Life Sciences 218 (2019) 147–152

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

USP14-mediated IκBα degradation exacerbates NF-κB activation and IL-1β-stimulated chondrocyte dedifferentiation

Ming Li[a,*], Jianqiang Zhao[b], Liang Jia[c]

[a] Department of Orthopaedic, the Second Hospital of Hebei Medical University, Shijiazhuang, Hebei 050005, China
[b] Department of Orthopaedic, Longyao County Hospital, Longyao County, Hebei 055350, China
[c] Rehabilitation Department, the Third Hospital of Xingtai City, Xingtai City, Hebei 054000, China

ARTICLE INFO

Keywords:
USP14
IκBα
NF-κB
IL-1β
Chondrocyte dedifferentiation

ABSTRACT

Osteoarthritis (OA) is an inflammatory joint disease. USP14, a deubiquitinating enzyme critical for ubiquitin-mediated proteasomal protein degradation, is implicated in inflammation regulation. However, its role and mechanism in OA are poorly understood. Here, we report that USP14 is upregulated in OA articular cartilage as well as in chondrocytes treated with IL-1β in vitro. USP14 upregulation depends on NF-κB pathway activation, since inhibition of this pathway by ACHP, a selective inhibitor of IKK-β, abolishes USP14 upregulation. We further show that USP14 in turn exacerbates NF-κB activation through promoting IκBα deubiquitination and degradation. Functionally, USP14 aggravates the dedifferentiation effect of IL-1β on chondrocytes, and NF-κB inhibition remarkably reverses this effect, highlighting an important role of NF-κB in mediating USP14 function. Collectively, our data reveal a previously unidentified feed-forward loop driven by USP14 and NF-κB pathway in promoting the dedifferentiation effect of IL-1β on chondrocytes. This mechanism might offer a useful hint for OA intervention.

1. Introduction

Osteoarthritis (OA) is the most common arthritis and is mainly characterized by the progressive degeneration of articular cartilage and bone remodeling [1]. Although, the etiology of this debilitating disease is not yet fully clarified, several predisposing factors, such as genetic background, obese, aging, abnormal loading and prior joint injuries, have been associated with OA pathogenesis and articular destruction [2]. However, to date, the effective therapies targeting the processes of OA pathogenesis are still lacking [3]. In past decade, a growing body of evidence has indicates that inflammation plays a critical role in OA pathogenesis, thus providing novel therapeutic approaches for modifying the progression of OA [4]. Among the regulators of inflammation, NF-κB attracts much attention as a potential target for OA treatment, since its activation increases the expression of an array of genes capable of inducing cartilage destruction, leading to OA initiation and progression [5].

NF-κB exists as an inactive form bound to IκB in the cytosol under unstimulated physiological conditions. Oppositely, upon stimulation of a variety of chemical and mechanical signals, such as proinflammatory cytokines, IκBα will be phosphorylated by IκB kinases (IKKs), resulting in its degradation through the ubiquitin-proteasome system, which is followed by the free translocation of NF-κB into the nucleus and expression induction of a wide-spectrum of molecules, such as cytokines, chemokines and proteases, etc., [6–8]. By so doing, NF-κB and its downstream effector molecules exert their functional roles in OA pathogenesis.

In addition to inflammation, OA is associated with the disruption of chondrocyte homeostasis and loss of differentiated phenotype, i.e., dedifferentiation (Speichert et al., 2018). IL-1β is a proinflammatory cytokine overexpressed in OA articular cartilage and also a critical mediator of cartilage destruction-associated processes, including the induction of chondrocyte dedifferentiation [9]. Nonetheless, the regulatory mechanisms underlying IL-1β-induced chondrocyte dedifferentiation are not fully understood.

USP14, the ubiquitin-specifc protease 14, is a deubiquitinase that controls the ubiquitin-mediated proteasomal degradation of proteins [10]. The abnormality of USP14 has been implicated in cancer [11], neurological disorders [12], and aging [13]. Recently, one study reported that USP14 overexpression in lung epithelial cells reduces IκB protein level and increases cytokine release [14]. Besides, another study showed that USP14 affects LPS-dependent NF-κB activation [15]. These

* Correspondence author at: Department of Orthopaedic, the Second Hospital of Hebei Medical University, No. 215 of Heping west road, Shijiazhuang, Hebei 050005, China.
E-mail address: limshhmu@163.com (M. Li).

https://doi.org/10.1016/j.lfs.2018.12.014
Received 11 October 2018; Received in revised form 2 December 2018; Accepted 10 December 2018
Available online 11 December 2018
0024-3205/

reports suggest that USP14 may have a role in the regulation of NF-κB-dependent inflammation. However, the regulation, function and underlying mechanism of USP14 involved in OA pathogenesis are not determined. In this study, we report a feed-forward loop existing between USP14 and NF-κB, and its role in IL-1β-induced chondrocyte dedifferentiation.

2. Materials and methods

2.1. Antibodies and reagents

The antibodies and reagents were purchased from the following sources: anti-USP14 (Invitrogen, PA5-30300), anti-β-Actin (Santa Cruz, sc-47778), anti-phospho-p65 (Ser536) (Cell Signaling, 3033), anti-p65 (Santa Cruz, sc-372), anti-IκBα (Cell Signaling, 4814), anti-COL1 (Santa Cruz, sc-59772), anti-COL2 (Santa Cruz, sc-52658), anti-Cox-2 (Santa Cruz, sc-1745), anti-ubiquitin (abcam, ab7780), goat anti-rabbit IgG-HRP (abcam, ab6721), goat anti-mouse IgG-HRP (abcam, ab6789), ACHP (Bio-Techne, 4547), MG-132 (Sigma, M8699), recombinant mouse IL-1β (R&D Systems, 401-ML).

2.2. Human articular cartilage sampling

Human OA articular cartilage of knee joints were sampled from 18 OA patients who received knee replacement at the Second Hospital of Hebei Medical University and met the American College of Rheumatology classification criteria for OA diagnosis [16]. Normal articular cartilage of knee joints were sampled from 22 patients with amputation from accidents. Patients in two groups were similar in age and sex. The informed consent was obtained from each patient, and the sampling procedure conforms to the protocols approved by the Medical Ethical Committee of the Second Hospital of Hebei Medical University.

2.3. Mice, chondrocyte isolation and culture

Twelve-week-old male C57BL/6 mice were used in this study and maintained under specific pathogen-free conditions. All experimental procedures complied with terms approved by the Institutional Animal Care and Use Committees of the Second Hospital of Hebei Medical University. Mouse primary articular chondrocytes were isolated and cultured as previously documented [17]. Briefly, mouse cartilage tissues were dissected from knee joint surface and rinsed in sterile PBS. Cartilage tissues were cut into small pieces with a sterile surgical blade in a biosafety cabinet, and incubated in 1.5 mg/ml pronase solution (Sigma) for 2 h at 37 °C and then digested in 2 mg/ml collagenase II solution (Sigma) overnight at 37 °C with agitation. The cell suspensions were filtered with a 70-μm size strainer (BD) and seeded with a density of 20,000 cells/cm^2 in a monolayer. Cells were cultured in DMEM/F12 medium (Invitrogen) supplemented with 10% FBS (Invitrogen), 1% penicillin-streptomycin (Invitrogen) and 1% L-glutamine (Invitrogen), and maintained at 37 °C in a humidified incubator with 5% CO_2. Cells were passaged with a 1:4 ratio when reached 80% confluence.

2.4. Treatment and overexpression in chondrocytes

When chondrocytes reached nearly 50% confluent, titrated concentrations of IL-1β or 2 μM ACHP were added for treatment. For overexpressing USP14 in chondrocytes, the coding sequence of mouse USP14 was cloned into the pCAG vector, and chondrocytes were transfected with pCAG-USP14 or vector control using Cytofect™ Cell Line Transfection Kit (Cell Applications, TF104K). Chondrocytes were harvested 72 h post-overexpression and confirmed by Western blot or qRT-PCR analysis.

2.5. RT-qPCR analysis

The total mRNA of chondrocytes were isolated using RNeasy Kit (Qiagen), and reversely transcripted into cDNA using RevertAid First strand cDNA Synthesis kit (ThermoFisher Scientific) according to manufacturer's instructions. mRNA quantification was performed using SYBR Green PCR Master Mix reagent kit (Applied Biosystems) and ABI7500 instrument (Applied Biosystems). *ACTB* was used as an endogenous control and data were analyzed using the comparative Ct method. Primer pairs for human or mouse genes are available upon request.

2.6. Western blot analysis

Chondrocytes were homogenized in RIPA lysis and extraction buffer (GBiosciences, 786–489) supplemented with protease inhibitor (Sigma) on ice for 20 min. Lysates were then centrifuged at 10000 × *g* for 20 min at 4 °C. Supernatants were collected and protein concentration was determined by BCA kit (Pierce, 23,225). Protein samples were denatured in SDS loading buffer for 10 min at 95 °C. Western blot was performed as described previously [18]. In brief, equal amount of proteins were loaded and subjected to 10% or 12% SDS-PAGE, followed by transfer onto PVDF membranes (Millipore). PVDF membranes were blocked, incubated sequentially with primary and secondary antibodies, and protein bands were detected with ECL reagent kit (ThermoFisher Scientific). The band intensity was quantified using ImageJ software.

2.7. Immunoprecipitation

The whole cell lysates of chondrocytes were obtained with IP lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% deoxycholate and 1% protease inhibitors). 50 μl samples were aspirated as input and stored at −80 °C, and the residual cell lysates were incubated with primary antibodies prior to being precipitated by protein A/G agarose overnight at 4 °C. Protein A/G agarose was washed 3 times with IP lysis buffer and immunoprecipitates were eluted with 1 × SDS loading buffer and denatured by boiling for 5 min. At last, the input and IP products were analyzed by Western blot.

2.8. Statistical analysis

Statistical analysis was performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA). All data are expressed as mean ± s.d. Statistical significance was calculated by unpaired two-tailed Student's *t*-test. $P < 0.05$ and $P < 0.01$ are considered statistically significant.

3. Results

3.1. USP14 is upregulated in OA articular cartilage and chondrocytes exposed to IL-1β

The expression change of USP14 occurs between normal and OA articular cartilage is unknown. To address it, we first compared its transcript level in clinical articular cartilage specimens from normal and OA knee joints. The result of qRT-PCR analysis showed that compared with that of normal control, the mRNA level of USP14 was significantly higher in OA cartilage [normal ($n = 22$) vs. OA ($n = 18$); $P < 0.01$] (Fig. 1A). Moreover, similar to this result, the protein level of USP14 was also found to be upregulated in OA cartilage (Fig. 1B). OA is featured by a catabolic and inflammatory joint environment, where proinflammatory cytokines, for example IL-1β, are excessively secreted and function as proarthritic stimuli [19]. We found that similar to UPS14 upregulation detected in OA cartilage, its expression level was induced by IL-1β treatment at both mRNA (Fig. 1C) and protein (Fig. 1D) levels in cultured mouse primary chondrocytes in vitro. Taken

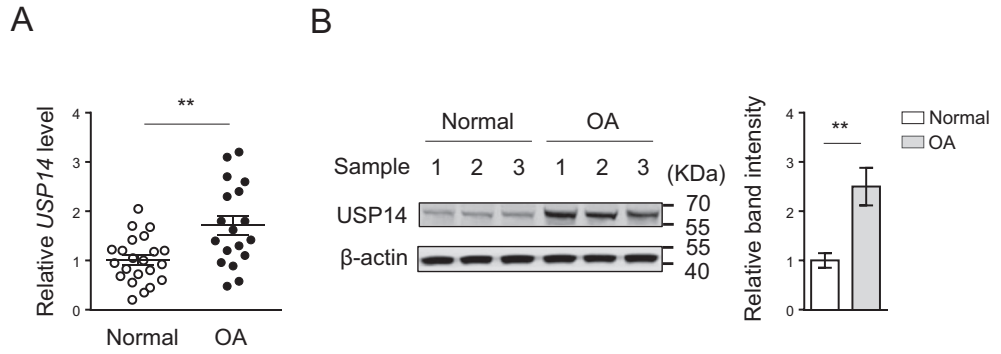
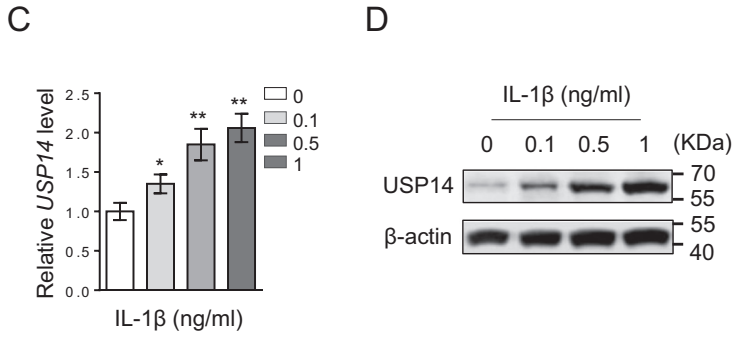


Fig. 1. USP14 is upregulated in OA articular cartilage and chondrocytes treated with IL-1β.
(**A–B**) mRNA level (A) and protein level (B) of USP14 in human knee cartilage from healthy subjects (normal) ($n = 22$) and OA patients ($n = 18$). Results relative to normal group are shown (**C–D**) mRNA level (C) and protein level (D) of USP14 in primary mouse articular chondrocytes treated with or without increasing concentrations of IL-1β for 24 h ($n = 3$). Results relative to vehicle are shown. β-Actin was used as a reference or loading control. Data are mean ± s.d. Student's *t*-test. $^{*}P < 0.01$; $^{**}P < 0.01$.



together, these data indicate that USP14 is upregulated in knee OA cartilage and also imply that this might be due to IL-1β stimulation in focal sites.

3.2. USP14 upregulation depends on NF-κB pathway activation

To further seek the regulatory mechanism by which IL-1β induces USP14 expression, we tended to focus on NF-κB, since it's a major intracellular regulator responding to the stimulation of proinflammatory cytokines [20]. In order to test the possible effect of NF-κB on the regulation of USP14 expression, ACHP, a small molecule specifically inhibiting IKKβ, was used to inhibit NF-κB activation and downstream effect [21]. As shown in Fig. 2A–B, at both the mRNA and protein levels in chondrocytes, the induced expression of USP14 by IL-β treatment was abolished by ACHP treatment, which efficiently inhibited NF-κB activation, as evidenced by the recovered phosphorylation level of p65. These results suggest that IL-1β induces USP14 expression in chondrocytes through the activation of NF-κB, at least in vitro.

3.3. USP14 exacerbates NF-κB activation through promoting IκBα deubiquitination and degradation

USP14 has been implicated in the regulation of LPS-induced NF-κB activation [15]. However, whether USP14 regulates NF-κB in chondrocytes is still unknown. We found that USP14 overexpression exacerbated the activation of NF-κB induced by IL-1β, as shown by phosphorylation level of p65, however, in chondrocytes unstimulated with IL-1β, USP14 overexpression had no similar effect (Fig. 3A), indicating that USP14 promotes IL-1β-induced NF-κB activation in chondrocytes. A previous study has shown that USP14 overexpression reduces IκB protein in lung epithelial cells [14]. IκBα is an essential negative regulator of NF-κB [22], therefore, to elucidate how USP14 regulates NF-κB activation, we tested the potential interaction between USP14 and IκBα in chondrocytes. The result showed that IκBα was co-immunoprecipitated by USP14 antibody in chondrocyte lysates, compared with IgG, and this co-immunoprecipitation was intensified when USP14 was overexpressed (Fig. 3B), pointing to an interaction between these two proteins. USP14 possesses deubiquitinating activity [23]. We

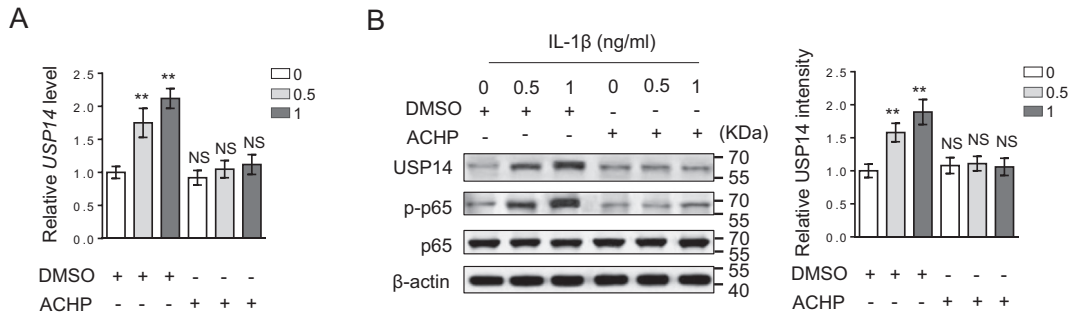


Fig. 2. USP14 upregulation is dependent on NF-κB activation.
(A–B) Relative mRNA level of USP14 (A) and protein level (B) in primary mouse articular chondrocytes treated with or without increasing concentrations of IL-1β in the presence or absence of 2 μM ACHP as indicated for 24 h (n = 3). DMSO was used as a treatment control. β-Actin was used as a reference or loading control. Data are mean ± s.d. Student's *t*-test. $^{**}P < 0.01$; NS, not significant.

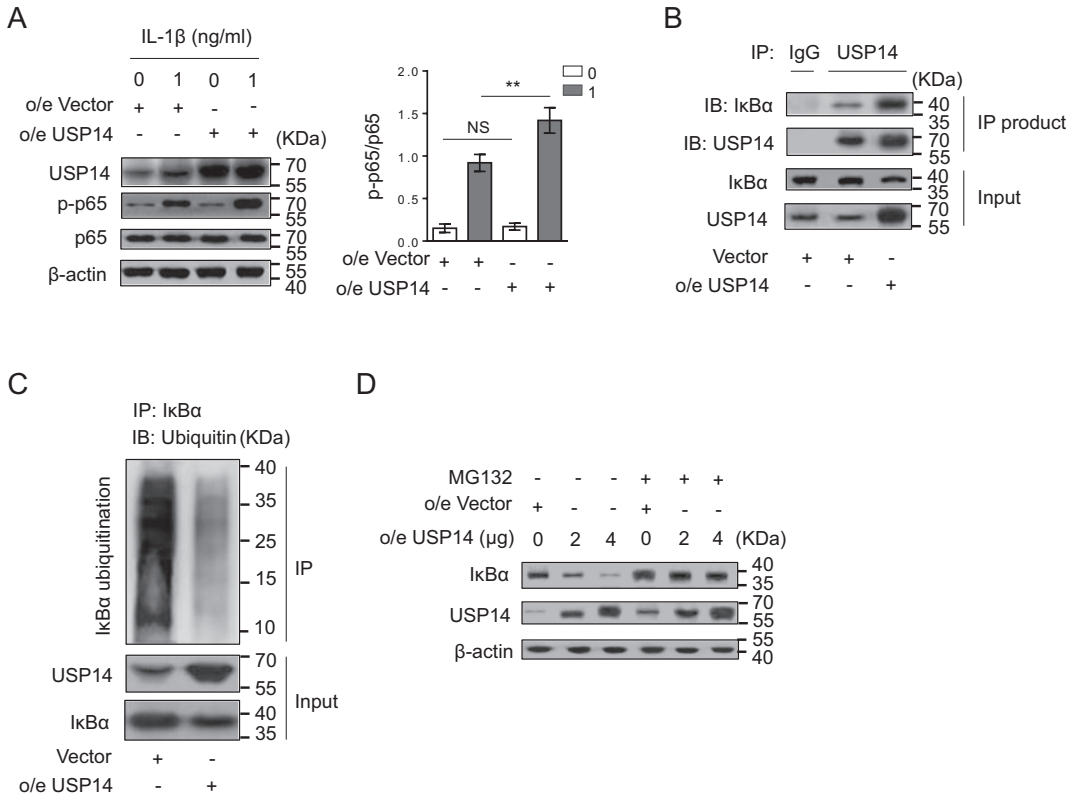


Fig. 3. USP14 ubiquitinates IκBα and exacerbates NF-κB activation
(A) Protein level in chondrocytes stably expressing vector or USP14 and treated with or without 1 ng/ml IL-1β for 24 h. p-p65/p65 is shown (right). (**B**) Lysates of chondrocytes stably expressing vector or USP14 were immunoprecipitated with IgG or USP14 antibody. The protein level was analyzed by Western blotting. (**C**) Lysates of chondrocytes stably expressing vector or USP14 were immunoprecipitated with IκBα antibody. The protein level was analyzed by Western blotting. (**D**) Chondrocytes transfected with increasing amount of USP14 plasmid were treated with or without 20 μM MG132. Protein level was analyzed by Western blotting. Experiments were conducted at least 3 times independently and representative images were shown. Data are mean ± s.d. Student's *t*-test. **,$P < 0.01$; NS, not significant.

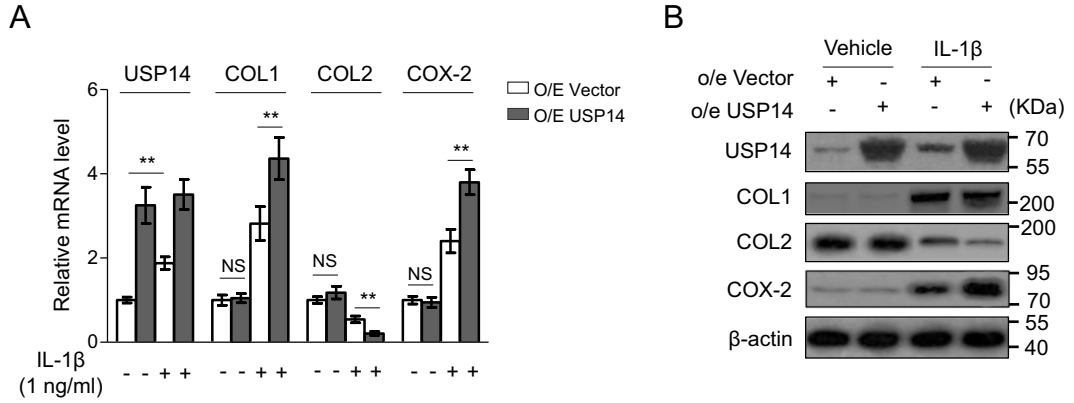


Fig. 4. USP14 aggravates the dedifferentiation effect of IL-1β on chondrocytes in vitro.
(**A**–**B**) mRNA level (**A**) and protein (**B**) level of USP14, COL1, COL2 and COX-2 in chondrocytes stably expressing vector or USP14 and treated with or without 1 ng/ml IL-1β for 24 h. β-Actin was used as a reference or loading control. Results relative to control group are shown. Experiments were conducted at least 3 times independently. Data are mean ± s.d. Student's *t*-test. **$P < 0.01$; NS, not significant.

next found that USP14 overexpression reduced IκBα ubiquitination (Fig. 3C). Together, these results indicate that USP14 binds with IκBα and mediates its deubiquitination in chondrocytes. USP14 reportedly enhances proteasome degradation of its target proteins [24]. To test whether USP14 promotes IκBα degradation via proteasome, we treated chondrocytes with MG132, a widely used proteasome inhibitor, to block proteasome-mediated protein degradation. The result showed that USP14 overexpression increasingly reduced IκBα protein level, however, this effect vanished in the presence of MG132 treatment (Fig. 3D), illustrating that USP14 accelerates proteasome-mediated degradation of IκBα. Collectively, these findings suggest that USP14 exacerbates IL-1β-induced NF-κB activation in cultured chondrocytes, which is related to the increased deubiquitination and accelerated proteasome-mediated degradation of IκBα.

3.4. USP14 aggravates the dedifferentiation effect of IL-1β on chondrocytes

IL-1β can induce the dedifferentiation of chondrocytes [25], which mimics the pathologic change of a loss of differentiated chondrocyte phenotype observed in OA patients [26]. It has been demonstrated that IL-1β-induced chondrocyte dedifferentiation is reflected by expression changes of biomarkers, such as decreased expression of type II collagen (COL2) and induced expressions of type I collagen (COL1) and cyclooxygenase 2 (COX-2) [27]. As shown by above results, USP14 is involved in IL-1β effect on chondrocytes, next, to learn more about the functional role of USP14, we tested whether it affects IL-1β-induced chondrocyte dedifferentiation. Expectedly, IL-1β treatment induced chondrocyte dedifferentiation, as shown by increased expressions of COL1 and COX-2, and concomitant decreased expression of COL2, both at mRNA (Fig. 4A) and protein (Fig. 4B) levels. Moreover, when USP14 was overexpressed in IL-1β-treated chondrocytes, these expression changes were further exacerbated (Fig. 4A-B), however, USP14 overexpression alone had no similar effect. Therefore, USP14 may participate in aggravating IL-1β-induced chondrocyte dedifferentiation.

3.5. Inhibition of NF-κB activation diminishes USP14 overexpression-induced enhancement of IL-1β dedifferentiation effect on chondrocytes

Many proarthritic effects of IL-1β are regulated by NF-κB, including IL-1β-induced chondrocyte dedifferentiation [28,29]. Since USP14 affects IL-1β-dependent NF-κB activation, we then examined whether this contributes to the promotive role of USP14 in chondrocyte dedifferentiation induced by IL-1β. Consistently, under IL-1β treatment, USP14 overexpression exacerbated NF-κB activation and chondrocyte dedifferentiation, as evidenced by increased levels of p65 phosphorylation, COL1 and COX-2, and decreased level of COL2 (Fig. 5A–B, **left half**). Whereas, when treated in combination with ACHP, in pace with the inhibited NF-κB activation, the expression changes of COL1, COX-2 and COL2 were remarkably recovered (Fig. 5A-B**, right half**), suggesting that NF-κB inhibition diminishes the promotive role of USP14 in chondrocyte dedifferentiation induced by IL-1β, and also highlighting the importance of NF-κB activation in regulating the pro-dedifferentiation effect of USP14 on IL-1β-treated chondrocytes.

4. Discussion

Except for a long-standing view of considering OA as a degenerative disease of cartilage, OA is now widely accepted as an inflammatory joint disease, in which chronic and low-grade inflammation, forged by the interplay of innate immune system and inflammatory mediators, play a fundamental role its pathogenesis [4]. Recent studies suggest that a deubiquitinase USP14 is a potential regulator of inflammation and cytokine production [14,15,30]. In our current study, by examining and comparing the clinical specimens between normal and knee OA cartilage, we found that USP14 was upregulated in OA cartilage at both mRNA and protein levels. This not only suggests a transcriptional activation of USP14 under this pathologic condition, but also hints a relevance of USP14 to OA pathogenesis. It is noteworthy that the size of recruited specimens is limited, and preferably, more surveys with larger size of clinical knee OA samples may be needed to strengthen this phenotype. Additionally, whether USP14 shows similar expression change in other anatomic sites with OA and the implicit meaning conferred by USP14 expression change for clinical OA diagnosis are unclear yet. We next noticed that USP14 exhibited analogical upregulation in cultured primary chondrocytes exposed to proinflammatory cytokine IL-1β in vitro. Given the clue that proinflammatory cytokines are upregulated and abundantly present in local OA cartilage [31], we propose that the stimuli derived from proinflammatory cytokines may serve as important factors for inducing USP14 expression in chondrocytes in OA cartilage. Future investigations on examining the correlation between USP14 expression and proinflammatory cytokine production in clinical OA samples may provide more direct evidence for explaining the expression change of USP14 in OA cartilage.

The mechanisms underlying the regulation of USP14 gene expression are barely investigated. Intriguingly, we reveal that the proinflammatory cytokine-induced USP14 upregulation is dependent on the activation of transcriptional factor NF-κB. Whether this regulatory circuitry is exclusively confined to the induction of USP14 gene expression stimulated by proinflammatory cytokine is uncertain. As a transcriptional factor, NF-κB is essential for activating the expressions of > 150 distinct target genes in almost all cell types in response to a broad range of specific physiological conditions, including inflammation, differentiation and survival, etc., [32,33]. Hence, the possibility that USP14 may be a direct target gene activated by NF-κB when chondrocytes are exposed to proinflammatory cytokines cannot be excluded. In addition to this possibility, other layers of mechanisms by which NF-κB may regulate USP14 include histone modification, post-translational modification, co-regulator interaction and synergy with other stimulus-induced transcriptional factors [34]. Further studies elucidating how NF-κB regulates USP14 expression at transcriptional level and beyond may be helpful for advancing our understanding of the control mechanisms

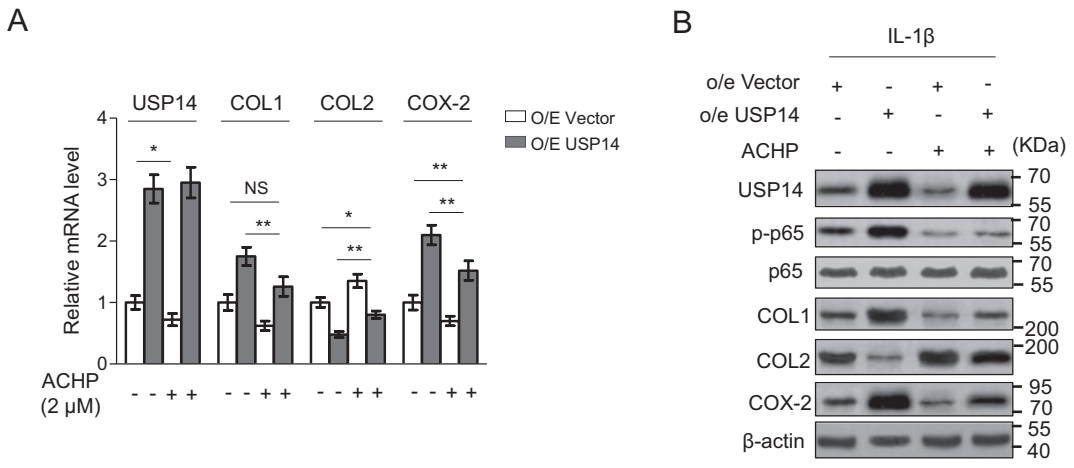


Fig. 5. NF-κB inhibition attenuates enhanced dedifferentiation effect of IL-1β on chondrocytes by USP14 overexpression.
(**A–B**) mRNA level (**A**) and protein level (**B**) of USP14, COL1, COL2 and COX-2 in chondrocytes stably expressing vector or USP14 and treated with 1 ng/ml IL-1β for 24 h in the presence or absence of 2 μM ACHP. β-Actin was used as a reference or loading control. Results relative to control group are shown. Experiments were conducted at least 3 times independently. Data are mean ± s.d. Student's *t*-test. $^{**}P < 0.01$; $^{*}P < 0.05$; NS, not significant.

of USP14 in OA disease. In an opposite direction, we discovered that USP14 in turn exacerbated NF-κB activation through promoting the deubiquitination of IκBα and accelerating its proteasome-dependent degradation, which coincides with a previous study showing that the overexpression of USP14 reduces IκB protein level in lung epithelial cells [14]. Combined with the aforementioned results that NF-κB activation induces USP14 upregulation, this newly unveiled reciprocal regulation between USP14 and NF-κB could be described as a feed-forward loop which is driven by these two molecules. In other words, this regulatory mechanism may result in a mutual promoting effect between USP14 and NF-κB in chondrocytes, at least when stimulated by IL-1β. Whether this applies to other circumstances or not still needs to be proved by solid evidence.

At any rate, in our following study, the reciprocal regulation between USP14 and NF-κB seems to exert certain functional effects on chondrocyte biology, as exemplified by USP14-aggravated dedifferentiation effect of IL-1β on chondrocytes in vitro, which was to a great extent recovered when NF-κB activation was attenuated. The dedifferentiation of chondrocytes is a huge obstacle for cell-based regeneration and repair of destructed articular cartilage in OA patients [35]. We believe that the synergized notorious effect of USP14 and NF-κB on IL-1β-induced dedifferentiation of chondrocytes revealed by this study may play a detrimental role in cartilage regeneration. Moreover, despite the impact of NF-κB pathway, other mechanisms are very likely responsible for the effect of USP14 on IL-1β-induced chondrocyte dedifferentiation, since NF-κB inhibition did not completely recover the effect of USP14 overexpression. Addressing this issue could help us to fully understand the role and mechanism of USP14 involved in the pathophysiology OA and chondrocytes.

In summary, this study associates USP14 expression change with knee OA and uncovers the novel role of USP14 in regulating chondrocyte dedifferentiation, in which a feed-forward loop existing between USP14 and NF-κB may play an important role. According to our findings, targeting NF-κB through USP14 manipulation might be of clinical benefit for OA intervention, especially for cartilage regeneration after destruction.

Disclosure of conflict of interest

None.

References

[1] A. Ioan-Facsinay, M. Kloppenburg, Osteoarthritis: inflammation and fibrosis in adipose tissue of osteoarthritic joints, Nat. Rev. Rheumatol. 13 (2017) 325–326.
[2] A. Mobasheri, M. Batt, An update on the pathophysiology of osteoarthritis, Ann. Phys. Rehabil. Med. 59 (2016) 333–339.
[3] P. Wehling, C. Evans, J. Wehling, W. Maixner, Effectiveness of intra-articular therapies in osteoarthritis: a literature review, Therapeutic Advances in Musculoskeletal Disease, 9 2017, pp. 183–196.
[4] W.H. Robinson, C.M. Lepus, Q. Wang, H. Raghu, R. Mao, T.M. Lindstrom, et al., Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis, Nat. Rev. Rheumatol. 12 (2016) 580–592.
[5] S. Rigoglou, A.G. Papavassiliou, The NF-kappaB signalling pathway in osteoarthritis, Int. J. Biochem. Cell Biol. 45 (2013) 2580–2584.
[6] J.A. DiDonato, M. Hayakawa, D.M. Rothwarf, E. Zandi, M. Karin, A cytokine-responsive IkappaB kinase that activates the transcription factor NF-kappaB, Nature 388 (1997) 548–554.
[7] E. Niederberger, G. Geisslinger, The IKK-NF-kappaB pathway: a source for novel molecular drug targets in pain therapy? FASEB J. 22 (2008) 3432–3442.
[8] K. Taniguchi, M. Karin, NF-kappaB, inflammation, immunity and cancer: coming of age, Nat. Rev. Immunol. 18 (2018) 309–324.
[9] M. Kapoor, J. Martel-Pelletier, D. Lajeunesse, J.P. Pelletier, H. Fahmi, Role of proinflammatory cytokines in the pathophysiology of osteoarthritis, Nat. Rev. Rheumatol. 7 (2011) 33–42.
[10] B.H. Lee, Y. Lu, M.A. Prado, Y. Shi, G. Tian, S. Sun, et al., USP14 deubiquitinates proteasome-bound substrates that are ubiquitinated at multiple sites, Nature 532 (2016) 398–401.
[11] P. D'Arcy, S. Brnjic, M.H. Olofsson, M. Fryknas, K. Lindsten, M. De Cesare, et al., Inhibition of proteasome deubiquitinating activity as a new cancer therapy, Nat. Med. 17 (2011) 1636–1640.
[12] S.V. Todi, H.L. Paulson, Balancing act: deubiquitinating enzymes in the nervous system, Trends Neurosci. 34 (2011) 370–382.
[13] J. Labbadia, R.I. Morimoto, The biology of proteostasis in aging and disease, Annu. Rev. Biochem. 84 (2015) 435–464.
[14] R.K. Mialki, J. Zhao, J. Wei, D.F. Mallampalli, Y. Zhao, Overexpression of USP14 protease reduces I-kappaB protein levels and increases cytokine release in lung epithelial cells, J. Biol. Chem. 288 (2013) 15437–15441.
[15] N. Liu, T. Kong, X. Chen, H. Hu, H. Gu, S. Liu, et al., Ubiquitin-specific protease 14 regulates LPS-induced inflammation by increasing ERK1/2 phosphorylation and NF-kappaB activation, Mol. Cell. Biochem. 431 (2017) 87–96.
[16] R. Altman, E. Asch, D. Bloch, G. Bole, D. Borenstein, K. Brandt, et al., Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association, Arthritis Rheum. 29 (1986) 1039–1049.
[17] J.H. Jonason, D. Hoak, R.J. O'Keefe, Primary murine growth plate and articular chondrocyte isolation and cell culture, Methods Mol. Biol. 1226 (2015) 11–18.
[18] Y. Zhang, J. Jia, S. Yang, X. Liu, S. Ye, H. Tian, MicroRNA-21 controls the development of osteoarthritis by targeting GDF-5 in chondrocytes, Exp. Mol. Med. 46 (2014) e79.
[19] G.M. van Buul, E. Villafuertes, P.K. Bos, J.H. Waarsing, N. Kops, R. Narcisi, et al., Mesenchymal stem cells secrete factors that inhibit inflammatory processes in short-term osteoarthritic synovium and cartilage explant culture, Osteoarthr. Cartil. 20 (2012) 1186–1196.
[20] C. Gasparini, M. Feldmann, NF-kappaB as a target for modulating inflammatory responses, Curr. Pharm. Des. 18 (2012) 5735–5745.
[21] S.C. Gupta, C. Sundaram, S. Reuter, B.B. Aggarwal, Inhibiting NF-kappaB activation by small molecules as a therapeutic strategy, Biochim. Biophys. Acta 1799 (2010) 775–787.
[22] Q. Zhang, M.J. Lenardo, D. Baltimore, 30 Years of NF-kappaB: a blossoming of relevance to human pathobiology, Cell 168 (2017) 37–57.
[23] C.L. Kuo, A.L. Goldberg, Ubiquitinated proteins promote the association of proteasomes with the deubiquitinating enzyme Usp14 and the ubiquitin ligase Ube3c, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E3404-E13.
[24] B.H. Lee, M.J. Lee, S. Park, D.C. Oh, S. Elsasser, P.C. Chen, et al., Enhancement of proteasome activity by a small-molecule inhibitor of USP14, Nature 467 (2010) 179–184.
[25] S. Speichert, K. El Bagdadi, A. Meurer, F. Zaucke, Z. Jenei-Lanzl, Role of noradrenalin in IL-1β-induced chondrocyte dedifferentiation, Osteoarthr. Cartil. 26 (2018) S87-S8.
[26] S.G. Hwang, S.S. Yu, H. Poo, J.S. Chun, c-Jun/activator protein-1 mediates interleukin-1beta-induced dedifferentiation but not cyclooxygenase-2 expression in articular chondrocytes, J. Biol. Chem. 280 (2005) 29780–29787.
[27] B. Ma, J.C. Leijten, L. Wu, M. Kip, C.A. van Blitterswijk, J.N. Post, et al., Gene expression profiling of dedifferentiated human articular chondrocytes in monolayer culture, Osteoarthr. Cartil. 21 (2013) 599–603.
[28] M. Shakibaei, T. John, G. Schulze-Tanzil, I. Lehmann, A. Mobasheri, Suppression of NF-kappaB activation by curcumin leads to inhibition of expression of cyclo-oxygenase-2 and matrix metalloproteinase-9 in human articular chondrocytes: implications for the treatment of osteoarthritis, Biochem. Pharmacol. 73 (2007) 1434–1445.
[29] M. Shakibaei, G. Schulze-Tanzil, T. John, A. Mobasheri, Curcumin protects human chondrocytes from IL-l1beta-induced inhibition of collagen type II and beta1-integrin expression and activation of caspase-3: an immunomorphological study, Ann. Anat. 187 (2005) 487–497.
[30] J. Wei, S. Dong, R.K. Bowser, A. Khoo, L. Zhang, A.M. Jacko, et al., Regulation of the ubiquitylation and deubiquitylation of CREB-binding protein modulates histone acetylation and lung inflammation, Sci. Signal. 10 (2017).
[31] T. Mabey, S. Honsawek, Cytokines as biochemical markers for knee osteoarthritis, World J. Orthop. 6 (2015) 95–105.
[32] T.D. Gilmore, Introduction to NF-kappaB: players, pathways, perspectives, Oncogene 25 (2006) 6680–6684.
[33] A. Panday, M.E. Inda, P. Bagam, M.K. Sahoo, D. Osorio, S. Batra, Transcription factor NF-kappaB: an update on intervention strategies, Arch. Immunol. Ther. Exp. 64 (2016) 463–483.
[34] S.T. Smale, Hierarchies of NF-kappaB target-gene regulation, Nat. Immunol. 12 (2011) 689–694.
[35] A.F. Steinert, S.C. Ghivizzani, A. Rethwilm, R.S. Tuan, C.H. Evans, U. Noth, Major biological obstacles for persistent cell-based regeneration of articular cartilage, Arthritis Res. Ther. 9 (2007) 213.