NUMB attenuates post-traumatic osteoarthritis by inhibitingBTRC and inactivating NF-κB pathway

Zhou Lv
Qingdao Municipal Hospital
Fubo Zhou
Laiyang Central Hospital
Xianqiang Cheng (cxq0535@163.com)
Laiyang Central Hospital

Research Article

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Abstract

Background

Post-traumatic osteoarthritis (PTOA) is closely related to the inflammatory response caused by mechanical injury, leading to joint degeneration. Herein, we aim to evaluate the role and the underlying mechanism of NUMB in PTOA progression.

Methods

Anterior cruciate ligament transection (ACLT)-induced rats and LPS-treated chondrocytes were used as in vivo and in vitro models of PTOA, respectively. NUMB overexpression plasmid (pcDNA-NUMB) were administered by intra-articular injection in PTOA model rats, and safranin O-fast green staining, the Osteoarthritis Research Society International (OARSI) Scoring System, and HE staining were used to evaluate the severity of cartilage damage. The secretion of inflammatory cytokines (TNF-α, IL-1β, IL-6) and chondrocyte-specific markers (MMP13, COL2A1) was detected by ELISA. Cell viability and apoptosis were evaluated by MTT assay and TUNEL staining.

Results

The expression of NUMB was lower expressed in ACLT-induced PTOA rats and LPS-treated chondrocytes. NUMB overexpression enhanced cell viability and reduced cell apoptosis, inflammation and cartilage degradation in chondrocytes stimulated by IL-1β. NUMB bound with BTRC to inhibiting p-IκBα expression, resulting in NF-κB pathway inactivation. BTRC overexpression reversed the promoting effect of NUMB overexpression on cell viability and the inhibitory effects of NUMB overexpression on apoptosis, inflammation and cartilage degradation in LPS-induced chondrocytes. Besides, overexpression of NUMB alleviated articular cartilage damage by repression of inflammation and cartilage degradation in ACLT-induced PTOA rats.

Conclusion

Our data indicated that NUMB negatively regulates BTRC regulated PTOA progression by BTRC/NF-κB pathway, which may be a viable therapeutic target in PTOA.

1. Introduction

Osteoarthritis (OA) is a common joint disease, which can cause chronic progressive joint swelling, pain, joint stiffness and even deformity, thus bringing a considerable burden of disability. Its pathogenesis is closely related to the degeneration and destruction of articular cartilage [1]. OA is mainly divided into two types: primary OA and secondary OA. Primary OA occurs gradually with age, but there is no specific

reason; Secondary OA is caused by some diseases, such as joint injury, autoimmune inflammatory arthritis, congenital joint malformation and so on [2]. Post-traumatic osteoarthritis (PTOA) is secondary to OA-induced joint injury, which mainly caused by trauma, bone fracture or overloading, resulting in cartilage or ligament injury [3, 4]. PTOA is prevalent in individuals of any age, but it is most common in young people following traumatic injury or in the context of unbalanced or excessive load-bearing [5]. Unfortunately, the results of clinical treatment are not ideal in PTOA. Although surgical intervention can reduce the symptoms of PTOA, the incidence of PTOA has not decreased, and there is no ideal measure to treat or prevent the occurrence and development of PTOA [6]. Therefore, it is of great significance for the effective and reasonable prevention and treatment of PTOA to clarify the mechanism of PTOA and to find key targets for intervention.

Growing number of studies revealed that cytokines play an important role in physiological metabolism and functional regulation of organisms. After the physical injury of articular cartilage caused by trauma, PTOA chondrocytes release a large number of inflammatory cytokines from focal tissues [7, 8]. These cytokines can regulate abnormal proliferation or apoptosis of joint chondrocytes, disrupt cartilage tissue metabolism, synthesis or degradation of extracellular matrix (ECM), denature cartilage matrix, eventually leading to change of joint cartilage structure or function, and interrupting joint activity [9, 10].

NUMB, a conserved membrane protein, is involved in cell-fate determination and differentiation. NUMB was originally identified as a mediator of tissue morphogenesis and patterning in the cleavage of Drosophila neurons [11]. A previous study revealed that NUMB can regulate the asymmetric mitosis of cells [12]. In addition, NUMB was reported to be tumor suppressor in various cancer. Silencing of NUMB significantly enhanced MCF-7 cell proliferation, invasion and migration by via β-cateni/Lin28 signaling pathway in breast cancer [13]. Shu et al. showed that the expression of NUMB was downregulated in intrahepatic cholangiocarcinoma tumor tissues and closely correlated with poor prognosis of patients with intrahepatic cholangiocarcinoma [14]. Surprisingly, Zhang et al revealed that NUMB is reported to be involved in chondrocyte apoptosis in OA [15]. However, the role and possible molecular mechanism of NUMB in PTOA have not been reported in vivo and vitro.

In this study, we aimed to elucidate the expression of NUMB in a rat or cell model of PTOA, and explore its possible mechanism in PTOA, in order to find a potential target for the treatment of PTOA.

2. Materials And Methods

2.1 Animals and the PTOA Model

Eight-week-old male Sprague-Dawley rats (n = 24, 220–250 g) were purchased from the Animal Center of Laboratory Animal Center of Shandong University. This animal experiment was approved by the Ethics Committee on Animal Experimentation of Qingdao Municipal Hospital. Rats were housed under controlled conditions (25 ± 2°C, 70% humidity and 12-hlight-dark periods) and fed a standard diet. As previously reported, anterior cruciate ligament transection (ACLT) was performed on the right knee to

cause joint instability, thereby inducing PTOA. The rats were randomized into the following four groups (n = 6/group): a sham group, an ACLT group, an ACLT + vector, and an ACLT + pcDNA-NUMB. Briefly, rats were anesthetized using 3% sodium pentobarbital via intraperitoneal injection. After sufficient disinfection, the medial skin of the patellar ligament was incised and the joint capsule was opened to dislocate the patellar bone. Flexing the knee joint exposed the anterior cruciate ligament and cut the anterior cruciate ligament. After rinsing with sterile saline, the wound was closed layer by layer and disinfected, and penicillin was injected intramuscularly to prevent infection. After 6 weeks, the rats were anesthetized with 3% sodium pentobarbital and sacrificed. The cartilage tissue was immediately taken out and embedded in paraffin for subsequent experiments. In sham control mice, a skin and capsule incision was instead conducted.

Safranin O/Fast Green staining

Cartilage tissue sections were dewaxed with 4% xylene and dehydrated with gradient alcohol. For Safranin O staining, tissues were stained with Safranin O solution (ScyTek, Logan, UT, USA). All staining was performed according to the manufacturer's instruction, and the morphological changes were observed with an optical microscope (BX51; Olympus Corp., Tokyo, Japan).

OARSI Scoring

Rat cartilage degeneration was graded for Safranin O/Fast Green-stained knee sections using the Osteoarthritis Research Society International (OARSI) scoring system. A subjective score of 0–6 was applied as previously described. Grading was performed by three blinded observers. The three grades for each section were then averaged, and the data for mice in each group were collated. Higher scores were indicative of more serious cartilage damage.

Hematoxylin-eosin (HE) staining

The cartilage tissues were taken from each rat in each group, fixed in 10% formalin, and then embedded in paraffin, and sequential serial sections were obtained. Afterward, the sections were stained with hematoxylin–eosin (HE) staining. Images were obtained using an optical microscope (BX51; Olympus Corp., Tokyo, Japan).

Cell culture

Human primary chondrocytes were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). The cells were cultured in complete Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), streptomycin (100 mg/ml) and penicillin (100 U/ml) at 37˚C in a humidified atmosphere containing 5% CO_2. Only cells within the fifth passage were used for the subsequent experiments.

Cell treatment

Chondrocytes were cultured in 96-well plates at a density of 10^4 cells /well and induced with various doses of IL-1β (1, 5, or 10 ng/ml) as a cell inflammatory model, and the normal chondrocytes (NC) were used as control.

Short-hairpin NUMB (sh-NUMB) plasmid, short-hairpin BTRC (sh-BTRC) plasmid, and their respective nontargeting sequence (sh-NC) as well as NUMB and BTRC overexpression plasmid (pcDNA-NUMB and pcDNA-BTRC) and their respective nontargeting sequence (vector) were synthesized by GenePharma (Shanghai, China). Chondrocytes were grown to 70%-80% confluency in 24-well plates and then transfected with Lipofectamine 3,000 reagents (Invitrogen, CA, USA) according to the manufacturer's instructions. Following transfection for 48 h, chondrocytes were collected for subsequent experiments.

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium Bromide (MTT) assay

Cell viability was tested via MTT assay analysis. After the indicated transfection or treatment, 1×10^4 chondrocytes were added into 96-well plates, and then incubated for 72 h, followed by incubating with 0.5 mg/mL MTT (Beyotime, Shanghai, China) for 4 h. Next, the optical density (OD) value was examined via a microplate reader (Molecular Devices, San Jose, CA, USA) with a wavelength of 570 nm.

TUNEL staining

After transfection, TUNEL staining was used to detect apoptosis in chondrocytes in the presence of IL-1β (10 ng/mL). Briefly, chondrocytes seeded on glass coverslips after fixation with acetone/methanol (vol/vol) for 5 min at − 20°C. Then chondrocytes were incubated with equilibration buffer for 10 s before adding TdT enzyme for 1 h at 37°C. Subsequently, chondrocytes were incubated with reaction mixture for 60 min and cell nuclei were stained with DAPI. Immunofluorescent images/slide were observed by using fluorescence microscope(Nikon, Tokyo, Japan). The results are presented as the apoptosis indices, which was quantified as (TUNEL-positive cells)/(total cells)×100%.

Western blotting

Cartilage tissues and chondrocytes were collected and lysed with RIPA buffer containing protease inhibitors (Beyotime, Jiangsu, China). Equal amounts of proteins were separated with 10% SDS-PAGE and then electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). After blocked in 5% nonfat milk, membranes were incubated with primary antibodies against NUMB (1:1,000), BTRC (1:1,000), p-p65 (1:500), p65 (1:1,000), IκBα (1:1,000), p-IκBα (1:1,000), and GAPDH (1:1,000) at 4°C for at least 12 h, followed by incubation with HRP-conjugated secondary antibody (1:5,000) for another 2 h at room temperature. Immunoblots were visualized by an enhanced chemiluminescence detection kit (ECL kit; Millipore, Billerica, MA) under a chemiluminescence imaging analysis system (Amersham Imager 600, GE, CT, USA). Relative integrated density values were calculated using Image J software.

Immunofluorescence staining

Chondrocytes were inoculated on polylysine-coated cover glass for 24 h, fixed with 4% paraformaldehyde for 20 min, and permeated with 0.2%Triton X-100 for 10 min. Then the cells were incubated with primary antibody (NF-κB p65) at 4°C overnight. After incubation with Alexa Fluor® 594 anti-mouse antibody (Molecular Probes, USA) for 1 h at room temperature, cells were counter-stained with DAPI (Invitrogen). Images were observed by Olympus fluorescence microscope (Nikon, Tokyo, Japan).

Enzyme-linked immunosorbent assay (ELISA)

The levels of TNF-α and IL-6 in serum and in samples of supernatants from cultured chondrocytes were measured with commercial ELISA kits (R&D Systems, MN, USA) following the instruction of manufacturer. The absorbance of samples was detected at 450 nm. Similarly, the levels of related secretory proteins such as MMP-13 and COL2A1 were also detected by ELISA kits (Sigma, St. Louis, MO, USA).

Co-immunoprecipitation (Co-IP) assay

Chondrocytes were lysed in RIPA buffer (Beyotime, Shanghai, China). Cell supernatant was collected and incubated with anti-NUMB antibody, anti-BTRC antibody or IgG at 4°C overnight. Then the mixture was incubated with 100 μL of protein A/G agarose beads (Takara Biotechnology, Dalian, China) overnight at 4°C. After incubation, the mixture was centrifuged at 3000 rpm for 5 min, and the beads were collected and washed with PBS for three times. The beads were boiled with loading buffer for 5 min and then centrifuged at 3000 rpm for 5 min to separate the proteins from the beads. The IP products were subsequently analyzed by using Western blotting.

Statistical analysis

Statistical analysis was performed with SPSS version 22.0 software. All data were presented as mean ± standard deviation (SD). Student's t-test was performed for the comparison between two groups, and analysis of variance (ANOVA) was performed for comparison among groups. $P < 0.05$ was considered as statistically significant difference.

3. Results

NUMB expression was increased in PTOA model of rats and IL-1β-induced chondrocytes

To test whether NUMB was implicated in PTOA progression, PTOA model was established in rats and in chondrocytes, respectively. Safranin O/fast green staining was used to evaluate articular cartilage, and the results showed that PTOA group had higher levels of knee cartilage loss and matrix degradation, compared to the sham group at different time points (2, 4, and 6 weeks), evidenced by markedly increased Osteoarthritis Research Society International (OARSI) scores (Fig. 1A). Western blotting analysis uncovered that NUMB expression was gradually elevated with time in PTOA group (Fig. 1B). Moreover, Western blotting results also showed that the expression of NUMB was significantly increased in chondrocytes after treatment of IL-1β in a dose-dependent manner (Fig. 1C).

Overexpression of NUMB suppressed IL-1β-induced chondrocyte injury

We first transfected NUMB overexpression plasmid (pcDNA-NUMB) or negative control (vector) in chondrocytes and incubated chondrocytes with IL-1β (10 ng/mL) for another 24 h. Western blotting revealed that NUMB levels were significantly higher in NUMB overexpressing cells relative to those transduced with vector (Fig. 2A). The results of MTT and TUNEL assays revealed that IL-1β caused significant decreasing of cell viability and increasing of cell apoptosis, while NUMB overexpression remarkably reversed these effects (Fig. 2B and C). In addition, IL-1β caused significant activation of inflammation and degradation of chondrocyte-specific markers. The secretion of IL-6, and TNF-α were significantly upregulated in chondrocytes in IL-1β group, and downregulated in IL-1β + pcDNA-NUMB group (Fig. 2D and E). NUMB overexpression reduced the promoting effect on MMP13 secretion and the inhibitory effect of on COL2A1 secretion (Fig. 2F and G).

NUMB bound with BTRC and positively regulated BTRC expression

To further investigate the function of NUMB, we predicted the potential binding proteins of NUMB by using the Genemania tool (http://genemania.org/). The result showed that there was a potential binding relationship between NUMB and BTRC (Fig. 3A). Co-IP assay showed that both NUMB and BTRC proteins could be detected by immunoprecipitation with NUMB antibody or BTRC antibody but not with IgG (Fig. 3B and C), suggesting that NUMB could bind with BTRC in chondrocytes. Besides, upregulation of NUMB significantly increased BTRC expression in chondrocytes, while silencing of NUMB deceased BTRC expression, indicating that NUMB bound with BTRC and positively regulated BTRC expression in chondrocytes (Fig. 3D).

Overexpression of NUMB suppressed IL-1β-induced chondrocyte injury by downregulation of BTRC

To explore whether NUMB exerted its function by binding with BTRC, chondrocytes were transfected with pcDNA-NUMB alone or together with pcDNA-BTRC. The results of Western blotting revealed that the expression of BTRC was upregulated by transfection of pcDNA-NUMB, which was restored by transfection of pcDNA-BTRC (Fig. 4A). NUMB overexpression elevated cell viability and cell apoptosis in chondrocytes, while BTRC overexpression reversed these effects (Fig. 4B and C). Additionally, BTRC overexpression significantly reversed the effects of NUMB overexpression on the secretion of IL-6, TNF-α, MMP13 and COL2A1 in chondrocytes (Fig. 4D-G).

NUMB inactivated NF-κB signaling pathway by BTRC/p-IκBα axis

BTRC is reported to be involved in p-IκBα degradation, resulting in nuclear translocation of NF-κB p65. To verify whether NUMB activated the NF-κB signaling pathway in a BTRC-dependent manner, chondrocytes were transfected with sh-NUMB or pcDNA-NUMB, respectively. Western blotting results showed that transfection of sh-NUMB reduced p-IκBα expression and increased p-p65 expression in chondrocytes, while transfection of pcDNA-NUMB increased p-IκBα expression and decreased p-p65 expression (Fig. 5A, B). Immunofluorescence staining revealed that nuclear localization of p65 was detected in chondrocytes

transfection of sh-NUMB, whereas cytoplasmic p65 was observed in chondrocytes transfection of pcDNA-NUMB (Fig. 5C).

Overexpression of NUMB alleviated articular cartilage degeneration in PTOA model of rats

Western blotting was performed to measure the expression of NUMB in knee joint tissue of rats. The expression of NUMB in ACLT + pcDNA-NUMB group was increased significantly compared with that in the ACLT + vector group (Fig. 6A). The results of Safranin O/fast green staining showed that NUMB overexpression significantly decreased the severity of articular cartilage degeneration in PTOA model of rats and significantly reduced cartilage OARSI grade scores (Fig. 6B and C). HE staining revealed that NUMB overexpression improved ACLT-induced chondrocyte damage and exfoliation (Fig. 6D). ELISA results showed a significant increasing of IL-6, TNF-α and MMP13 and decreasing of COL2A1 in ACLT and ACLT + vector group compared with sham group, whereas these changes were significantly reversed in the ACLT + pcDNA-NUMB (Fig. 6E-H). Besides, NUMB overexpression efficiently inhibited the expression of BTRC and p-p65 and promoted the expression of p-IκBα (Fig. 6I-K).

4. Discussion

PTOA is mainly caused by trauma, accounting for ~ 12% of OA, and the knee joint is the most common site of traumatic injury in the body [16, 17]. With the development of PTOA, disability develops and may seriously affect the quality of life of patients. In this study, we established a rat model of PTOA via conducting ACLT of the right knee joint and a cell model of PTOA via treating IL-1β, and found that NUMB expression was decreased in knee joint tissue of rats and chondrocytes. Overexpression of NUMB remarkably promoted the proliferation and reduced apoptosis of chondrocytes. Besides, a large amount of evidence revealed that the content of IL-6 and TNF-α in knee joint fluid is positively related to the severity of PTOA [18]. Matrix metalloproteinase-13 (MMP-13) is an important extracellular matrix degrading enzymes, which can efficiently degrade type II collagen, lead to cartilage degradation and promote the progress of PTAO or OA progression [19, 20]. Herein, our results showed that the levels of TNF-α and IL-6 were higher in ACLT-induced rats and IL-1β-induced chondrocytes. Results also revealed a significant increasing of MMP13 expression and a decreasing of collagen type II alpha 1 chain (COL2A1) expression. Some studies have shown that NUMB participated in regulating the production of pro-inflammatory cytokines [21]. NUMB exerted protective effects in cisplatin-induced acute kidney injury, manifested by reducing cisplatin-induced tubular apoptosis and ameliorating tubular necrosis and renal inflammation [22]. Zhou et al. demonstrated that Astaxanthin inhibited neuroinflammation via regulating microglia M1 activation by upregulation of NUMB in Lipopolysaccharide (LPS)-treated BV2 cells and mice [23]. Recently, there is new evidence that the expression of NUMB is significantly downregulated in cartilage of rat knees after ACLT surgery, and NUMB was a target of miR-146a-5p, which could inhibit cell viability and promote apoptosis and inflammatory factor expression in chondrocyte after IL-1β treatment [15, 24]. The above results suggested that NUMB has a positive regulatory effect on PTOA. However, the specific mechanism of NUMB in PTOA needs further study.

In current study, we revealed that beta-transducin repeat containing E3 ubiquitin protein ligase (BTRC) is a potential target of NUMB, and Co-IP analysis confirmed the targeting relationship between BTRC and NUMB. At present, the research on BTRC is mostly related to tumor. BTRC, a member of the F-box and WD40 repeat family of proteins, play an important role in epithelial-mesenchymal transition (EMT) progression [25]. Zhou et al. revealed that BTRC mediated the snail's ubiquitination in cancer and BTRC inhibition contributed to the upregulation of snails, which induces EMT [26]. In triple-negative breast cancer, LINC00511 interacted with BTRC to maintaining the stability of Snail, thus promoting triple-negative breast cancer cell growth and invasion [27]. Furthermore, recent studies reported that BTRC mediated the ubiquitination of phosphorylated IκBα (p-IκBα), thereby triggering translocation of NF-κB to the nucleus and activation of target genes [28, 29]. Lim et al. showed that WBP2 promoted ubiquitin-mediated proteasomal degradation of IκBα, an inhibitor for NF-κB, via enhancing mRNA stability of BTRC to promote TNBC cell migration and invasion [30]. TSPAN15 was a novel oncogene, which enhanced oesophageal squamous cell carcinoma metastasis through BTRC/NF-KB signal pathway [31]. Another study showed that miR-10a could accelerate IκBα degradation and NF-κB activation by upregulation of BTRC, thus increasing the excessive secretion of NF-κB-mediated inflammatory cytokines and the proliferation and migration of fibroblast-like synoviocytes in rheumatoid arthritis [32]. NF-κB was reported to mediate the production of various inflammatory factors and played a key role in the pathogenesis of PTOA. Inhibition of NF-κB pathway was able to attenuate cartilage degeneration in a murine model of PTOA [33, 34]. Herein, we found that NUMB activated the NF-κB signaling pathway in a BTRC-dependent manner. Overexpression of NUMB reduced BTRC expression and increased p-IκBα expression, resulting in translocation of NF-κB to the cytoplasm. While, knockdown of NUMB showed the opposite effects. BTRC overexpression reversed the effects of NUMB on cell viability, cell apoptosis and inflammation.

In summary, our studies demonstrated that NUMB is lower expressed in knee joint tissue of rats after ACLT surgery and IL-1β-induced chondrocytes. NUMB downregulates BTRC and inactivates the NF-κB pathway to reduce chondrocyte damage in PTOA. Our results suggest that NUMB may become a further research object for the treatment and intervention of PTOA.

Declarations

Acknowledgements

No applicable.

Founding

No applicable.

Declaration of conflicting interests

No conflict of interest exits in the submission of this manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

All animal care and experimental procedures in this study were approved by Animal Care and Use Committee of Qingdao Municipal Hospital.

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Figures

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Figure 1

The expression of NUMB in knee joint tissue of rats and chondrocytes with IL-1β treatment. (A) Safranin O/fast greens staining of the sagittal sections of the knee joint tissue at different time points (2, 4, and 6 weeks). (B) The degree of cartilage loss and matrix degradation from 2–6 weeks (scale bar: 500 mm) was calculated using the Osteoarthritis Research Society International (OARSI) scoring system. (C and D) Relative expression of NUMB protein in knee joint tissue of rats and IL-1β-induced chondrocyte was detected by Western blotting. Data were presented as mean ± SD. *P<0.05.

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Figure 2

Effects of NUMB overexpression on chondrocyte **cell viability, apoptosis and inflammation.** Chondrocyte were transfected with pcDNA-NUMB (20 nM) and its corresponding negative controls, respectively. (A)

The expression of NUMB in chondrocyte was measured with Western blotting. (B) Cell viability was evaluated with MTT assay. (C) TUNEL staining was performed to detect the apoptosis of chondrocyte. (D-G) The secretion of IL-6, TNF-α, MMP13 and COL2A1 was examined with ELISA. Data were presented as mean ± SD. *P<0.05.

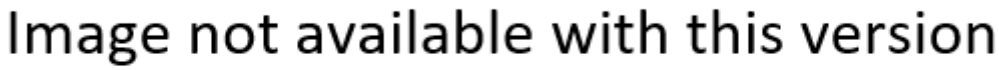

Figure 3

NUMB bound with BTRC and promoted BTRC expression. (A) The online Genemania tool was used to predict the potential interacting proteins of NUMB. (B and C) The binding between NUMB and BTRC was detected by Co-IP assay. (D) pcDNA-NUMB (20 nM), sh-NUMB (20 nM) and their negative controls were transfected into chondrocyte, respectively. The expression of BTRC protein was measured with Western blotting. Data were presented as mean ± SD. *P<0.05.

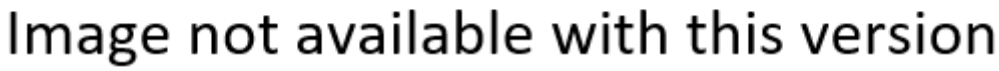

Figure 4

NUMB promoted cell viability and inhibited apoptosis and inflammation by inhibiting BTRC expression in chondrocyte. Chondrocyte were transfected with pcDNA-NUMB (20 nM) alone or together with pcDNA-BTRC (20 nM). (A) The expression of BTRC in chondrocyte was measured with Western blotting. (B) Cell viability was evaluated with MTT assay. (C) TUNEL staining was performed to detect the apoptosis of chondrocyte. (D-G) The secretion of IL-6, TNF-α, MMP13 and COL2A1 was examined with ELISA. Data were presented as mean ± SD. *P<0.05.

Figure 5

NUMB inactivated NF-κB signaling pathway by BTRC/p-IκBα axis. pcDNA-NUMB (20 nM), sh-NUMB (20 nM) and their negative controls were transfected into chondrocyte, respectively. (A and B) The expression of p-IκBα and p-p65 in chondrocyte was measured with Western blotting. (C) The nuclear localization of p65 was detected in chondrocytes with Immunofluorescence staining. Data were presented as mean ± SD. *P<0.05.

Figure 6

Overexpressing NUMB inhibited articular cartilage degeneration in a rat model of ACLT-induced PTOA. On day 3 post-ACLT, the affected knee joints of rats were injected with a lentivirus encoding a control or NUMB overexpression vector (vector and pcDNA-NUMB). At 8 weeks post-ACLT, rats were euthanized. Sham-operated animals served as negative controls. (A) NUMB protein levels in knee joints of mice were assessed by Western blotting. (B) Sagittal tibial articular cartilage sections were subjected to safranin O-fast green staining. Scale bar = 200 μm or 50 μm. (C) The OARSI scoring system was used to grade mouse cartilage degeneration. (D) The chondrocyte damage was detected with HE staunung. (E-H) The secretion of IL-6, TNF-α, MMP13 and COL2A1 was examined with ELISA. (I-K) Cartilage expression of BTRC, p-IκBα and p-p65 was evaluated with Western blotting. Data were presented as mean ± SD. *P<0.05.