USF1 promotes the development of knee osteoarthritis by activating the NF-κB signaling pathway

XIANDONG SONG1, MIN ZHU2, HAO LI2, BO LIU1, ZHAOWEI YAN1, WEICAN WANG1, HONGYI LI2, JIPING SUN2 and SHIXING LI2

1Department of Orthopedics, Hongqi Hospital Affiliated with Mudanjiang Medical University; 2Department of Radiology, Hongqi Hospital Affiliated with Mudanjiang Medical University, Mudanjiang, Heilongjiang 157011, P.R. China

Received January 10, 2018; Accepted July 19, 2018

DOI: 10.3892/etm.2018.6608

Abstract. The current study mainly aims to evaluate the expression pattern and underlying mechanism of upstream stimulating factor 1 (USF1) in the muscle tissues of knee osteoarthritis (KOA) patients. In accordance with previous findings, our data showed that muscle strength was significantly decreased in KOA patients compared with controls. Furthermore, several inflammatory factors, including tumor necrosis factor α (TNFα), IL-8, IL-6, and MCP-1, were associated with reduced muscle strength in KOA patients. Not surprisingly, NF-κB signaling was significantly activated in the muscle tissues of KOA patients compared with control individuals. Furthermore, we showed that USF1 was increased in the muscles of KOA patients compared with controls. More importantly, overexpression of USF1 in primary human skeletal muscle cells significantly increased the activation of NF-κB signaling as well as the levels of pro-inflammatory factors. In summary, we showed novel data that the upregulation of USF1 promoted NF-κB activation-induced inflammatory responses in muscle tissues of KOA patients.

Introduction

Knee osteoarthritis (KOA) is a major cause of disability among the elderly (1). Inflammation of the synovial joint plays a key role in the progression of KOA and also results in pain and disability (2). Furthermore, increased inflammation and pain can decrease the use of the knee extensor muscles thereby relieving the joint load related to knee function (3,4). Thus, it is common for muscle atrophy to occur beginning at the onset of knee OA (5). It has been well established that the knee extensor muscles mainly function as a key regulators in the maintenance of daily walking activities (6). However, the underlying mechanism by which inflammation is modulated in the skeletal muscles (knee extensors) of KOA patients is poorly understood.

In response to insult and/or injury, inflammation is induced and participates in cell injury (7). Abnormal expression of inflammatory factors, including interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα), has been widely reported in KOA patients (8). Within muscle, p65 NF-κB signaling is one of the key signaling pathways that upregulates cytokine gene expression, including TNFα, IL-1β, IL-6, and MCP-1 (7,9). Undoubtedly, activation of p65 NF-κB signaling in muscle may decrease muscle strength and function (10,11). Upstream stimulating factor 1 (USF1), which is a 43-kDa protein, is a key member of the eukaryotic evolutionarily conserved basic helix-loop-helix-leucine zipper transcription factor family (12). USF1 has been reported to be involved in multiple biological processes, including cell proliferation and lipogenesis, by binding the E-box regulatory elements (CANNTG) (13-15). The mechanism of the problem of muscle movement caused by KOA is not clear. Previous studies have shown that upstream stimulatory factor (USFI) plays a key role in various muscle cells. For instance, USF1 is shown to regulate human cGMP-dependent protein kinase I gene expression in vascular smooth muscle cells, thereby maintaining smooth muscle cell relaxation, growth, and differentiation (16). Furthermore, USF1 is demonstrated to modulate the expression of osteopontin in cultured vascular smooth muscle cells and might promote initial osteopontin expression observed post carotid injury in vivo (17). In skeletal muscle, USF1 is shown to increase PGC-1alpha promoter activation (18). However, whether USF1 is abnormally expressed in the muscle tissues of KOA patients has never been explored.

The current study mainly aims to evaluate the expression pattern and underlying mechanism of action of USF1 in the muscle tissues of KOA patients, which may shed light on the prevention and treatment of KOA.

Materials and methods

Patient samples. In the current study, twenty patients (10 men and 10 women) with diagnosed KOA and five control individuals (3 men and 2 female) were recruited from Hongqi...
Hospital Affiliated with Mudanjiang Medical University. These patients were scheduled for knee replacement surgery and able to walk at least forty-five meters independently (without the use of walking aids). Patients were excluded if they had uncontrolled systemic disease (non-musculoskeletal conditions that would make testing difficult and uncomfortable for the participants, such as chronic obstructive airway disease or congestive heart failure) or a preexisting neurologic or other orthopedic condition affecting walking. The study protocol was approved by the Human Research Ethics Committees of Hongqi Hospital Affiliated with Mudanjiang Medical University. All of the participants were informed about the nature of the study and signed a consent form prior to participation. The details for all participants are listed in Table I.

**Cell culture.** Primary human skeletal muscle cells were purchased from Procell (CP-H095, Wuhan, China, http://www.procell.com.cn/view/2244.html). The cells were cultured in specific complete medium for human skeletal muscle cells (CM-H095; Procell, Wuhan, China) supplemented with 10% heat-inactivated fetal calf serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 100 U/ml penicillin and streptomycin in 25-cm² culture flasks at 37°C in a humidified atmosphere with 5% CO₂.

**Determination of muscle strength.** The strength of the knee extensor muscle group was determined in the effected legs of the 20 patients with KOA and in the leg from which the muscle biopsy specimen was obtained in the 7 control subjects. A portable nonextendable strain gauge (load cell) was used to measure muscle strength for this study. The strain gauge was attached to the subject’s leg using a webbing strap with a Velcro fastener. The subject sat in a tall chair with a strap around the lower leg 10 cm above the ankle joint, and the hip and knee joint angles were positioned at 90 degrees. The distance from the knee joint to the strap around the ankle was measured with a tape measure and was used for the calculation of torque force (N) x distance (m). Each subject exerted maximal force against the strap assembly for 3 sec. Three trials were recorded for each subject, and the highest score was used for the analysis.

**Muscle biopsy.** Resting muscle samples were isolated from the vastus lateralis, as previously described (19). In brief, the muscle samples from KOA patients were collected during their knee replacement surgery ~5 cm proximal to the suprapatellar pouch. The biopsies were taken after the skin was incised and prior to knee joint capsule incision with no trauma to the muscle or the joint at that time (19).

**Protein extraction and western blot analysis.** Skeletal muscle (30 mg) was extracted using RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and collected following centrifugation at 12,000 x g for 30 min at 4°C. A bicinchoninic protein assay kit (Pierce; Thermo Fisher Scientific, Inc.) was used to determine the protein concentration. A total of 15 µg protein was loaded per lane, separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 8% non-fat dry milk at 4°C overnight. Following three washes with PBS with Tween 20 (5 min/wash), the membranes were incubated with a following primary antibodies at 4°C overnight: p-p65 (3033, 1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA), p65 (8242, 1:1,000; Cell Signaling Technology, Inc.), anti-IkBα (#4812, 1:1,000; Cell Signaling Technology, Inc.) USF1 (ab125020, 1:1,000; Abcam, Cambridge, MA, USA) and GAPDH (cat. no. 5174; 1:1,000; Cell Signaling Technology, Inc.). Following several washes with TBST, the membranes were incubated with horseradish-peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG) or HRP-conjugated mouse antigoat IgG (ZF-0311, all 1:5,000; Zhongshan Gold Bridge Biological Technology Co., Beijing, China) for 2 h at room temperature and then washed followed by detection with enhanced chemiluminescent substrate (EMD Millipore, Billerica, MA, USA). GAPDH was used as an internal control. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used for density analysis.

**Adenoviral vector construction.** The adenovirus vectors overexpressing USF1 (Ad-USF1) or negative control (NC) (Ad-NC) were constructed by GenChem (Shanghai, China). For the transfection of adenovirus vectors into primary human skeletal muscle cells, the cells were seeded at a density of 10⁴ cells/well in 6-well plate. At 80% confluence, Ad-USF1 and Ad-NC were transfected into primary human skeletal muscle cells at 30 multiplicity of infection (MOI) for 48 h. Then, the cells were collected for further study.

**Enzyme-linked immunosorbent assay (ELISA).** Muscle tissue or cell lysates were centrifuged at 16,000 x g for 15 min at 4°C, and supernatants were used to quantify the levels of TNF-α (cat no. DTA00C; Human TNF-α Quantikine ELISA kit), IL-6, (cat no. D6050; Human IL-6 Quantikine ELISA kit), IL-1β (cat no. DLB50; Human IL-1 beta/IL-1F2 Quantikine ELISA kit), and IL-8 (cat no. D8000C; Human IL-8/CXCL8 Quantikine ELISA kit) by way of a sandwich ELISA following the manufacturers’ protocols (R&D Systems, Minneapolis, MN, USA). Samples were read at a 450 nm wavelength using a microplate reader (Model 3550; Thermo Fisher Scientific, Inc.).

**Immunohistochemistry.** Muscle tissues samples from KOA patients or control were cut into 5 µm. Then, the slices were fixed in 4% phosphate-buffered neutral formalin at room

Table I. Basic physical characteristics of KOA patients and healthy controls.

| Characteristics | Control | KOA | P-value |
|-----------------|---------|-----|---------|
| Age (years)     | 67.8±5.6 | 65.8±9.4 | >0.05   |
| Height (cm)     | 168.3±8.7 | 171.3±10.9 | >0.05  |
| Weight (kg)     | 67.8±20.33 | 73.2±48.7 | >0.05   |
| BMI (kg/m²)     | 27.6±1.3 | 28.9±2.4 | >0.05  |
| Muscle strength (Nm) | 143.5±26.5 | 83.5±11.5 | <0.001  |

BMI, body mass index; KOA, knee osteoarthritis.
temperature for 20 min, embedded in paraffin and cut into 5-µm thick sections, followed by deparaffinization, descending alcohol series of rehydration, and microwave-heating in sodium citrate buffer (Solarbio Science & Technology Co., Ltd.) at 100°C for 30 min for antigen retrieval. Sections were subsequently incubated with 0.3% hydrogen peroxide/phosphate-buffered saline for 30 min. The sections were incubated with a primary anti-p-p65 antibody (#3033; Cell Signaling Technology, Inc.) or anti-IκBα (#4812, Cell Signaling Technology, Inc.) at a 1:50 dilution and 4°C overnight. Detection of the primary antibody was performed via incubation with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (ZDR-5036, Zhongshan Gold Bridge Biological Technology Co.,) for 1 h at room temperature and visualized with a 3,3’-Diaminobenzidine substrate. Stained cells were counted in 5 random fields using light microscopy (magnification, 40x, Olympus CK40; Olympus Corporation, Tokyo, Japan).

Immunofluorescence. Primary human skeletal muscle cells (~1x10⁶) were cultured in a 6-well plate for 24 h with glass coverslips. After that, the cells were transfected with Ad-NC or Ad-USF1 for 48 h. Then, the cells on the coverslips were fixed in 4% paraformaldehyde for 30 min at room temperature. The samples were washed three times in PBS for 5 min and fluorescence intensity was examined using a fluorescence microscope (Olympus Corporation) at a magnification of x100.

Statistical analysis. Data are presented as the mean ± standard deviation. To compare the two groups, two-tailed unpaired Student’s t-test was performed. For multiple group comparisons, one-way analysis of variance followed by Tukey’s post hoc test were used. Statistical tests were performed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Decreased muscle strength was identified in the muscle tissues of KOA patients. The basic physical characteristics are shown in Table I. No significance was found in the age, height, weight and BMI of patients with KOA or healthy controls. By contrast, lower muscle strength was identified in KOA patients than in healthy controls (Table I).

Increased inflammatory factors in the vastus lateralis muscle tissues of KOA patients. Next, we determined the inflammatory factors in the vastus lateralis muscle tissues of KOA patients and control individuals. ELISA showed that the levels of IL-6, MCP-1, IL-8 and TNFα were significantly increased in the muscle tissues of KOA patients compared with control individuals (Fig. 1).

NF-κB signaling is activated in the vastus lateralis muscle tissues of KOA patients. p65 NF-κB signaling is a key signaling pathway that upregulates cytokine gene expression, including TNFα, IL-1β, IL-6, and MCP-1, in muscle tissues (7,9). Thus, we analyzed p65 NF-κB activation in the muscle tissues of KOA patients and control individuals. Western blot assays indicated that p65 NF-κB was significantly increased in the muscle tissues of KOA patients compared with control individuals, while IκBα, an inhibitor of NF-κB, was shown to be decreased in the muscle tissues of KOA patients (Fig. 2). We also analyzed histological changes of p-p65 and IκBα in skeletal muscle. In line with the findings of western blot, p-p65 was found to be enhanced in the muscle tissues of KOA patients compared with control individuals, but IκBα was decreased in the muscle tissues of KOA patients (Fig. 2B and C).

Upregulation of USF1 in the vastus lateralis muscle tissues of KOA patients. Furthermore, we evaluated the expression of USF1 in muscle tissues of KOA patients. Compared with control individuals, the protein levels of USF1 were significantly enhanced in the muscle tissues of KOA patients (Fig. 3).

USF1 activates NF-κB signaling in primary human skeletal muscle cells. To further explore whether USF1 activates NF-κB signaling in primary human skeletal muscle cells, adenovirus vectors overexpressing USF1 or NC were transfected into primary human skeletal muscle cells for 48 h.

Figure 1. Inflammatory factors were increased in the vastus lateralis muscles of KOA patients. ELISA showed that the levels of IL-6 (A), MCP-1 (B), IL-8 (C) and TNFα (D) were significantly increased in the muscle tissues of KOA patients compared with those of control individuals. P<0.05 and **P<0.001 vs. control. KOA, knee osteoarthritis; ELISA, enzyme-linked immunosorbent assay; TNFα, tumor necrosis factor α.
As shown in Fig. 4A, the transfection efficiency was similar between Ad-NC or Ad-USF1 in in primary human skeletal muscle cells. Western blot assays indicated that overexpression of USF1 significantly induced the transcription of p65 NF-κB signaling (Fig. 4B). Moreover, an ELISA assay revealed upregulation of inflammatory factors, including TNFα (Fig. 4C), IL-8 (Fig. 4D), IL-6 (Fig. 4E) and MCP-1 (Fig. 4F).

Discussion

The progression of KOA is accompanied by injury of the entire joint structure and increased inflammation in the joint (20,21). Impaired muscle strength and dysfunction are common features in the affected legs and likely decrease the quality of life among patients with knee OA (22,23). Thus, it is important to improve inflammation-induced impairments in muscle strength among patients with KOA.

Muscle weakness is a typical characteristic in patients with KOA (24,25). In accordance with previous findings, our data showed that muscle strength was significantly decreased in KOA patients compared with controls. Increasing evidence has indicated that inflammatory responses can induce significant changes in the cellular microenvironment that then result in the survival, repair and maintenance of muscle cells (21,26). In KOA patients, it has been reported that the levels of pro-inflammatory cytokines are significantly increased and muscle mass is obviously decreased (27,28). Our data showed that several inflammatory factors, including TNFα, IL-8, IL-6 and MCP-1, were associated with reduced muscle strength in KOA patients. These observations suggest
that the enhancement of proinflammatory molecules within the muscle tissues may impair physical function among KOA patients.

Increased NF-κB activity in injured muscle fibers is widely reported to diminish the myogenic potential of their associated satellite cells (29). Furthermore, the p105/p50 subunit in NF-κB knockout mice has been demonstrated to be partially resistant to muscle atrophy (30). Thus, we evaluated the activation of NF-κB signaling in the vastus lateralis muscle tissues of KOA patients compared with controls. Not surprisingly, NF-κB signaling was significantly activated in the muscle tissues of KOA patients compared with control individuals. Thus, it is of great importance to elucidate the underlying cellular mechanisms that regulate inflammatory signaling in the muscle tissues of KOA patients, thereby providing a novel therapeutic method for treating KOA.

In skeletal muscle, the transcription of the mouse type Iα (Rlα) subunit of the cAMP-dependent protein kinase begins at the alternative noncoding first exons 1a and 1b (31). A previous study has indicated that the regulation of the promoter upstream of exon 1a (Pa) depends on two adjacent E boxes (E1 and E2) in intact muscle (31). More importantly, USF1 is an important transcription factor that binds the E-box elements in the promoter region of muscle-specific genes (32,33). However, the expression pattern of USF1 in the muscle tissues of KOA patients has never been reported. For the first time, we showed that USF1 was increased in the muscle tissues of KOA patients compared with control. More importantly, overexpression of USF1 in primary human skeletal muscle cells significantly increased the activation of NF-κB signaling as well as the levels of pro-inflammatory factors. Thus, our data showed that USF1 activated NF-κB signaling in muscle tissues of KOA patients, which was then involved in inflammation-induced muscle weakness.

To our knowledge, this is the first study to explore a relationship between USF1 and NF-κB activation-induced inflammatory responses in muscle tissues of KOA patients, with findings aimed at improving the inflammatory response and preventing physical disability. However, we have to admit that some limitations exist in the current study. For instance, how the expression of USF1 was upregulated in the muscle tissues of KOA patients. In addition, whether other signaling pathways are involved in the correlation between USF1 and inflammation response in muscle tissues of KOA patients deserves further exploration. In the future, we will carry out deep research on the above questions thereby fully elucidating the underlying mechanism by which USF1 is modulated in the progression of KOA.

Figure 4. USF1 activates NF-κB signaling in primary human skeletal muscle cells. (A) Fluorescence assay showed the transfection efficiency of Ad-NC or Ad-USF1 in primary human skeletal muscle cells. Scale bar, 20 μm. (B) Western blot assays indicated that the overexpression of USF1 significantly induced the transcription of p65 NF-κB signaling. An ELISA assay revealed upregulation of inflammatory factors, including TNFα (C), IL-8 (D), IL-6 (E) and MCP-1 (F). *P<0.05, **P<0.01 and ***P<0.001 vs. control. USF1, upstream stimulating factor 1; ELISA, enzyme-linked immunosorbent assay; TNFα, tumor necrosis factor α.
Acknowledgements

Not applicable.

Funding

The present study was supported by a grant from Mudanjiang Medical University (MDJ-20160432).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

XS performed the experiments and analyzed the data. HL, BL, ZY, WW, HL, JS and SL performed the IHC staining and western blot experiments. MZ designed the experiments, analyzed the data and gave final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Research Ethics Committee of Mudanjiang Medical University (Mudanjiang City, China) and all the patients have provided written informed consent for this study.

Patient consent for publication

Informed consent for participation in the study or use of their tissue was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

References

1. Michael JW, Schlüter-Brust KU and Eyssel P: The epidemiology, etiology, diagnosis, and treatment of osteoarthritis of the knee. Dtsch Arztebl Int 107: 152-162, 2010.
2. Richter F, Natura G, Löser S, Schmidt K, Viisanen H and Schaible HG: Tumor necrosis factor causes persistent sensitization of joint nociceptors to mechanical stimuli in rats. Arthritis Rheum 62: 3806-3814, 2010.
3. Park SK, Kobas D and Ferber R: Relationship between lower limb muscle strength, self-reported pain and function, and frontal plane gait kinematics in knee osteoarthritis. Clin Biomech (Bristol, Avon) 38: 68-74, 2016.
4. Macías-Hernández SI, Miranda-Duarte A, Ramírez-Mora I, Cortés-González S, Morones-Alba JD, Olsacoaga-Gómez A, Coronado-Zarco R, Soria-Bastida MLA, Nava-Bringas TI and Cruz-Medina E: Knee muscle strength correlates with joint cartilage T2 relaxation time in young participants with risk factors for osteoarthritis. Clin Rheumatol 35: 2072-2092, 2016.
5. Ruhdorfer A, Wirth W and Eckstein F: Association of knee pain with a reduction in thigh muscle strength—a cross-sectional analysis including 4553 osteoarthritis initiative participants. Osteoarthritis Cartilage 25: 658-666, 2017.
6. Farrokh S, Voycheck CA, Gustafson JA, Fitzgerald GK and Tashman S: Knee joint mechanics during downhill gait and its relationship with varus/valgus motion and muscle strength in patients with knee osteoarthritis. Knee 23: 49-56, 2016.
7. Sarkar D and Fisher PB: Molecular mechanisms of aging-associated inflammation. Cancer Lett 236: 13-23, 2006.
8. Peake J, Delia Gatta P and Cameron-Smith D: Aging and its effects on inflammation in skeletal muscle at rest and following exercise-induced injury. Am J Physiol Regul Integr Comp Physiol 298: R1485-R1495, 2010.
9. Russell AP: Molecular regulation of skeletal muscle mass. Clin Exp Pharmacol Physiol 37: 378-384, 2010.
10. Buch A, Carmeli E, Boker LK, Marcus Y, Shefer G, Kis O, Berner Y and Stern N: Muscle function and fat content in relation to sarcopenia, obesity and frailty of old age—an overview. Exp Gerontol 76: 25-32, 2016.
11. Mishra SK and Misra V: Muscle sarcopenia: An overview. Acta Myol 22: 43-47, 2003.
12. Zhang L, Handel MV, Schartner JM, Hagar A, Allen G, Curet M and Badié B: Regulation of IL-10 expression by upstream stimulating factor (USF-1) in glioma-associated microglia. J Neuroimmunol 188: 197-207, 2007.
13. Cheung E, Mayr P, Coda-Zabetta F, Woodman PG and Boam DS: DNA-binding activity of the transcription factor upstream stimulatory factor (USF-1) is regulated by cyclin-dependent phosphorylation. Biochem J 344: 145-152, 1999.
14. Naukkarinen J, Gentle M, Soro-Paavonen A, Saarelä J, Koistinen HÅ, Pajukanta P, Taskinen MR and Peltonen L: USF1 and dyslipidemias: Converging evidence for a functional intronic regulatory element. Hum Mol Genet 14: 2505-2602, 2005.
15. Rada-Iglesias A, Ameer U, Kapranov P, Enroth S, Komorowski J, Gingeras TR and Wadelius C: Whole-genome maps of USF1 and USF2 binding and histone H3 acetylation reveal new aspects of promoter structure and candidate genes for common human disorders. Genome Res 18: 380-392, 2008.
16. Sellak H, Choi C, Browner N and Lincoln TM: Upstream stimulatory factors (USF-1/USF-2) regulate human cGMP-dependent protein kinase I gene expression in vascular smooth muscle cells. J Biol Chem 280: 18425-18433, 2005.
17. Malyankar UM, Hanson R, Schwartz SM, Ridall AL and Giachelli CM: Upstream stimulator factor 1 regulates osteopontin expression in smooth muscle cells. Exp Cell Res 250: 535-547, 1999.
18. Ircher I, Ljubicic V, Kirwan AF and Hood DA: AMP-activated protein kinase-regulated activation of the PGC-1alpha promoter in skeletal muscle cells. PLoS One 3: e3614, 2008.
19. Gustafsson T, Osterlund T, Flanagan JN, von Waldén F, Trappe TA, Linnehan RM and Tesch PA: Effects of 3 days unloading on molecular regulators of muscle size in humans. J Appl Physiol (1985) 109: 721-727, 2010.
20. Fiehn JL, Landerman LR, Somers TJ, Keefe JF, Guilak F, Blumenthal JA, Caldwell DS and Kraus VB: Exploratory secondary analyses of a cognitive-behavioral intervention for knee osteoarthritis demonstrate reduction in biomarkers of adipocyte inflammation. Osteoarthritis Cartilage 24: 1528-1534, 2016.
21. Neogi T, Guermazi A, Skezai A, Roemer F, Nevitt MC, Arendt-Nielsen L, Woolf C, Niu J, Bradley LA, Quinn E and Law LF: Association of joint inflammation with pain sensitization in knee osteoarthritis: The multicenter osteoarthritis study. Arthritis Rheumatol 68: 654-661, 2016.
22. Henriedsdotter C, Ellegaard K, Klokker L, Bartholdy C, Bandak E, Bartels EM, Bliddal H and Henriksen M: Changes in ultrasound assessed markers of inflammation following intra-articular steroid injection combined with exercise in knee osteoarthritis: Exploratory outcome from a randomized trial. Osteoarthritis Cartilage 24: 814-821, 2016.
23. Vescovi F, Giavacesi G, Maglio M, Scotto d'Abusco A, Politi L, Scandurra R, Olivetto E, Grigolo B, Borzi RM and Fini M: Chondroprotective activity of N-acetyl phenylalanine glucosamine derivative on knee joint structure and inflammation in a murine model of osteoarthritis. Osteoarthritis Cartilage 25: 589-599, 2017.
24. van Putten E, Volpato S, Furrer Z, Heikkinen E, Fried LP and Guralnik JM: Handgrip strength and cause-specific and total mortality in older disabled women: Exploring the mechanism. J Am Geriatr Soc 51: 636-641, 2003.
25. Hoeksa AF, ter Steeg AM, Nellissen RG, van Ouwerkerk WJ, Lankhorst GJ and de Jong RA: Neurological recovery in obstetric brachial plexus injuries: an historical cohort study. Dev Med Child Neuro 46: 76-83, 2004.
26. Franceschi C, Capri M, Monti D, Guanda S, Oliveri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scotti M, et al: Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev 128: 92-105, 2007.
27. Taekema DG, Westendorp RG, Frölich M and Gussekloo J: High innate production capacity of tumor necrosis factor-alpha and decline of handgrip strength in old age. Mech Ageing Dev 128: 517-521, 2007.

28. Gopinath SD and Rando TA: Stem cell review series: Aging of the skeletal muscle stem cell niche. Aging Cell 7: 590-598, 2008.

29. Cai D, Frantz JD, Tawa NE Jr, Melendez PA, Oh BC, Lidov HG, Hasselgren PO, Frontera WR, Lee J, Glass DJ and Shoelson SE: IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 119: 285-298, 2004.

30. Hunter RB and Kandarian SC: Disruption of either the Nfkb1 or the Bcl3 gene inhibits skeletal muscle atrophy. J Clin Invest 114: 1504-1511, 2004.

31. Barradeau S, Imaizumi-Scherrer T, Weiss MC and Faust DM: Muscle-regulated expression and determinants for neuromuscular junctional localization of the mouse RIalpha regulatory subunit of cAMP-dependent protein kinase. Proc Natl Acad Sci USA 98: 5037-5042, 2001.

32. Whetstine JR, Witt TL and Matherly LH: The human reduced folate carrier gene is regulated by the AP2 and sp1 transcription factor families and a functional 61-base pair polymorphism. J Biol Chem 277: 43873-43880, 2002.

33. Apone S and Hauschka SD: Muscle gene E-box control elements. Evidence for quantitatively different transcriptional activities and the binding of distinct regulatory factors. J Biol Chem 270: 21420-21427, 1995.

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