European Review for Medical and Pharmacological Sciences 2020; 24: 5914-5924

FBW7 regulates HIF-1α/VEGF pathway in the IL-1β induced chondrocytes degeneration

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Abstract. – OBJECTIVE: The aim of the study was to observe the effect of F-box/WD repeat-containing protein 7 (FBW7) on hypoxia-inducible factor-1α (HIF-1α)/vascular endothelial growth factor (VEGF) pathway in chondrocytes (CHs) under IL-1β induced degeneration.

PATIENTS AND METHODS: We explored the levels of FBW7, HIF-1α, and VEGF in degenerated cartilage from osteoarthritis (OA) and chondrocytes (CHs) treated by IL-1β. Meanwhile, we regulated HIF-1α and FBW7 expression in IL-1β treated CHs and observed the effects FBW7 of the HIF-1α/VEGF pathway.

RESULTS: FBW7 expression was significantly decreased along with the increased HIF-1α and VEGF expression both in OA cartilage and IL-1β induced degenerated CHs. Additionally, suppression of HIF-1α decreased VEGF level, which contributed to the production of collagen II, aggrecan and SOX-9, and inhibited collagen I and Runx-2 expression. Furthermore, FBW7 suppressed HIF-1α/VEGF pathway and promoted the integration of collagen II, aggrecan, and SOX-9, but inhibited the collagen I and Runx-2 expression.

CONCLUSIONS: FBW7 negatively regulates HIF-1α/VEGF pathway and plays a protective role in the IL-1β induced CHs degeneration.

Key Words:
FBW7, HIF-1α, VEGF, Chondrocyte degeneration, IL-1β.

Introduction

The degeneration of articular chondrocytes (CHs) caused by multiple factors is the primary pathological basis of osteoarthritis (OA). As OA develops, CHs degeneration leads to the occurrence of articular cartilage fibrosis, ulcer formation, and subchondral bone hyperplasia, which are the main features of OA. The articular cartilage in a hypoxic environment is an avascular tissue, and the oxygen partial pressure drops from 6% of the cartilage surface to 1% of the deep layer[1]. Articular CHs are hypoxic cells, and hypoxia-inducible factor-1α (HIF-1α) is a hypoxia regulator, which plays a vital role in regulating the metabolism and survival of CHs[2,3]. The level of HIF-1α combining with other abnormal gene expressions may affect the normal morphology and function of cartilage[4,5], one of which is vascular endothelial growth factor (VEGF)[6,7]. VEGF is a target gene to HIF-1α and is currently recognized as the most potent pro-angiogenic factor. During cartilage development, VEGF functions as a mediator to promote neovascularization and maintain CH survival[8].

However, during the development of OA, VEGF is enhanced by the regulation of HIF-1α with increased angiogenesis and osteogenic factor expression, resulting in vascularization of articular cartilage and subchondral osteogenesis which are the main pathological features of advanced OA, as well[9,10]. Therefore, there is still a lot of ambiguity about the function of HIF-1α and VEGF in OA. F-box and WD repeat domain-containing7 (FBW7) are classified from the F-box protein family, and it recognizes the substrate protein of the SCF-type ubiquitin ligase complex and regulates the degradation of various proteins *via* ubiquitin-proteasome pathway[11]. It has been confirmed that FBW7 takes part in cell cycle regulation, cell proliferation, differentiation, apoptosis, and tumor metastasis through the negative control of Mcl-1, Notch, HIF-1α, Cyclin E, KLF5, NF-κB2 and other proteins in cell biological processes[12,13]. FBW7 recognizes the phosphorylated HIF-1α

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and mediates its ubiquitination, and the ubiquitinated HIF-1α is eventually degraded by the proteasome. High expression of FBW7 accelerates HIF-1α degradation and inhibits tumor angiogenesis, while the the expression of HIF-1α is increased after the suppression of FBW7, which promotes angiogenesis and stimulates the growth and metastasis of tumor cells[14].

In addition to hypoxic conditions, the inflammatory factors can also induce the accumulation of HIF-1α[15]. Yudoh et al[16] found that IL-1β can increase the gene expression of HIF-1α in CHs. Besides, Zhu et al[17] found that IL-1β can upregulate the level of HIF-1α in articular cartilage. The activation of HIF-1α induced by IL-1β may have a complex regulation mechanism for CH metabolism in degenerative bone and joint diseases[18]. For different views of the HIF-1α/VEGF in OA, we firstly used the IL-1β treated CHs model to detect the function of FBW7 in the regulation of HIF-1α/VEGF pathway, which hopefully provides a novel strategy to control the development of CHs degeneration in OA.

Patients and Methods

Articular Cartilages Collection

OA articular cartilages were obtained from 5 patients (average age: 57 years, 2 males and 3 females) undergoing total knee joint replacement. Besides, knee cartilages from 5 patients undergoing amputation for malignant bone neoplasms of femur proximal end were collected as a control group. The control group was selected at the condition without a personal history of OA. This study was supported by the Ethics and Research Committee of Jiaozhou Central Hospital of Qingdao. All patients signed an informed consent letter before surgery. The protein or RNA extraction were frozen in liquid nitrogen, and tissues for CHs isolation were conserved in Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12; Invitrogen, Carlsbad, CA, USA) culture medium immediately after cutting from patients.

CHs Isolation and Treatments

The articular specimens were placed in a sterile petri dish containing DMEM/F12 culture medium, cut the cartilage into small pieces from the surface of articular without the deep ossified cartilage. The pieces were digested with 0.25% type II collagenase (Sigma-Aldrich, St. Louis, MO, USA) at 37°C overnight, and got CH pellets after filtration by cell sieve. All CHs used for the experiment were passaged to the first or two generations. CHs were treated with IL-1β (5 ng/mL, Sigma-Aldrich, St. Louis, MO, USA) to induce degeneration as the previous describe[19]. The specific inhibitor of HIF-1α, 2-Methoxyestradiol (2-Me, 10 μM, Selleck, Houston, TX, USA), was used to suppress the HIF-1α expression of CHs[20]. CHs were transfected with FBW7-plasmid or siRNA (purchased from Invitrogen, Carlsbad, CA, USA) to upregulate or downregulate the expression of FBW7 in CHs *in vitro*.

Western Blot Analysis (WB)

Total protein in the articular cartilage and CHs were extracted by radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China), separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membranes were then blocked with 5% milk for 1; membranes were incubated with primary antibodies: FBW7 (ab109617, Abcam, Cambridge, MA, USA), HIF-1α (ab51608, Abcam, Cambridge, MA, USA), VEGF (ab69479, Abcam, Cambridge, MA, USA), collagen I (ab34710, Abcam, Cambridge, MA, USA), collagen II (ab34712, Abcam, Cambridge, MA, USA), and beta-actin (ab8226, Abcam, Cambridge, MA, USA) at 4°C overnight. Finally, the membranes were treated with secondary antibody for 1 h. The bands of the membranes were detected by enhanced chemiluminescence (ECL; Beyotime, Shanghai, China) and analyzed using Image J software (NIH, Bethesda, MD, USA).

Immunofluorescence (IF)

CH was seeded at 5×10^4/ml on cell coverslips in a 24-well plate. Before staining, CHs were fixed with 4% paraformaldehyde, permeabilized by 0.05% Triton X, and blocked in 5% bovine serum albumin (BSA). Then, they were incubated with primary antibodies: collagen II (ab34712, Abcam, Cambridge, MA, USA), FBW7 (ab109617, Abcam, Cambridge, MA, USA), and HIF-1α (ab51608, Abcam, Cambridge, MA, USA) overnight at 4°C. CHs were then incubated with Alexa Fluor 647 or 488 for 1 h in the dark. All these antibodies were purchased from Abcam (Cambridge, MA, USA). The staining intensity was measured using the fluorescence microscope.

Quantitative Real Time-PCR Analysis (qRT-PCR)

Total RNA of cartilage tissue and CHs were isolated with TRIzol kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, complementary deoxyribose nucleic acid (cDNA) was reversed with a reverse transcription kit and finally amplified with the SYBR Green Master kit (TaKaRa, Otsu, Shiga, Japan). All these reagents were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The qRT-PCR was performed to analyze mRNA levels of SOX-9, Runx-2, collagen I, FBW7, HIF-1α, VEGF, aggrecan, and collagen II. The primers used for qRT-PCR are listed in Table I. mRNA expression was calculated by normalization to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) according to the method of $2^{-\Delta\Delta Ct}$.

Enzyme-Linked Immunosorbent Assay (ELISA)

The content of FBW7, HIF-1α, and VEGF secreted by CHs in the supernatant was measured by an ELISA kit (Biocompare, South San Francisco, CA, USA) according to the manufacturer's instructions.

Transfection

CHs were planted at 1.5×10^5 cells/ml in 6-well plates until the cell fusion rate reached 60%. Cells were transfected with FBW7 coded siRNA (Catalog#HSS124318, Invitrogen, Carlsbad, CA, USA) and plasmid (Catalog#82878, Invitrogen, Carlsbad, CA, USA) in Lipofectamine 2000 (10 μmol/mL; Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After a 12-hour transfection, the medium was replaced.

Cell Viability Assay

The viability of CHs was measured *via* the Cell Counting Kit-8 (CCK8) test. CHs were seeded at 1×10^4 cells per well in a 96-well plate and treated with specific drugs as described for indicated time points. Next, CHs were incubated with CCK-8 reagent (Biovision, Milpitas, CA, USA) according to the manufacturer's instructions. The intensity of the CCK-8 product was determined at 450 nm, using a microplate reader (MS, Helsinki, Finland). The result was shown as relative to non-treated value.

Statistical Analysis

All data were assessed and presented by GraphPad Prism (v8.0.2.263, La Jolla, CA, USA). Data are expressed as the means ± standard deviation (SD). The differences between the two groups were analyzed using the Student's *t*-test. A comparison between multiple groups was made using One-way ANOVA test followed by post-hoc test (Least Significant Difference). A value of $p<0.05$ was considered statistically significant between groups.

Results

Levels of FBW7, HIF-1α, and VEGF in OA Cartilage

To determine the FBW7, HIF-1α, and VEGF levels of OA joint tissues, we isolated total protein and mRNA from both healthy and OA cartilage. As shown in Figure 1A, the healthy cartilage showed a smooth, even, and shiny surface and the CHs isolated from healthy cartilage appeared small and round shape. However, OA cartilage looked like much uneven and abrasive, and the CHs isolated from OA cartilage seemed to be

Table I. Primer sequences of the genes for RT-PCR.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
Collagen II	TGGACGATCAGGCGAAACC	GCTGCGGATGCTCTCAATCT
Aggrecan	ACTCTGGGTTTTCGTGACTCT	ACACTCAGCGAGTTGTCATGG
Collagen I	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
SOX-9	AGCGAACGCACATCAAGAC	CTGTAGGCGATCTGTTGGGG
Runx-2	TGGTTACTGTCATGGCGGGTA	TCTCAGATCGTTGAACCTTGCTA
FBW7	GGCCAAAATGATTCCCAGCAA	ACTGGAGTTCGTGACACTGTTA
HIF-1α	CATCACGCCGTCCTATGTCG	CGTCAAAGACCGTGTTCTCG
VEGF	AGGGCAGAATCATCACGAAGT	AGGGTCTCGATTGGATGGCA
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

RT-PCR, quantitative reverse-transcription polymerase chain reaction.

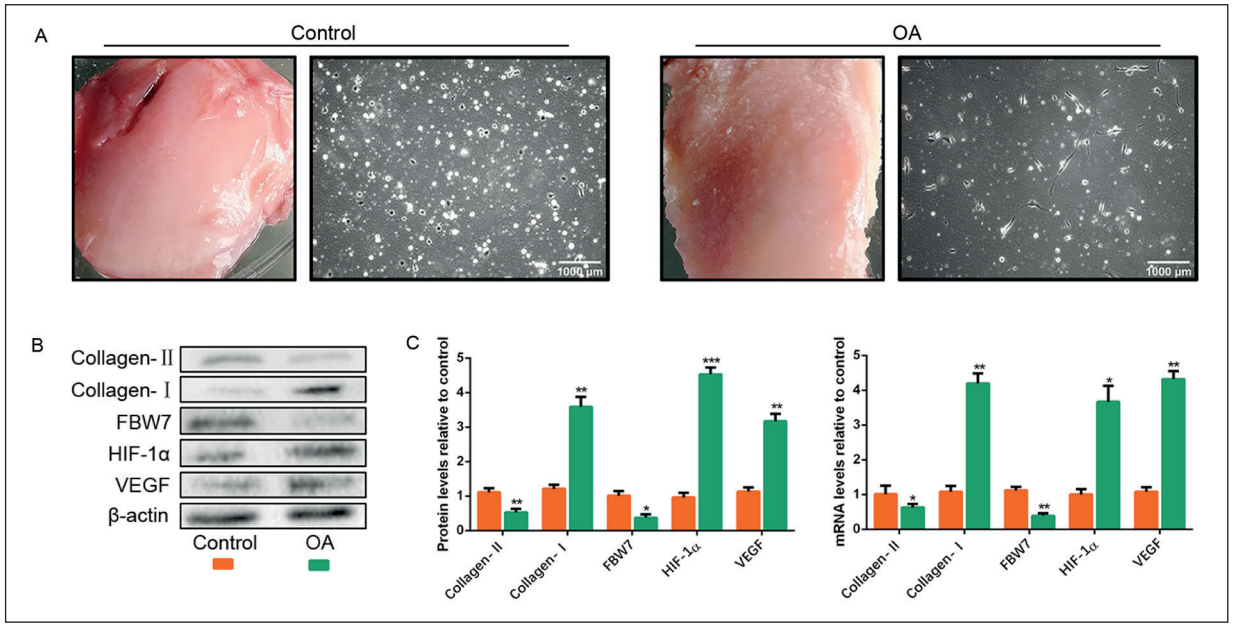

Figure 1. Levels of FBW7, HIF-1α, and VEGF in OA cartilage from patients. **A,** Pictures of the cartilage and the CHs isolated from the cartilage, separately (magnification: 400×). **B,** The protein levels of collagen II, collagen I, FBW7, HIF-1α, and VEGF were determined by WB and (**C**) quantification analysis. **D,** mRNA levels of collagen II, collagen I, FBW7, HIF-1α, and VEGF were determined by RT-PCR. The values are mean ± SD of three independent experiments. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control).

the oval and longer shape. The collagen II synthesized by CH was decreased, and collagen I as a hypertrophic marker of CH was increased in OA cartilage compared with the control both in protein and mRNA levels (Figure 1B-1D). In addition, we observed a significant reduction of FBW7 expression in OA condition compared with the controls, along with a rise of HIF-1α and VEGF expression (Figure 1B-1D). The data indicated that the levels of FBW7, HIF-1α, and VEGF were possibly related to the progress of OA.

Levels of FBW7, HIF-1α, and VEGF in IL-1β Treated CHs In Vitro

To confirm the levels of FBW7, HIF-1α, and VEGF changes in IL-1β-induced CHs degeneration model, we cultured CHs with 5 ng/ml IL-1β from 1 day to 3 days. The cells without treatment were set as a control group. The shape of CH changed from round to long and protrusive with the prolonged stimulation time (Figure 2A). The mRNA expression of chondrogenic genes, such as collagen II, aggrecan, and SOX-9 were significantly decreased under the treatment of IL-1β, especially 3 days later compared with the control. Besides, CHs de-differentiated makers, such as collagen I and Runx-2, were increased, resulting from IL-1β treatment, especially 3 days later (Figure 2B). The result of CCK-8 suggested the viability of CHs was also affected due to IL-1β (Figure 2C). All data above showed a negative effect of IL-1β that caused CHs degeneration. We further determined endogenetic and exogenetic FBW7, HIF-1α, and VEGF levels using qRT-PCR and ELISA methods. Both of them indicated that IL-1β suppressed FBW7 but promoted HIF-1α and VEGF expression with a time-dependent manner (Figure 2D, 2E). These results suggest the degeneration of CHs affects FBW7, HIF-1α, and VEGF expression.

Suppression of HIF-1α Results in a Reduction of VEGF and Protects IL-1β-Induced CHs Degeneration In Vitro

To determine the role of HIF-1α in the regulation of VEGF in the IL-1β-induced CHs degeneration *in vitro*, two concentrations of 2-Me was used to co-culture with IL-1β for the suppression of HIF-1α expression. The IF staining showed that 2-Me inhibited HIF-1α expression and contributed to increasing the collagen II in a dose-dependent, especially under the IL-1β stimulation (Figure 3A, 3B and 3C). Western blot result indicated 2-Me also resulted in a decrease

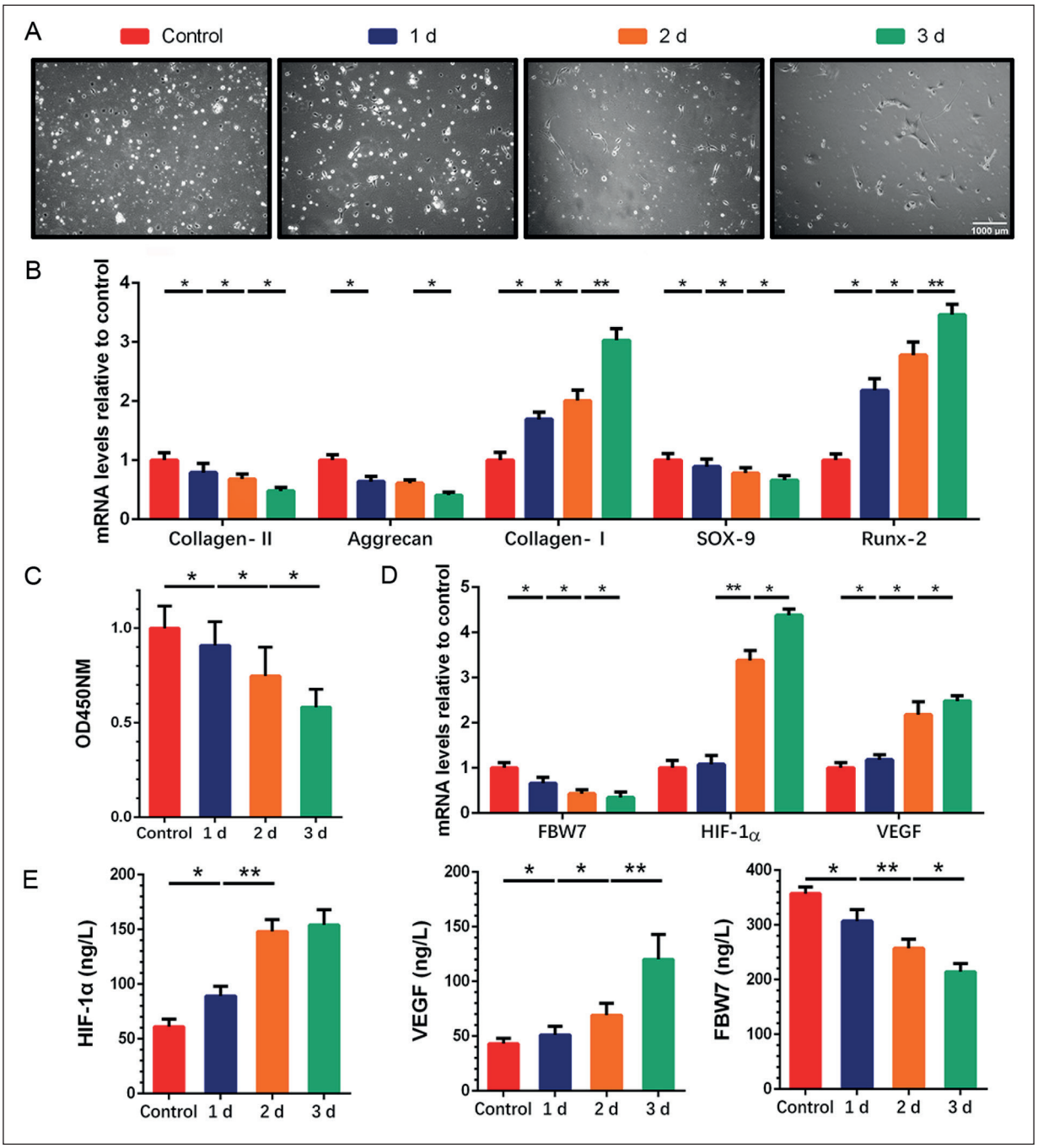

Figure 2. Levels of FBW7, HIF-1α, and VEGF in IL-1β treated CHs. CHs isolated from the control cartilage were cultured with IL-1β (5 ng/mL) form 1 d to 3 d. **A,** Pictures of CHs after stimulation (magnification: 400×). **B,** The mRNA expression levels of collagen II, aggrecan, collagen I, SOX-9, and Runx-2 were assayed by qRT-PCR. **C,** Cell viability was measured by CCK-8 assay. **D,** The mRNA expression levels of FBW7, HIF-1α, and VEGF were assayed by qRT-PCR. **E,** The levels of FBW7, HIF-1α, and VEGF in the supernatant of the culture medium was determined by ELISA. The values are mean ± SD of three independent experiments. (*$p < 0.05$, **$p < 0.01$).

of VEGF expression, and with a dose-dependent in the condition of IL-1β treated compared with the control group (Figure 3D, 3E). CCK-8 assay demonstrated that 2-Me protected the CHs viability under the negative function of IL-1β (Figure 4A). Apart from these, we also measured the mRNA expression of collagen II, aggrecan, SOX-9, collagen I, Runx-2, FBW7, HIF-1α, and

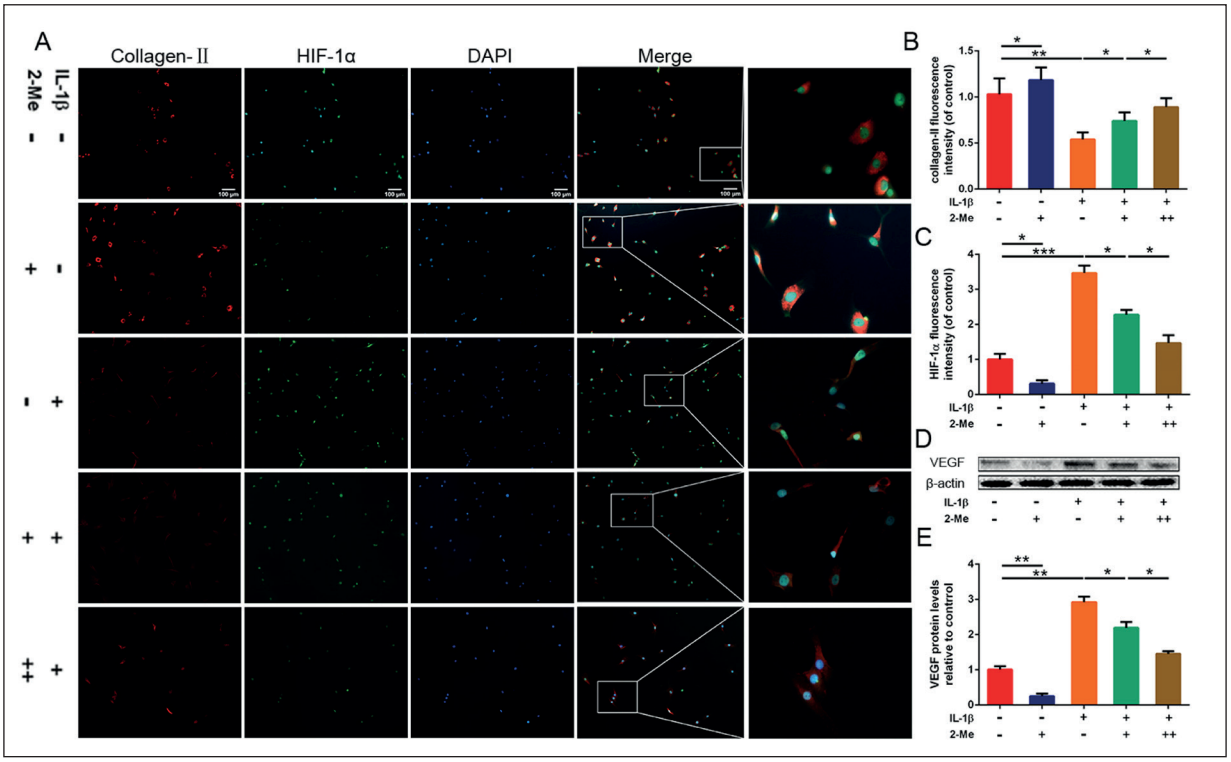

Figure 3. 2-Me suppressed VEGF expression and protected IL-1β-induced CHs degeneration. CHs were pretreated with IL-1β (5 ng/mL) for 3 d and treated with 2-Me (10 μM) for another 24 h. **A,** The protein expression level of collagen II and HIF-1α were determined by IF (magnification: 400×) (**B, C**,) quantification analysis. **D,** The protein expression level of VEGF was determined by WB and (**E**) quantification analysis. The values are mean ± SD of three independent experiments. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

VEGF *via* RT-PCR, and the quantitative result was shown as Figure 4B, 4C, and 4D. These data suggested the inhibitory effect of HIF-1α in turned a suppression of VEGF and a protective function to CHs.

FBW7 Regulates HIF-1α/VEGF Pathway in IL-1β Treated CHs In Vitro

To determine the function of FBW7 in the regulation of HIF-1α/VEGF pathway in the IL-1β-induced CHs degeneration *in vitro*, we mediated the FBW7 gene expression by transfecting with siRNA or plasmid targeting FBW7. As shown in Figure 5A-5C, IF showed that FBW7 deficiency did not affect the collagen II expression, but that FBW7 overexpression contributed to the synthesis of collagen II even under the IL-1β stimulation. We determined the protein levels of HIF-1α and VEGF by WB, the data indicated that FBW7 overexpression led to the decrease of HIF-1α and VEGF expression, and FBW7 downregulation caused an increase of HIF-1α and VEGF expression compared with the control group (Figure 5D, 5E, 5F). CCK-8 assay demonstrated that FBW7 overexpression protected the CHs viability under the negative function of IL-1β (Figure 6A). Finally, we measured the mRNA levels of aggrecan, Runx-2, SOX-9, collagen I, collagen II, FBW7, HIF-1α, and VEGF *via* qRT-PCR, and the quantitative result was shown as Figure 6B, 6C, and 6D. These results indicated the silencing of FBW7 caused an upregulation of HIF-1α and VEGF; however, the overexpression of FBW7 suppressed the HIF-1α/VEGF pathway in the IL-1β-induced CHs degeneration. FBW7 is a protective factor in the metabolism of CHs, and it can regulate the HIF-1α/VEGF pathway under IL-1β treatment.

Discussion

In the progress of OA, cartilage suffers from hypoxia, mechanical stress, and local inflammation, and the expression of HIF-1α is increased to regulate the activity and metabolism of CHs through the expression of downstream factors,

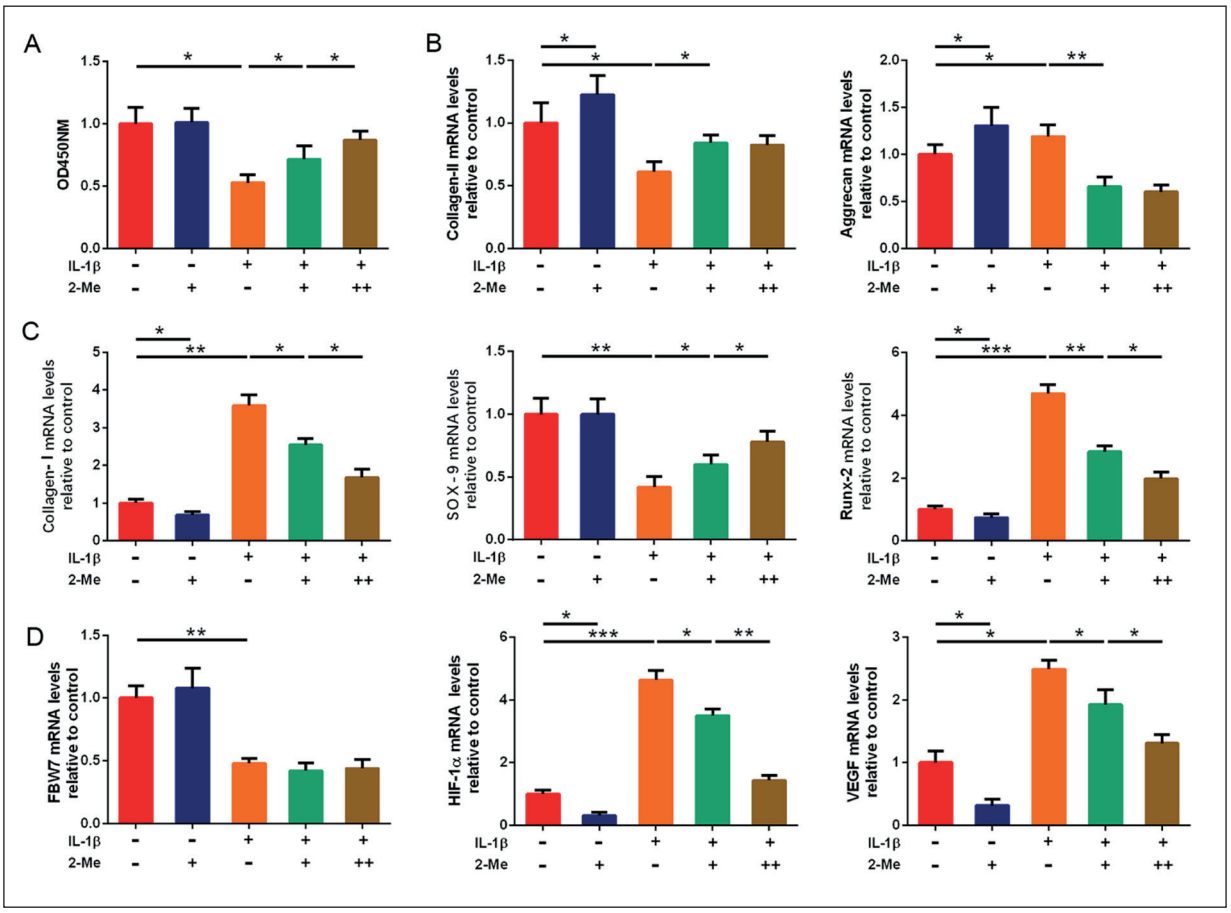

Figure 4. 2-Me suppressed VEGF expression and protected IL-1β-induced CHs degeneration. CHs were pretreated with IL-1β (5 ng/mL) for 3 d and treated with 2-Me (10 μM) for another 24 h. **A,** Cell viability was measured by CCK-8 assay. The mRNA levels of (**B**) collagen II, aggrecan, (**C**) collagen I, SOX-9, Runx-2, (**D**) FBW7, HIF-1α, and VEGF were assayed by qRT-PCR. The values are mean ± SD of three independent experiments. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

such as VEGF[21]. In this study, we found that the amount of HIF-1α and VEGF was increased from OA cartilage compared with the healthy cartilage. Meanwhile, the expression of FBW7 significantly decreased. Like the previous studies, the progress of OA accompanies a changed FBW7, HIF-1α, and VEGF level, each of which has been confirmed to participate in the development of OA. However, the relationship between FBW7 and HIF-1α/VEGF in the metabolism of CHs to the effect of OA is still unknown. Chronic inflammation results in symptoms and progression of OA. Oxidative stress, inflammatory, and matrix catabolic processes in CHs. The complexity of inflammation responses involves repair responses, autophagy, oxidative stress, and matrix catabolic processes in CHs[22]. FBW7 has been reported to have the ability to regulate the inflammatory responses in various cells[23,24]. FBW7 enhances the suppression of NF-κB in the 1,25-vitamin D signaling of colon carcinoma, prostate cancer cells, and neck squamous carcinoma cell lines[25]. Besides, FBW7 plays an essential role in the cerebellar development, and FBW7 deficiency affects the cerebellar size, Purkinje cell number, and axonal arborisation[26]. Whereas, the research about the role of FBW7 in CHs inflammation is limited.

CHs were isolated from the healthy cartilage and used IL-1β as an inducer of inflammatory factor resulting in the degeneration of CHs. We observed that CHs dedifferentiated into fibrotic and hypertrophic CHs under the stimuli of IL-1β with an increased expression of collagen I and Runx-2 and decreased expression of collagen II, aggrecan, SOX-9. Additionally, the levels of FBW7, HIF-1α, and VEGF was reduced both in the CHs and the supernatant from the secretion

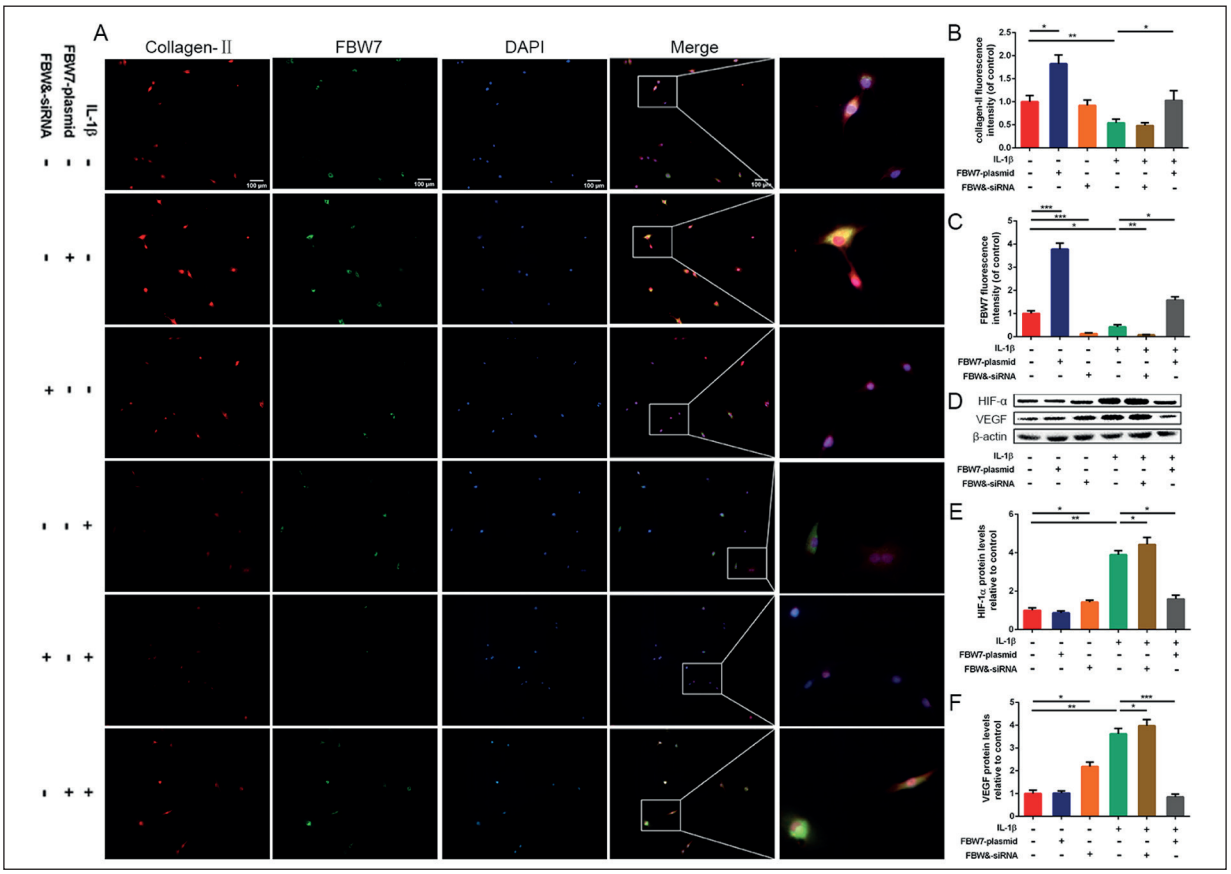

Figure 5. FBW7 suppressed HIF-1α/VEGF pathway and protected IL-1β-induced CHs degeneration. CHs were pretreated with IL-1β (5 ng/mL) for 3 d, and transfected with siRNA or plasmid targeting FBW7 for 12 h. **A,** The protein expression level of collagen II and FBW7 were determined by IF (magnification: 400×) (**B, C**) quantification analysis. **D,** The protein expression level of HIF-1α and VEGF were determined by WB and (**E, F**) quantification analysis. The values are mean ± SD of three independent experiments. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

of CHs. Therefore, we believe that FBW7, HIF-1α, and VEGF played a role in the degeneration of CHs both *in vivo* and *in vitro*, which was also mentioned in the previous study by Pfander et al[18]. To indicate the existence of the HIF-1α/VEGF pathway in the IL-1β treated CHs, we used a classical HIF-1α inhibitor to treat CHs and gained almost a 30% inhibition efficiency. The suppression of HIF-1α contributed a protective effect to CHs in viability, chondrogenic gene expression, but no significant difference to FBW7. Notably, the level of VEGF was decreased as the reduction of HIF-1α with a dose-dependent. Similarly, Cheng et al[27] found that HIF-1α/VEGF signaling inhibits the differentiation of articular cartilage progenitors. Miao et al[28] elucidated fatty acid-induced proinflammatory cytokine activates of HIF1-α pathway and stabilizes VEGF in mice CHs. VEGF has chemotactic effects on monocytes, enhances fibroblast proliferation, and promotes the secretion of inflammatory mediators, which forms a positive feedback regulation network and further encourages the development of OA[29]. All studies prove that not only hypoxia but also inflammation can activate the expression of HIF1-α in the degeneration of CHs along with an increased level of VEGF.

Finally, to determine the role of FBW7 in the HIF-1α/VEGF pathway in CHs, we regulated the FBW7 expression in IL-1β induced CHs by silencing and overexpressing FBW7 gene. Li et al[30] found that FBW7 suppresses the HIF-1α axis in the inhibition of colorectal cancer metastasis. Flügel et al[14] reported that FBW7 degrades HIF-1α in the regulation of cell migration, angiogenesis, and growth. In our research, IL-1β suppressed the expression of FBW7 but increased

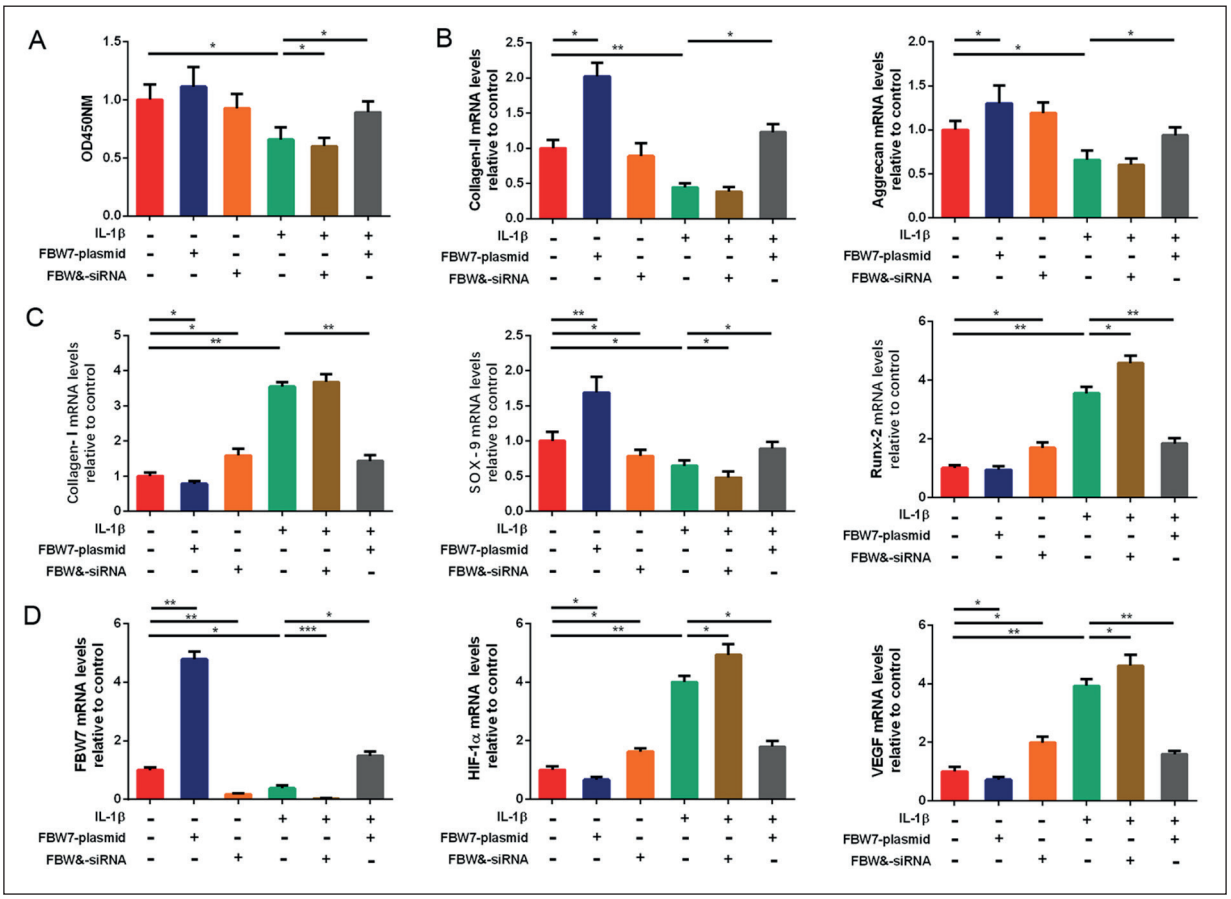

Figure 6. FBW7 suppressed HIF-1α/VEGF pathway and protected IL-1β-induced CHs degeneration. CHs were pretreated with IL-1β (5 ng/mL) for 3 d, and transfected with siRNA or plasmid targeting FBW7 for 12 h. **A,** Cell viability was measured by CCK-8 assay. The mRNA levels of (**B**) collagen II, aggrecan, (**C**) collagen I, SOX-9, Runx-2, (**D**) FBW7, HIF-1α, and VEGF were assayed by RT-PCR. The values are mean ± SD of three independent experiments. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

HIF-1α expression; in addition, the upregulation of FBW7 resulting in the reduction of HIF-1α, and downregulation of FBW7 caused a higher level of HIF-1α. Furthermore, the VEGF level always turned out to be positively related to HIF-1α expression. IL-1β induced CHs degeneration in different aspects. However, the overexpression of FBW7 partly conversed the negative effect of IL-1β brought to CHs, and the downregulation of FBW7 aggravated the degenerated phenotype caused by IL-1β, which is potentially related to the HIF1-α/VEGF pathway.

Conclusions

In brief, the role of HIF-1α and VEGF in OA is ambiguous under different circumstances, such as hypoxia, oxidative stress, and inflammation. Despite this, our study, for the first time, confirmed that FBW7 regulates the HIF1-α/VEGF pathway participating in the progress of IL-1β induced CHs degeneration *in vitro*, which is meaningful to the pathology of OA. The novelty of this study lies in the upregulation of FBW7 suppresses HIF1-α/VEGF pathway and protects the stability of the chondrogenic phenotype. Since FBW7 recognizes and binds to target proteins and participates in the ubiquitin-proteasome pathway, in the next experiment, we will continue to explore the specific mechanism of whether FBW7 regulates HIF-1α expression through ubiquitin-proteasome or another pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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