both reduced FNDC5, along with the increase of HIF-1α. Moreover, the decline of area and diameter of myotubes caused by hypoxia were rescued by inhibiting HIF-1α via YC-1. and Conclusions: Collectively, our research indicated that FNDC5/irisin was negatively regulated by HIF-1α and could participate in the regulation of muscle atrophy caused by hypoxia.

New & Noteworthy

FNDC5/irisin is negatively regulated by HIF-1α and involves in the regulation of muscle atrophy caused by hypoxia

Background

Skeletal muscles account for more than 40% of the body and are responsible for converting chemical energy into mechanical energy to generate force and power, maintaining posture, and producing movement (1). Long-term exposure to a high-altitude environment can cause skeletal muscle atrophy, especially above 5,000 m (2) for the first time (3). Although studies have reported that ambient hypoxia in the high-altitude region can be the main reason for skeletal muscle atrophy, the mechanism is still unclear.

As the central regulator in sensing and responding to hypoxia, hypoxia-induced factor -1 α (HIF-1α) plays a vital role in regulating skeletal muscle atrophy (4), via controlling the expression and distribution of skeletal muscle fibers (5) and formation of multinucleated myotubes (4, 6).

Also, recent studies have brought to light that irisin, mainly produced by skeletal muscle after exercise (7), can be related to muscle atrophy (8). Irisin is a 112- amino-acid (aa) secreted hormone and has been regarded as a biomarker of sarcopenia (9). Injecting recombinant irisin into skeleton muscles rescued skeletal muscle atrophy caused by denervation (10) or hindlimb unloading (11). Previous studies have also found that hypoxia inhibits irisin expression in cardiomyocytes (12) and serum of volunteers who participated in the Alps climbing (13). Nevertheless, the potential mechanism is still not clear.

Based on the documented studies, we investigated the relationship of HIF-1α and irisin in muscle atrophy caused by hypoxia in vivo and in vitro and identified that irisin is negatively regulated by HIF-1α.
Materials And Methods

Mice and hypoxic exposure

All mice involved in this procedure were performed in the light of animal protocol guidelines described by the Northwestern Polytechnical University Institutional Animal Care and Use Committee. 60 male C57Bl/6J mice aged 11-13 weeks were purchased from the Air Force Military Medical University. These mice were randomly divided into two groups: control and hypoxia. In the control group, 30 mice were housed in a normoxic environment with an altitude of 399 m, the rest of the mice were firstly placed in a hypoxic chamber (China, FLYDWC50-C) with 15.4% (3000 m altitude simulation) for 3 days to adapt the environment, then housed in 11.6% O₂ (altitude of 5300 m). During the whole experiment, all mice were free to access to food and water, and the light and dark cycle was 12 h:12 h.

Body composition and grip strength measurement

Body composition of mice was detected by Dual-energy X-ray assess (DEXA, South Korea, I-BMD), at the day before hypoxic exposure and the end of 2 and 4 weeks of hypoxic exposure. The grip strength of mice was detected by grip force instrument (Jinan Yiyan Technology, YLS-13A) at the end of 2 and 4 weeks of hypoxic treatment.

Treadmill experiment

The mice were subjected to a single treadmill exhaustive exercise to detect the muscle fatigue strength according to a previous protocol (14) at a speed of 14m/min on a 5° slope by using a Mouse treadmill machine (Anhui Zhenghua Biological Instruments, ZH-PT) at the end of 4 weeks of hypoxic exposure.

ELISA for determining irisin and myostatin concentration in plasma/muscle homogenate

Blood samples of anesthetized mice were harvested into EDTA-containing tubes, immediately after the exercise programme, then centrifuged at 3000 rpm for 15 min at 4 °C, the plasma was collected (15). Quadriceps were harvested and then rinsed with pre-cooled PBS at 1:9 weight-volume ratio and then further fully grinded with protease inhibitor (Beyotime; P1010; 1:100). Finally, the homogenate was centrifuged at 5000 g for 10 min, and the supernatant was obtained to get the quadriceps homogenate. The level of irisin and myostatin in plasma/muscle homogenate were quantified using an ELISA kit according to the protocol of the manufacturer (irisin, Phoenix Pharmaceuticals, NO. EK-067-29/ myostatin, Jianglai, JL50880).

Muscle fiber cross-sectional area (CSA) measurement

Transverse sections (4 µm) were cut from the mid-belly area of paraffin-embedded gastrocnemius and soleus muscle and then stained with Gill’s hematoxylin followed by 1% eosin (servicebio; H&E; G1005). The slides were scanned using Digital Slice Scanner (KFBIO, KF-PRO-020), CSA of more than 20 muscle fibers from 6 random fields were analyzed (n = 6 mice per treatment group) by using ImageJ software.
Cell culture and hypoxic treatment

C2C12 murine myoblasts were cultured in the growth medium (GM) made of Dulbecco’s modified Eagle’s medium (DMEM; Gibco; 12800-017) with high glucose concentration supplemented with 10% (v/v) fetal bovine serum (FBS; Bi; C04001-500) and 1% penicillin/streptomycin (Beyotime; C0222) at 37 °C in a 5% CO₂ and 95% air-humidified atmosphere. When C2C12 myoblasts were grown up to 70-80% confluency, the medium was replaced to differentiation medium (DM) containing DMEM supplemented with 2% (v/v) horse serum (HS; Gibco; 26050088) (Valle-Tenney, Rebolledo, Lipson, & Brandon, 2020). Two methods were used to simulate hypoxia in vitro. Chemical hypoxia was induced by CoCl₂ treatment with a concentration of 10, 50, 100, and 200 μM in DM, while ambient hypoxia was mimicked by being cultured in a cell culture chamber with a gas mixture containing 1% O₂, 5% CO₂, and 94% N₂.

Cell viability assay

The effect of CoCl₂ or YC-1 treatment on cell viability was detected by cell kit counting-8 (TargetMol, Boston, MA, USA; C0005). Briefly, 2.5 × 10⁴ C2C12 murine myoblasts were seeded in a 96-well plate, after 70-80% confluency, cells were cultured in DM for another 48 h, and then incubated with CoCl₂ (10, 50, 100, and 200 μM) or YC-1 (1, 5, 10, 20, 50 μM) and CCK-8 regent (10 μl for each well) for 24 h. The luminescence signal was detected by Multifunction Microplate Reader (Bio-tek; Synergy HT).

RNA extraction and real-time quantitative PCR (qPCR)

Total RNA was isolated from gastrocnemius muscle by using Trizol (AG; AG21102), total RNA (1 μg) was reverse-transcribed to cDNA by using HiScript II Q RT SuperMix (Vazyme; R223-01), Quantitative real-time polymerase chain reactions (qPCR) were performed in 10 μl final volume system, respectively, 4.6 μl of cDNA template, 0.2 μl primers and 5 μl 2 × ChamQ SYBR qPCR Master Mix (Vazyme; Q311-02). GAPDH was used as the housekeeping gene control. Relative mRNA expression was performed in the comparative dCt method (2⁻ΔΔCt). The primer sequences were synthesized by Sangon Biotech and shown in Table 1.

Table 1. Primer sequences
| Primers | Forward primer sequences | Reverse primer sequences |
|---------|--------------------------|--------------------------|
| PGC-1α  | 5’-CAACAATGAGCCTGCGAACA-3’ | 5’-CTTCATCCACGGGGAGACTG-3’ |
| FNDC5   | 5’-GGACCTGGAGGAGGACAGAATA-3’ | 5’-CTGGCGCGAGAAGAGAGCTATAA-3’ |
| Mstn    | 5’-GGATGGCAAGCCCAATGTT-3’ | 5’-GATTCAGGTGTGGTGGAGCCA-3’ |
| GAPDH   | 5’-CGGTGCTGAGTATGCTGGG-3’ | 5’-ATGAGCCCTTCCACAATGCC-3’ |
| ADAM10  | 5’-GGAAGCTTTAGTCATGGGTCTG-3’ | 5’-CTCCTTCCTCTACTCCAGTCAT-3’ |
| Myod1   | 5’-TACGACACCCGCTACTACAGTG-3’ | 5’-GTGGTGCATCTGCCAAAAG-3’ |
| Myf5    | 5’-CTGTCTCTGGAGGAAAC-3’ | 5’-TGGAGAGGAGGAGCTGTGT-3’ |
| MyoG    | 5’-GCAATGCACTGGAGTTCG-3’ | 5’-ACGATGGACGTAAGGGAGTG-3’ |
| Mrf4    | 5’-TGCTAAGGAAGGAGGACAA-3’ | 5’-CCTGCTGGTGGAGAATGTT-3’ |

**Western blotting**

Gastrocnemius muscle tissues or C2C12 myotubes were lysed in RIPA enhancer buffer (Beyotime; P0013B) and supplemented with protease inhibitor cocktail (Beyotime; P1005; 1:100). After electrophoresis and transfer, the PVDF membrane was incubated with primary antibody, the following primary antibodies: anti-HIF-α (CST; #36169; 1:1000), anti-FNDC5 (Abcam; ab174833; 1:2000), anti-β-tubulin (Abcam; ab6046; 1:2000), anti-GAPDH (Abcam; ab8245; 1:1000) and anti-PGC-1α (Proteintech; 20658-1-AP; 1:2000) were incubated overnight at 4 °C. The HRP-coupled secondary antibody (Immunoway; RS0001; 1:10000) was incubated for 1 h at room temperature (RT). Protein bands were displayed via clarity™ western ECL substrate (BIO-RAD; #170-5060) by using Chemiluminescence Apparatus (Shanghai; Tanon; T5200).

**Immunofluorescence staining**

C2C12 myotubes were exposed to 1% ambient hypoxia with YC-1 (50 μM) for 12h, and then washed with phosphate saline buffer (1 x PBS) and fixed for 10 min in 4% (w/v) paraformaldehyde at RT. After being washed 3 times with PBS containing 0.1% Tween 20, myotubes were permeabilized in Triton X-100 (Beyotime; P0096) for 10 min at RT, and then washed 3 times and incubated with 1% BSA for 30 min. Mouse anti-myosin (Myosin heavy chain; MF-20; DSHB) diluted 1:200 in PBST was added to incubate at 4 °C overnight. The cells were washed three times in PBS for 5 min each wash. Alexa Fluor™ 488 goat anti-mouse antibody (Invitrogen; R37120) in 1% BSA was added for 1 h a light-proof box at RT. Cell nuclei were stained with Hoechst 33258 (Beyotime; C1011) for 10 min. Myotube fusion index (an index reflecting the degree of muscle differentiation, which refers to the total number of nuclei in the myotube with more than 2 nuclei/the total number of nuclei in the same field of vision), myotube diameter (Feret’s diameter) and area were measured by ImageJ software.

**Statistical Analyses**
All assays were performed at least 3 replicates, and the quantitative experimental data were presented as mean ± standard deviation (SD). The statistical analysis was performed with GraphPad Prism 8.0 (GraphPad Software; United States). The Student’s t-test or One-Way ANOVA was used to determine the significance value. P-value < 0.05 was considered to be significant.

Results

Hypoxia reduced the lean weight and fat weight of mice

The body composition of mice was detected by DEXA after 2 or 4 w of hypoxic exposure (Fig1. A). Body weight (Fig1. B), lean weight (Fig1. C), and fat weight (Fig1. D) were significantly reduced, while not fat in tissue (Fig1. E). However, hypoxia had a slight effect on bone-related parameters (Fig1.F-I), only bone mineral content (BMC) and bone volume were decreased by 2 w of hypoxic treatment, but recovered to the control level at the end of 4 weeks of hypoxia (Fig1.G & H). In general, hypoxia significantly reduced lean weight and fat weight, which could be the main reason for body weight loss.

4 w of hypoxic exposure induced muscle atrophy of mice

The skeletal muscle function of mice was judged by the grip strength and the duration time of exercise. The grip strength (Fig2. A) was significantly reduced at the end of 2 and 4 w of hypoxia (P<0.01). Also, a significant reduction of 59.6% (P<0.01) in the duration time of treadmill exercise was observed in mice exposed to 28 days of hypoxia (Fig2. B). In addition, the relative weight of skeletal muscles (gastrocnemius, soleus, quadriceps, and triceps) was normalized to their tibia lengths, the results showed that gastrocnemius (Gas) and quadriceps (Quad) muscle weight were reduced after 4 w of hypoxic exposure, while the soleus (Sol) and triceps (Tric) were not changed (Fig2. C). Moreover, the muscle fibers were stained by H&E, the CSA and diameter of the Gas muscles were significantly reduced after hypoxic treatment (Fig2. E-H), while Sol muscles were not changed (Fig2. I-L). The expression of myogenic factors was detected by q-PCR in the Gas muscle of mice. The results showed that expression of Mrf4 and myogenin were significantly reduced, while the expression of Myf5, Myod1, and myostatin (Mstn) were not affected by hypoxic treatment (Fig2. M).

Hypoxiasignificantly reducedthe expression of FNDC5/irisin in mice

The results of the treadmill experiment showed that 4 w of hypoxic exposure significantly increased the expression of HIF-1α in Gas muscle (Fig3. A, B), meanwhile decreasing the expression of FNDC5 (Fig3. C) in Gas muscle. Also, irisin expression in plasma was reduced (Fig3. D). However, in the Gas muscle both FNDC5 and ADAM10 (splicing FNDC5 to irisin) mRNA expression were not changed (Fig3. E & F). And PGC-1α expression was not affected by hypoxia both in protein and mRNA level (Fig3. G, H&I). Also, myostatin concentration in plasma (Fig3. J) or quadriceps muscle (Fig3. H) was not changed by hypoxic treatment.

Inhibition of HIF-1α induced by CoCl₂ significantly increased FNDC5 expression in C2C12 cells
The relationship between HIF-1α and irisin in hypoxia was investigated. C2C12 myotubes were treated with CoCl$_2$ at a concentration of 10, 50, 100, 200 μM. The cell viability was detected by the CCK-8 kit. We found that CoCl$_2$ did not affect the activity of C2C12 myotubes (Fig4. A), even at the concentration of 200 μM. The expression of HIF-1α showed a dose-dependent increasing trend with the increase of CoCl$_2$ (Fig4. B, C), and correspondingly the expression of FNDC5 (precursor of irisin) was decreased in a CoCl$_2$ concentration-dependent manner (Fig4. D). However, quantitative analysis showed there was no difference in the expression of PGC-1α (Fig4. E). Additionally, FNDC5 protein correlated negatively with HIF-1α ($r = -0.627$, $p = 0.0135$, Fig4. F), and no statistically significant correlation was observed in the protein expression between FNDC5 and PGC-1α ($r = 0.055$, $p = 0.843$, Fig4. G).

Since HIF-1α is negatively related to the expression of FNDC5 in hypoxia, whether inhibiting HIF-1α could rescue the expression of FNDC5? Here we used Lifeciguat (also named YC-1) as an effective HIF-1α inhibitor and observed the expression of FNDC5. First, through the cell viability test (CCK-8), 1 and 50 μM YC-1 had no effects on cell viability (Fig4. H), so 50 μM was chosen for the next experiment (Sakurai et al., 2010). On the 5thday of differentiation, cells were added with 50 or 100 μM CoCl$_2$ and 50 μM YC-1 and incubated for another 24 h (Fig4. I). The results showed that both 50 μM and 100 μM CoCl$_2$ significantly increased the expression of HIF-1α, and YC-1 reversed the increase only in 100 μM of CoCl$_2$ treatment (Fig4. J). In the same way, YC-1 reversed the decrease of FNDC5 expression caused by 100 μM of CoCl$_2$ (Fig4. K). However, the expression of PGC-1α did not change during the whole experiment (Fig4. L).

Inhibition of HIF-1α induced by 1% O$_2$ ambient hypoxia increased FNDC5 expression and myotube formation in C2C12 cells

After 5 days of differentiation, C2C12 cells were placed in a 1% O$_2$ hypoxic chamber with 50 μM YC-1, and cultured for 6 h, 12 h, and 24 h respectively (Fig5. A). We found that the expression of HIF-1α significantly increased in ambient hypoxia at 12 h and 24 h, which was abrogated by YC-1 treatment (Fig5. B). Also, YC-1 treatment rescued the decrease of FNDC5 after 12 h of hypoxia (Fig5. C). The expression of PGC-1α after 12 h of hypoxia was significantly higher than that of 6 h (Fig5. D).

To investigate whether increasing the expression of irisin in hypoxia can improve hypoxia-induced skeletal muscle atrophy, the C2C12 myotubes were exposed to hypoxia with YC-1 for 12 h, and the diameter and area were detected after the C2C12 myotubes were stained by MyHC (Fig5. E). We found that hypoxia significantly reduced the area (Fig5. F) and diameter (Fig5. G) of C2C12 myotubes, which was reversed by inhibition of HIF-1α by treatment with YC-1. There was no obvious difference was observed in the myotube fusion rate (Fig5. H).

Discussion

Irisin has been identified as a pro-myogenic factor for ameliorating muscle atrophy, but whether it plays some role in hypoxia-induced muscle atrophy was not investigated. In the present study, we found that
irisin expression was negatively regulated by HIF-1α, which may be an important reason for skeleton atrophy caused by hypoxia.

We found that the weight loss caused by hypoxia is related to the decrease in fat and muscle mass. Here, we mainly focused on the reason for the decrease in muscle mass, which was manifested by decreased grip strength and run duration time of mice, as well as decrease of CSA and diameter of muscle fiber. To date, the cellular mechanism of hypoxia-induced skeletal muscle atrophy has been concerned with the disorder of protein synthesis and degradation (16–18) and hormone secretion (19) in skeletal muscle, etc. But the mechanism is still not clear.

In this study, the decrease in muscle mass was only observed in Gas muscle, not in Sol muscle. We thought that may be due to the difference in fiber composition of Gas and Sol. Gas muscle is mainly composed of type I muscle fiber, while Sol muscle is mainly composed of type II muscle fiber, which is thought more insensitive than type II fiber to hypoxia in a previous study (20). The level of circulating irisin has been regarded as a newly identified biomarker for muscle weakness and atrophy (21). The concentration of irisin was lowered in sarcopenia and pre-sarcopenia groups compared with non-sarcopenic participants (9, 22), but ascent when these participants increased skeletal muscle mass or enhanced skeletal muscle function through high (23) or low (24) intensity exercise. Reza M.M, et al have found that irisin increases myogenic differentiation and myoblast fusion by activating IL-6 signaling and further improves injured skeletal muscle by promoting protein synthesis (10). Moreover, Chang JS et al have revealed that irisin prevents dexamethasone-induced atrophy in C2C12 myotubes (25). Irisin comes from FNDC5 (type I membrane protein5) encoded by fibronectin type III domain containing 5 (fndc5) and cleaved by splicing enzyme A Disintegrin and Metalloproteinase Domain 10 (ADAM10) (26). ADAM10 was reported to be modulated by HIF-1α (27, 28), which could be induced by hypoxia. Then for hypoxia-induced skeletal muscle atrophy, does irisin play a role?

Our results showed that along with skeletal muscle atrophy caused by hypoxia, both irisin in plasma and FNDC5 in Gas muscle was reduced. Also, irisin precursor FNDC5 was reduced in C2C12 myotubes both by ambient hypoxia and CoCl₂-induced hypoxia, in vitro. This is consistent with the discovery of decreased irisin in humans after 2 w of climbing on the Alps (13). Moscoso, I et al. showed that FNDC5/irisin expression was also lowered in H9C2 cardiomyocytes by 0.1% O₂ hypoxia (12). Thus, the expression of FNDC5/irisin probably is a biomarker in hypoxia-induced muscle atrophy. Moreover, we found that the reduction of FNDC5/irisin expression induced by hypoxia can be reversed by inhibition of HIF-1α, which was confirmed by that YC-1 rescued the diameter and area of myotubes in 12 h of hypoxia in vitro. We also found that 6 h of hypoxia didn’t affect the expression of HIF-1α and FNDC5, which could be explained that the time of hypoxic exposure was too little to cause the response. That was in keeping with the result from Moscoso, I., et al (12) who have found that FNDC5 expression is decreased in hypoxia at least after 8 h of treatment. Maybe there is a window phase for myotubes to respond to hypoxia (29). Because 6 h of exposure did not cause the change of HIF-1α and FNDC5, and after 24 h of exposure, the response was also lower than that of 12 h. A future further experiment is required to explain the result.
Myostatin was regarded as the upstream regulator of FNDC5 (30). Shan et al have revealed that myostatin stimulates the expression of FNDC5/irisin through the AMPK/PGC-1α pathway (15) in muscle. After that, Ge et al have found that myostatin post-transcriptionally inhibits Fndc5 expression in both myoblasts and adipocytes via the miR-34a pathway (31). Conversely, in this study, though FNDC5/irisin expression was decreased by hypoxia, the concentration of myostatin neither in plasma or quadriceps was changed. Which was similar to Sliwicka E et al’ nding in humans that the concentration of myostatin was not changed after 2 w of Alps climbing (13) when irisin was lowered. As well, previously the transcriptional coactivator PGC-1α has reported an increase in C2C12 myotubes in severe hypoxic conditions such as 0.2% (32) or 0.5% O₂ (33). Whereas, there were also some opposite results that PGC-1α was reduced by 35% in muscles after 66 d of exposure at an altitude beyond 6400 m (34). Our results showed that PGC-1α was unchanged by hypoxia compared with normoxia. These competing results showed that PGC-1α may be regulated by many factors, which can't be seen as a key marker factor in hypoxia-induced muscle atrophy.

Although these discoveries were revealed in this study, there are also still some limitations. The specific mechanism by which HIF-1α regulates irisin expression was not explained. Of note, we only focused on the role of irisin in the initial stage of hypoxia on C2C12 myotubes, the effect of long-term chronic hypoxia on C2C12 myotubes still requires further exploration.

To summary, our findings showed that FNDC5/irisin was negatively regulated by HIF-1α and participated in the regulation of muscle atrophy caused by hypoxia. Irisin could be a new challenge and opportunity to treat and improve hypoxia-induced skeletal muscle atrophy in the future.

Declarations

All mice involved in this procedure were performed in the light of animal protocol guidelines described by the Northwestern Polytechnical University Institutional Animal Care and Use Committee. Data availability statements are “not applicable”. Also the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Shiqiang L and Pengyu F were mainly involved in experimental design, data collation, and manuscript writing, Huiyun X provided major theoretical knowledge and relevant suggestions, and Kaiting N, Rui W, Baoqiang Y, and Jiahui C made nal checks on the manuscript and data.

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Figures
Figure 1

Hypoxia reduced lean weight and fat weight of mice. Representative morphology (left) and dual X-ray digital images (right) of mice after 4 w of hypoxia treatment (A). DEXA results showed that body weight (B), lean weight (C), and fat weight (D) were significantly reduced after 14 d and 28 d of hypoxia, while fat in tissue (E), bone area (F), BMC (Bone mineral content) (G), bone volume (H) and BMD (Bone mineral density) (I) were not affected. Values represented means ± SD. *P < 0.05, **P < 0.01. n = 6-8.
Figure 2

4 w of hypoxic exposure induced muscle atrophy of mice. The grip strength (A) of mice and run duration time (B) in the treadmill experiment were significantly reduced after 4 w of hypoxia. The weight of gastrocnemius (Gas) and quadriceps (Quad) muscles were reduced, but not soleus (Sol) and triceps (Tric), which were all normalized to the tibia length (C). (D) showed the representative images of H&E staining. Hypoxia significantly decreased the cross-sectional area (CSA) and diameter (feret's diameter) in Gas muscles (E-H), but not in Sol muscle (I-L). The relative expression of myogenic regulatory factors in Gas muscles were detected (M). Values represented means ± SD. *P < 0.05, **p < 0.01, ***P < 0.001, ****P<0.0001. n = 6-8. Scale bar, 50 μm.
Figure 3

Hypoxia reduced the expression of FNDC5/irisin in mice. Hypoxia treatment significantly increased HIF-1α expression (A, B) and reduced FNDC5 expression (C, n ≥ 4) after treadmill exercise in Gas muscles. Irisin expression in plasma (D) of mice was reduced in hypoxia (n = 8-10), and the mRNA level of FNDC5 (E) and ADAM10 (F) in Gas muscle were not changed. Also, PGC-1α expression was not changed both in protein (G-H) and mRNA level (I, n = 5). Myostatin concentration in both plasma (J) and quadriceps muscle (K) was not affected by hypoxia (n ≥ 4). Values represented means ± SD. *P < 0.05, **P < 0.01.
Figure 4

Inhibition of HIF-1α induced by CoCl2 increased FNDC5 expression in C2C12 myotubes. Chemical hypoxia induced by CoCl2 treatment (10, 50, 100, and 200 µM) did not affect the viability of C2C12 myotubes (A). CoCl2 treatment all significantly increased the expression of HIF-1α (B & C), meanwhile decreased FNDC5 expression (D) and did not affect PGC-1α expression (E). The expression of FNDC5 negatively correlated with HIF-1α and no correlation with PGC-1α in hypoxia (F & G). Inhibition of HIF-1α by YC-1 (1, 5, 10, 20, and 50 µM) mildly affected the viability of C2C12 myotubes (H). 50 µM of YC-1 treatment abrogated the increase of HIF-1α (I) and the decrease of FNDC5 (K) induced by CoCl2 (G). PGC-1α expression was not affected by both CoCl2 (E) and YC-1 (L). Values represented means ± SD. *P < 0.05, **P < 0.01. n = 3.
Figure 5

Inhibition of HIF-1α in 1% O2 ambient hypoxia increased FNDC5 expression and myotube formation in C2C12 myotubes. HIF-1α was increased significantly in hypoxia for 12h (A), which was rescued by YC-1 (B), meanwhile, YC-1 reversed the decrease of FNDC5 (C). The expression of PGC-1α was not affected by both hypoxia and YC-1 (D). Hypoxia significantly reduced the area and diameter of C2C12 myotubes, and which were rescued by YC-1 treatment (E-G), while myotube fusion index was not influenced (H). Values represented means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. Green, MyHC; blue, Hoechst. Scale bar, 50 µm.