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Eupatilin protects chondrocytes from apoptosis via activating sestrin2-dependent autophagy

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ABSTRACT

Cartilage degradation is the main characterization of osteoarthritis (OA). Accumulating evidence suggests that chondrocyte apoptosis and autophagy are associated with cartilage degradation. Thus, we investigated the protective effect and underlying mechanism of eupatilin for treating OA. IL-1β was used to simulate OA in vitro. Data show that eupatilin treatment attenuated IL-1β-induced apoptosis of chondrocytes. Autophagy was also activated by eupatilin in a dose-dependent manner. Then, pretreatment with chloroquine (CQ), an autophagic inhibitor, decreased eupatilin-induced autophagy and increased apoptosis in the chondrocytes. To investigate the mechanism of eupatilin, the expressions of sestrin2 and mTOR were measured using Western blot; eupatilin upregulated sestrin2 but downregulated mTOR phosphorylation. The administration of sestrin2-siRNA significantly decreased autophagy and reversed the protective effect of eupatilin against chondrocyte apoptosis and degradation of the cartilage matrix. Thus, eupatilin can inhibit IL-1β-induced apoptosis via sestrin2-dependent autophagy in chondrocytes.

1. Introduction

Osteoarthritis (OA) is a chronic disease that causes joint pain and stiffness, and its prevalence increases with age [1]. The chief articular cartilage component is the extracellular matrix (ECM), which is maintained in dynamic equilibrium via regulation of synthesis and degradation. However, this equilibrium is disrupted in OA and cartilaginous tissue is continuously lost [2,3].

Articular cartilage lacks mesenchymal stem cells and a blood supply. Chondrocytes, which are separated by an interterritorial matrix, are the only cell type within articular cartilage, and this means that chondrocyte viability is critical for cartilage repair. Areas lacking chondrocytes directly reduce the ability of cartilage to maintain and repair the ECM. Studies indicate that chondrocyte apoptosis is the key to cartilage degeneration [4,5]. Chondrocyte apoptosis is linked to the severity of cartilage destruction and degradation of the cartilage matrix [6], so understanding how cell death occurs in cartilage and attenuating chondrocyte apoptosis may help treat OA.

Autophagy is a catabolic process that is important to homeostasis maintenance and cell stress responses [7]. This process regulates lysosomal degradation of dysfunctional organelles and macromolecules in the autophagosome-lysosomal pathway; increased autophagy during OA pathology suggests a relationship between the two [8]. Also, reduced autophagy and increased apoptosis occurred in late-stage OA. Autophagy activation protected chondrocytes from death, suggesting a protective role of autophagy in chondrocyte death and cartilage degeneration [9]. Thus, an impaired autophagosome-lysosomal pathway may be a track for reducing cartilage degeneration. Mammalian target of rapamycin (mTOR), a conserved serine/threonine protein kinase, is an important cell survival pathway that regulates autophagy. mTOR is associated with many clinical disorders and the activation of mTOR occurred in OA cartilage, while the inhibition of mTOR reduced cartilage degradation and apoptosis [10]. Zhang's group showed that mTOR was overexpressed in OA, and this was associated with increased chondrocyte apoptosis and decreased expression of key autophagic genes during OA. In addition, an mTOR KO mouse was used to study mTOR/autophagy signaling during OA pathophysiology and they observed reduced chondrocyte apoptosis and reduced disease progression in OA models [11].

Sestrin2, a stress sensor protein kinase, can help with homeostasis and protect cells from stress [12,13]. Reports suggest that sestrin2 can enhance autophagy by inhibition of mTOR [14]. Recently, Lotz's group

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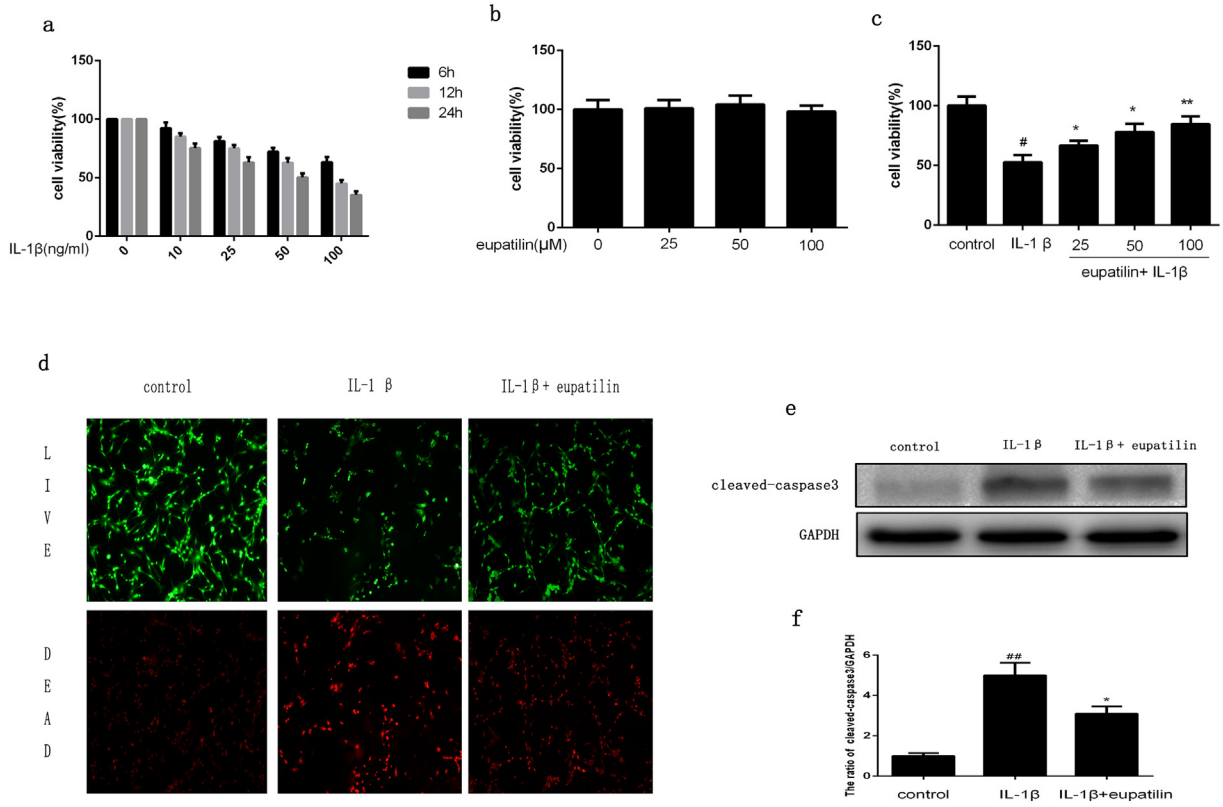

Fig. 1. Treatment of eupatilin decreases apoptosis IL-1β-induced apoptosis in chondrocytes. (a) Chondrocytes were incubated for 6, 12 or 24 with IL-1β at concentrations ranging from 10 to 100 nM. Cell viability was tested by Cell Counting Kit-8 (CCK-8). (b) CCK-8 results of chondrocytes treated with increasing concentrations of eupatilin for 24 h. (c) CCK-8 results of eupatilin -pretreated chondrocytes induced by IL-1β. (d) Calcein "AM/PI" fluorescence-quenching assay of chondrocytes treated with IL-1β and IL-1β plus eupatilin. (e–f) The Western blots and quantification data of cleaved caspase 3 for chondrocytes treated with IL-1β and IL-1β plus eupatilin. Data are expressed as mean ± SD. Significant differences are indicated as *P < 0.05 compared with the IL-1β group. ##P < 0.01 compared with the control group.

found that sestrin expression was suppressed in OA affected cartilage [15] and sestrin overexpression could regulate mTOR activity and upregulate autophagy. However, there are only a few studies of sestrin and OA and mechanistic studies are lacking.

Eupatilin (5,7-dihydroxy-3,4,6-trimethoxyflavone), a bioactive compound extracted from Artemisia, may have anti-apoptotic, anti-inflammatory, and neuroprotective activity [16–18]. It may also have antioxidant properties via sestrin-2-dependent autophagy in HepG2 cells [19]. Recent studies suggest that eupatilin may be useful for arthritis [20,21], but how it may work is not clear. Thus, we studied the therapeutic potential of eupatilin on OA in vitro and attempted to understand its mechanism of action.

2. Materials and methods

2.1. Reagents

Eupatilin (purity ≥98%), autophagy inhibitor chloroquine (CQ), and collagenase II were obtained from Sigma Chemical. (St. Louis, MO). A cell counting Kit-8 (CCK-8) was purchased from Dojindo (Kumamoto, Japan). Primary antibodies for mTOR and p-mTOR came from CST (Framingham, MA, USA). GAPDH, sestrin2, cytochrome *c*, MMP1, MMP 3, MMP13, ADAMTS-5, Bcl2, Bax, cleaved caspase-3, LC-3, Beclin-1, and p62 antibodies were acquired from Abcam (Cambridge, UK). Dulbecco's modified Eagle's medium (DMEM)/F12, and FBS were purchased from Healthcare Life Sciences (Hyclone; Logan, UT).

2.2. Immunohistochemical examination

Specimens in paraffin were deparaffinized and rehydrated in graded ethanol. Sections were microwaved in 10 mM sodium citrate for 15 min for antigen unmasking. Then, slides were blocked with 5% BSA for 30 min at room temperature. Next, primary antibody to cleaved-caspase3 (1:100), Bax (1:100), Bcl2 (1:100), sestrin2 (1:100), and LC3 (1:100) were applied and incubated overnight at 4 °C. Finally, the sections were incubated with HRP-conjugated secondary antibody (Santa Cruz Biotechnology, Dallas, TX) and counterstained with H&E. Images were visualized with Image-Pro Plus software, version 6.0 (Media Cybernetics, Rockville, MD), and integral absorbance values were used as indicators of Bax, Bcl2, cleaved caspase-3, sestrin2, and LC3 expression. At least three sections from each specimen were assessed for protein expression.

2.3. Isolation and culture of chondrocytes

In our study, the primary chondrocytes were obtained from the knee joints of 1–2-week-old Sprague-Dawley (SD) rats (Animal Center of Wenzhou Medical University). Articular cartilage were isolated from knee joints under sterile conditions, and cut into small pieces, which were then digested with 0.25% Trypsin-EDTA solution for 30 min, and then with 0.1% collagenase II in DMEM/F12 at 37 °C with 5% CO_2 for 4 h. Extracted chondrocytes were cultured in 75-cm^2 culture flasks in DMEM/F12 containing 10% FBS, and they were incubated in an atmosphere of 95% air and 5% CO_2 at 37 °C. Media were changed every 2–3 days. Chondrocytes were passaged at a ratio of 1:3 when confluence reached 80–90%. Passages of 2–4 chondrocytes were used in our study.

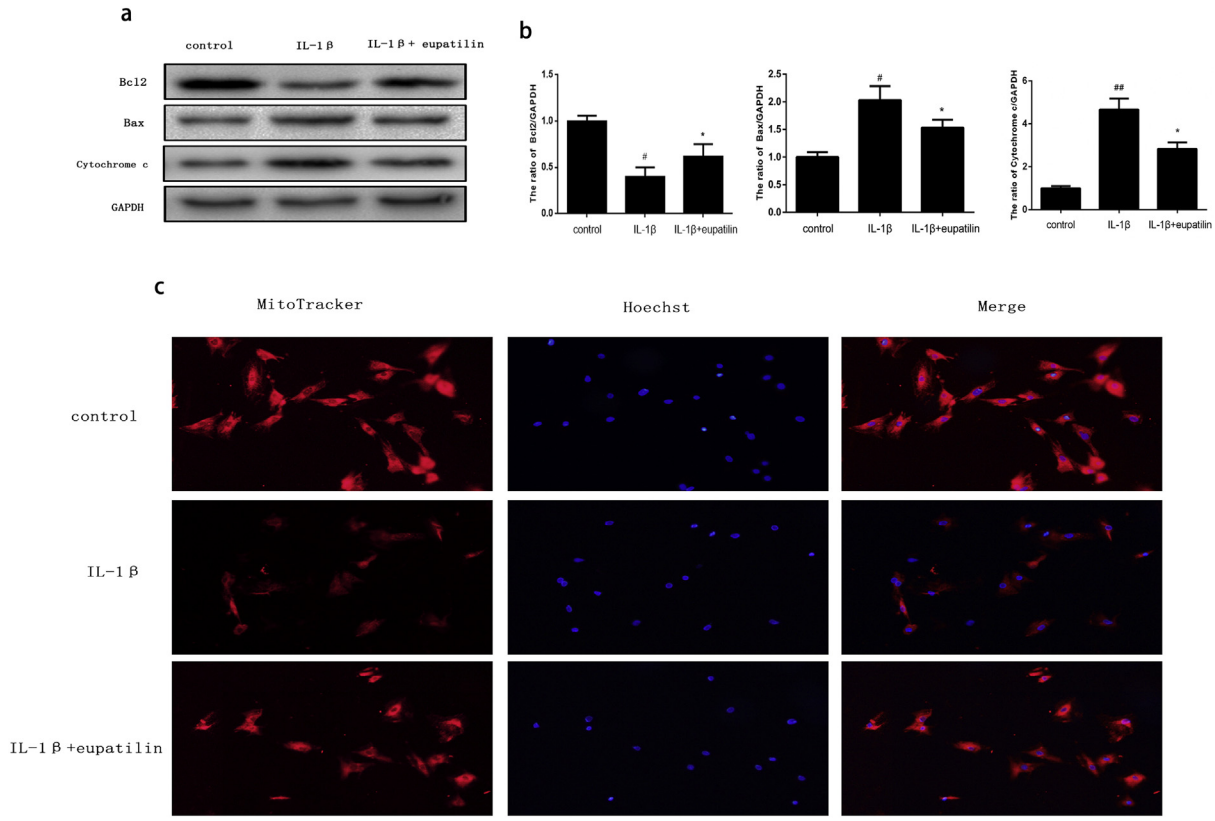

Fig. 2. Eupatilin inhibits IL-1β-induced mitochondrial dysfunction in chondrocytes. Chondrocytes were pretreated with eupatilin (100 μM), followed by 24 h of IL-1β treatment. (a–b) After treatment, the expression of Bax, Bcl-2 and cytochrome *c* were determined by Western blot and quantification analysis. (c) MitoTracker was used to assess the mitochondrial function of chondrocytes. Data are expressed as mean ± SD. Significant differences are indicated as $^{*}P < 0.05$ compared with the IL-1β group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the control group.

2.4. Cell viability

Cell viability was measured using a CCK-8 kit according to the manufacturer's instructions. Chondrocytes were seeded on 96-well plates (5000/well) for 24 h, and then treated with eupatilin, IL-1β, or both. Subsequently, 100 μl DMEM/F12 containing 10 μl of CCK-8 solution was added to each well at 37 °C for an additional 1 h. Absorbance was read at 450 nm using a microplate reader (Leica Microsystems, Germany).

2.5. Western blot

Chondrocyte protein was extracted using RIPA and PMSF, and protein was quantified using a BCA protein assay kit (Beyotime). The same amount of protein (40 μg) was resolved with SDS-PAGE and transferred to PVDF membranes (Bio-Rad). Membranes were blocked with 5% non-fat dry milk for 2 h at room temperature, and subsequently washed three times for 5 min each time in Tris buffered saline with Tween-20 (TBST). Then, membranes were incubated with primary antibodies against Bcl 2 (1:1000), Bax (1:1000), cleaved caspase3 (1:1000), LC3 (1:1000), Becline-1 (1:1000), p62 (1:1000), mTOR (1:1000), p-mTOR (1:1000), Sestrin 2 (1:1000), GAPDH (1:5000), MMP1 (1:1000), MMP 3 (1:1000), MMP13 (1:1000), and ADAMTS-5 (1:1000) overnight at 4 °C. After washing with TBST three times for 5 min, the membranes were incubated with their respective peroxidase-conjugated secondary antibodies for 2 h at room temperature. Finally, bands were visualized with ECL and Image Lab 3.0 software (Bio-Rad).

2.6. Immunofluorescence microscopy

Collagen II and LC3 expression was measured using immunofluorescence assays. Chondrocytes were cultured on slices in 6-well plates. Samples were fixed with 4% paraformaldehyde for 30 min and permeabilized with 0.5% Triton X-100 for 10 min at room temperature. Cells were blocked with 5% goat serum for 1 h and then incubated with primary antibodies against collagen II and LC3 (1:200) overnight at 4 °C. Then, the cells were incubated with fluorescein-conjugated goat anti-rabbit IgG antibody (1:400) for 1 h and labeled with DAPI for 1 min. Finally, three fields of each slide were captured randomly under a fluorescence microscope (Olympus Inc., Tokyo, Japan).

2.7. siRNA transfection

Chondrocytes were transfected with Sestrin2 siRNA (GenePharma, Shanghai, China) or a non-targeting control siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Transfected cells were confirmed and then used in subsequent studies.

2.8. MitoTracker

Fluorescence staining for mitochondria transmembrane potential (MMP) was performed by MitoTracker Red CMXRos according to the manufacturer's instructions. After treatment, the chondrocytes were stained with 50 nM MitoTracker and Hoechst for 30 min at 37 °C. Red fluorescent images of at least three random microscopic fields were acquired per slide for microscopic observation with a fluorescent microscope (Olympus Inc., Tokyo, Japan), and fluorescent intensity was measured using Image J software 2.1 (Bethesda, MD) by observers who were blinded to experimental groups.

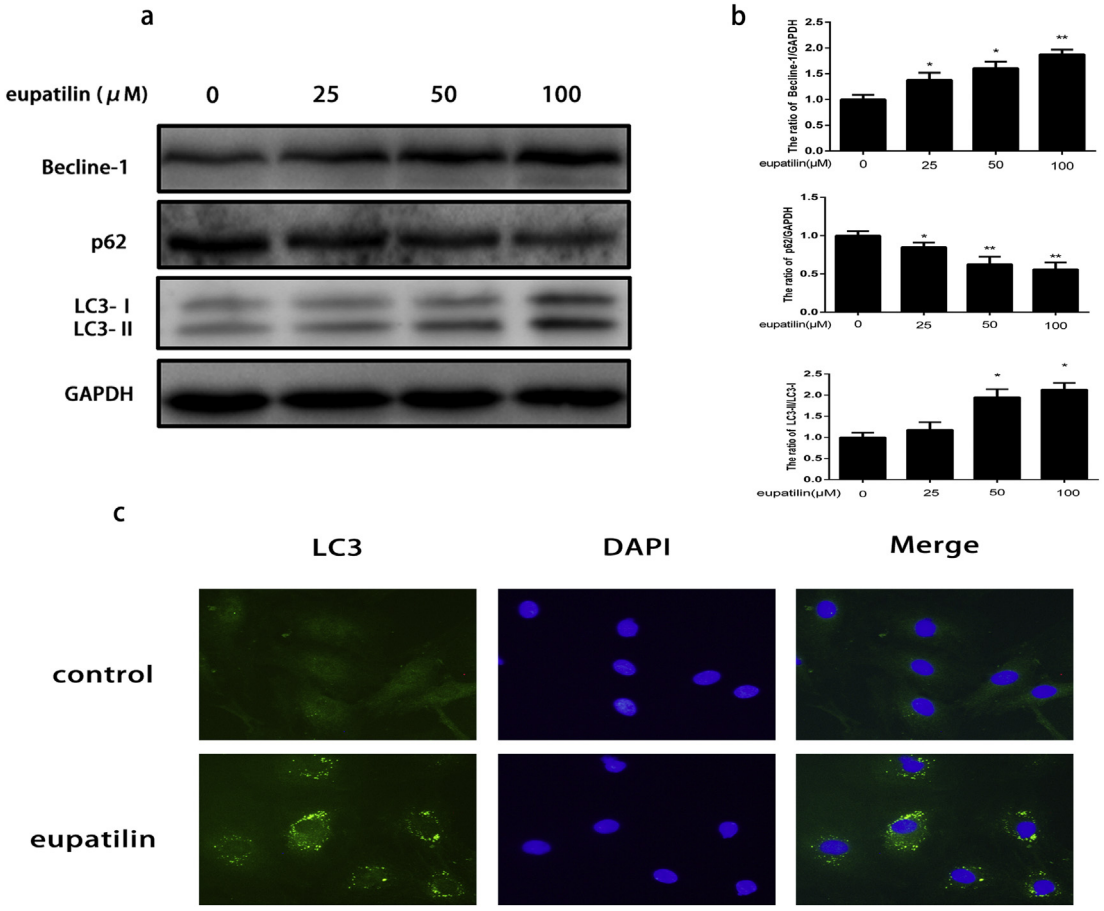

Fig. 3. Eupatilin has the ability to activate autophagy in chondrocytes. (a–b) Chondrocytes were incubated with 0, 25, 50, 100 μM eupatilin for 24 h. Protein levels of LC3, Beclin-1 and p62 of chondrocytes were determined by Western blot and quantification analysis. (c) Immunofluorescence staining of LC3 were evaluated after treatment with eupatilin (100 μM) or not for 24 h. Data are expressed as mean ± SD. Significant differences are indicated as *P < 0.05, **P < 0.01 compared with the control group.

2.9. TUNEL method

TUNEL was used to measure apoptosis of chondrocytes that were cultured on slices in 6-well plates. Samples were fixed with 4% paraformaldehyde for 30 min and permeabilized in 3% H_2O_2 and 0.1% Triton X-100 for 10 min at room temperature. Then, the cells were stained using an In Situ Cell Death Detection Kit and DAPI. Images were visualized under a fluorescent microscope (Olympus).

2.10. Live/dead assays

Live and dead cells were counted with a calcein AM/PI fluorescence-quenching assay according to the manufacturer's instructions. After treatment of chondrocytes as described, the cells were treated with 2 μM calcein AM and 20 μg/ml PI, and incubated for 30 min at 37 °C. Changes were visualized under a fluorescent microscope (Olympus).

2.11. Statistical analysis

Data are means ± standard deviation (SD). Statistical significance was assessed using one-way ANOVA, and $P < 0.05$ was considered to be statistically significantly relevant.

3. Results

3.1. Eupatilin inhibits apoptosis of chondrocytes induced by IL-1β

Fig. 1a shows that cell viability decreased after IL-1β treatment in a dose- and time-dependent manner. We selected 100 ng/ml of IL-1β for 24 h for experimental conditions because other concentrations had no effect (Fig. 1b). To investigate the cytoprotective effects of eupatilin, chondrocytes were pretreated with eupatilin and treated with IL-1b 24 h later. Eupatilin was protective against IL-1β-induced cell damage (Fig. 1c). Calcein AM/PI fluorescence-quenching data showed that pretreatment with eupatilin reduced the IL-1β-induced cell death (Fig. 1d). To study whether eupatilin could inhibit apoptosis of chondrocytes due to IL-1β, we measured the expression of apoptosis-related protein (cleaved caspase-3) using Western blot. Chondrocytes were treated as described and IL-1β significantly increased cleaved caspase-3 but pretreatment with eupatilin reduced this effect induced by IL-1β (Fig. 1e, f).

3.2. Eupatilin protects chondrocytes from IL-1β-induced mitochondrial dysfunction

Chondrocytes were pretreated with eupatilin as indicated and then with IL-1β. The overexpression of Bax induced by IL-1β, which causes opening of mitochondrial pores and release of cytochrome *c*, was measured. IL-1β downregulated anti-apoptotic protein (Bcl-2), so the mitochondrial apoptosis pathway was activated by IL-1β. These responses were recovered with eupatilin (Fig. 2a, b). Mitochondrial

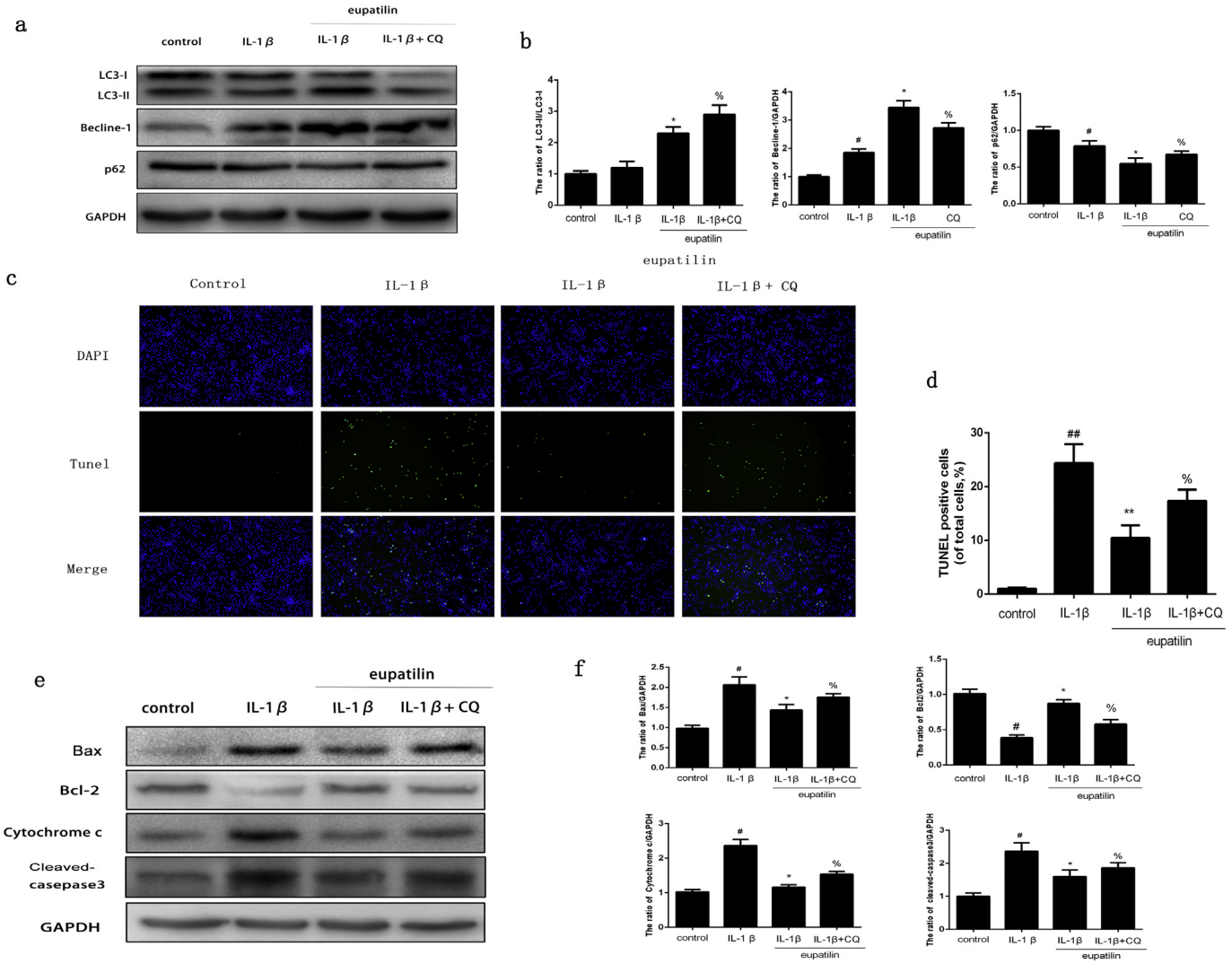

Fig. 4. Eupatilin protects chondrocytes against IL-1β-induced apoptosis through the activation of autophagy. Chondrocytes were treated with IL-1β alone, or treated with eupatilin (100 μM) and IL-1β in the presence or absence of the autophagy inhibitor CQ (50 nM). (a–b) The protein expressions of LC3, Beclin-1 and p62 of chondrocytes were determined by Western blot and quantification analysis. (c–d) TUNEL assay was performed in chondrocytes. (e–f) The protein expressions of cleaved caspase 3, Bax, Bcl-2 and cytochrome *c* were determined by Western blot and quantification analysis. Data are expressed as mean ± SD. Significant differences are indicated as $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the control group, $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the IL-1β group, $^{\%}P < 0.05$ compared with the IL-1β + eupatilin group.

function was assessed by membrane potential-dependent MitoTracker and data showed that IL-1β helped with mitochondrial recovery from dysfunction (Fig. 2c).

3.3. Eupatilin induces autophagy in chondrocytes

We measured indicators of autophagy (LC3-II/LC3-I ratio, Beclin-1, and p62) using Western blot. Chondrocytes were treated as indicated and data showed that eupatilin increased the ratio of LC3-II/LC3-I and Beclin-1 and decreased the expression of p62, suggesting activation of autophagy (Fig. 3a–b). To confirm this, immunofluorescent analysis of LC3 was performed and the data showed that eupatilin activated autophagy (Fig. 3c).

3.4. Autophagy protects chondrocytes against IL-1β-induced apoptosis

As shown in Fig. 4a–b, treatment with eupatilin (100 μM) for 3 h activated autophagy-related protein. To investigate how autophagy interacts with anti-apoptotic effects of eupatilin, CQ was used to block lysosomal function in chondrocytes. CQ pre-treatment (50 nM) significantly increased the expression of LC3-II/-I and p62 and decreased the expression of Beclin-1. Meanwhile, the protective effect of eupatilin against apoptosis was blunted with the pretreatment of CQ and the TUNEL assay confirmed these data (Fig. 4c–d). As shown in Fig. 4e–f, eupatilin significantly decreased IL-1β-induced apoptosis but pretreatment with CQ inhibited this protective effect. Thus, eupatilin attenuated apoptosis and this effect may be related to the activation of autophagy.

3.5. Eupatilin and expression of Sestrin2 and mTOR

To understand how sestrin2 may regulate mTOR, we measured the effect of eupatilin on the expression of sestrin2 and mTOR in chondrocytes. Chondrocytes were treated with eupatilin (25, 50, or 100 μM) and sestrin2, and mTOR protein was quantified using Western blot. Fig. 5a–b shows that sestrin2 significantly increased after treatment with eupatilin and the p-mTOR/mTOR ratio was dose-dependently decreased.

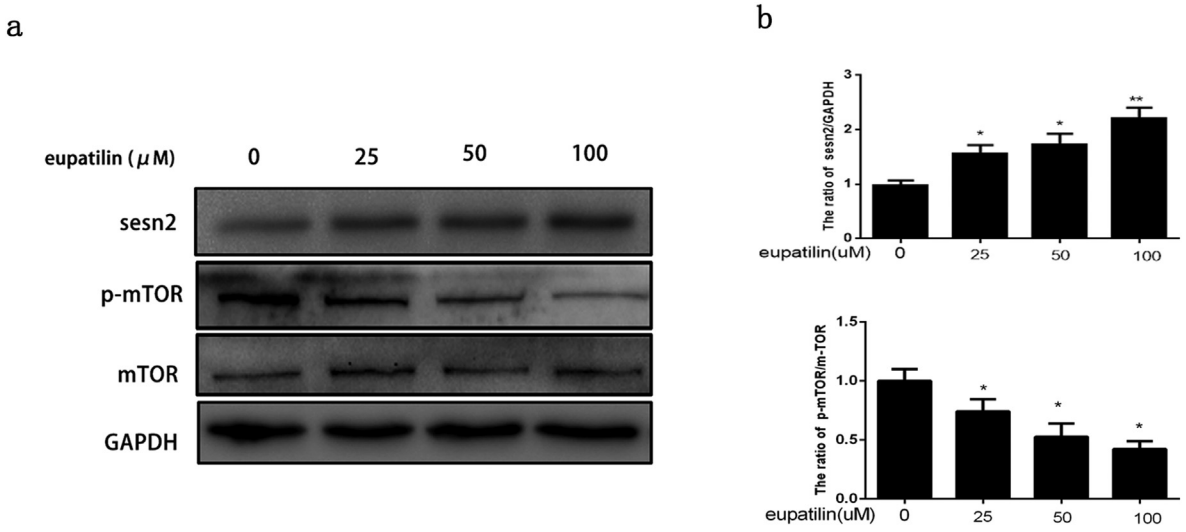

Fig. 5. Eupatilin promotes the expression of Sestrin2 and mTOR. Chondrocytes were treated with different concentration of eupatilin (25, 50, 100 μM). (a–b) The protein expressions of Sestrin2, p-mTOR and mTOR were determined by Western blot and quantification analysis. Data are expressed as mean ± SD. Significant differences are indicated as *P < 0.05, **P < 0.01 compared with the control group.

3.6. Inhibition of sestrin2 by siRNA significantly attenuated eupatilin-induced autophagy and increased apoptosis in chondrocytes

To confirm the role of sestrin2 in eupatilin protection, cells were transfected as indicated and Fig. 6a–b shows that siRNA for sestrin2 significantly decreased its expression compared with the controls and control siRNA. Then, we studied the influence of sestrin2-siRNA on eupatilin with regard to apoptosis and autophagy. Western blot data showed that eupatilin activated autophagy in the chondrocytes treated with 100 ng/ml IL-1β but sestrin2-siRNA markedly reduced the ratio of LC3-II/LC3-I and beclin-1 and increased the ratio of p-mTOR/mTOR and p62 (Fig. 6c–d). IL-1β significantly downregulated Bcl-2 protein but increased Bax and cleaved caspase-3 protein. When chondrocytes were pretreated with eupatilin, the upregulation of apoptosis was weakened. However, sestrin2-siRNA significantly reversed the anti-apoptotic effects of eupatilin (Fig. 6e–f).

3.7. Protective effect of eupatilin on ECM degeneration

MMPs and ADAMTS are major catabolic enzymes in OA pathogenesis. Excess MMP and ADAMTS activity can destroy the ECM composition of collagen and proteoglycans. To determine the effects of eupatilin for treating OA in vitro, MMP-1, MMP-3, MMP-13, and ADAMTS-5 protein were measured with Western blot (Fig. 7a–b). Chondrocytes were treated as described and eupatilin inhibited increased MMP-1, MMP-3, MMP-13, and ADAMTS-5 induced by IL-1β treatment. However, this protective effect was attenuated by sestrin2-siRNA. Immunofluorescent data showed that eupatilin suppressed protein degradation of collagen-II induced by IL-1β, but sestrin2-siRNA significantly reversed this effect of eupatilin (Fig. 7c).

4. Discussion

OA is a chronic joint disease that is characterized by the degradation of articular cartilage and subchondral bone remodeling. Previous studies have shown that pro-inflammatory cytokines, such as IL-1β, contribute to the pathogenesis of OA [22]. Excessive production of IL-1β contributes to the release of catabolic enzymes, such as matrix metalloproteinases (MMPs) and ADAMTS, which degrade the cartilage matrix. In addition, IL-1β was also reported to induce chondrocyte apoptosis and suppress the expression of ECM components, such as collagen and proteoglycans [23]. Therefore, we used IL-1β to simulate OA.

Plant-derived compounds are of interest because many studies have suggested that they may have activity against some diseases [24]. Eupatilin, a component of DA-9601, was thought to protect the gut against mucosal injury and it has been briefly studied for safety [25]. Other studies suggest that eupatilin may have activity against OA and protect cardiomyocytes from ischemia/reperfusion injury via anti-inflammatory and anti-apoptotic activity [26,27]; however, how this compound may work is not clear. Few studies describe the effects of eupatilin on OA, so we looked at eupatilin's effect on apoptosis and degeneration of chondrocytes and investigated the underlying mechanisms.

We report that IL-1β increased apoptotic-related proteins and MMP-1, MMP-3, MMP-13, and ADAMTS-5 were upregulated. Also, type II collagen decreased after stimulation with IL-1β. These data were consistent with previous studies [28]. However, catabolic enzyme production and protein degradation of collagen-II induced by IL-1β was inhibited by eupatilin, suggesting a protective effect of eupatilin on the ECM. But this protective effect was attenuated by sestrin2-siRNA. In the present study, we examined that eupatilin induced autophagy in chondrocytes. However, inhibition of sestrin2 by siRNA significantly suppressed eupatilin-induced autophagy. So we demonstrated that the inhibition of autophagy may account for the catabolic enzyme production or changes of collagen II expression. The inference is consistent with some previous studies [29,30].

Recent studies suggest that chondrocyte death was correlated with the severity of cartilage degeneration [31]. More apoptosis was found with OA cartilage and amelioration of chondrocyte apoptosis may be a novel treatment for OA [32]. The mitochondrial pathway is a classical one for apoptosis upregulation of the Bax/Bcl-2 ratio, which causes permeabilization of the mitochondrial membrane, leading to release of cytochrome *c*. Then, the caspase family, which is considered a final executor of apoptosis, is activated. We found that mitochondrial apoptotic-related proteins measured using Western blot were reduced and anti-apoptotic proteins were increased. MitoTracker data showed that eupatilin could reduce IL-1β-induced mitochondrial membrane potential collapse. Our in vitro data were confirmed with immunohistochemistry in an OA rat model. Thus, eupatilin suppressed IL-1β-induced chondrocyte apoptosis, and this could reduce mitochondrial dysfunction.

Autophagy, an endogenous process to remove damaged organelles, maintains essential cellular homeostasis. Autophagy is associated with

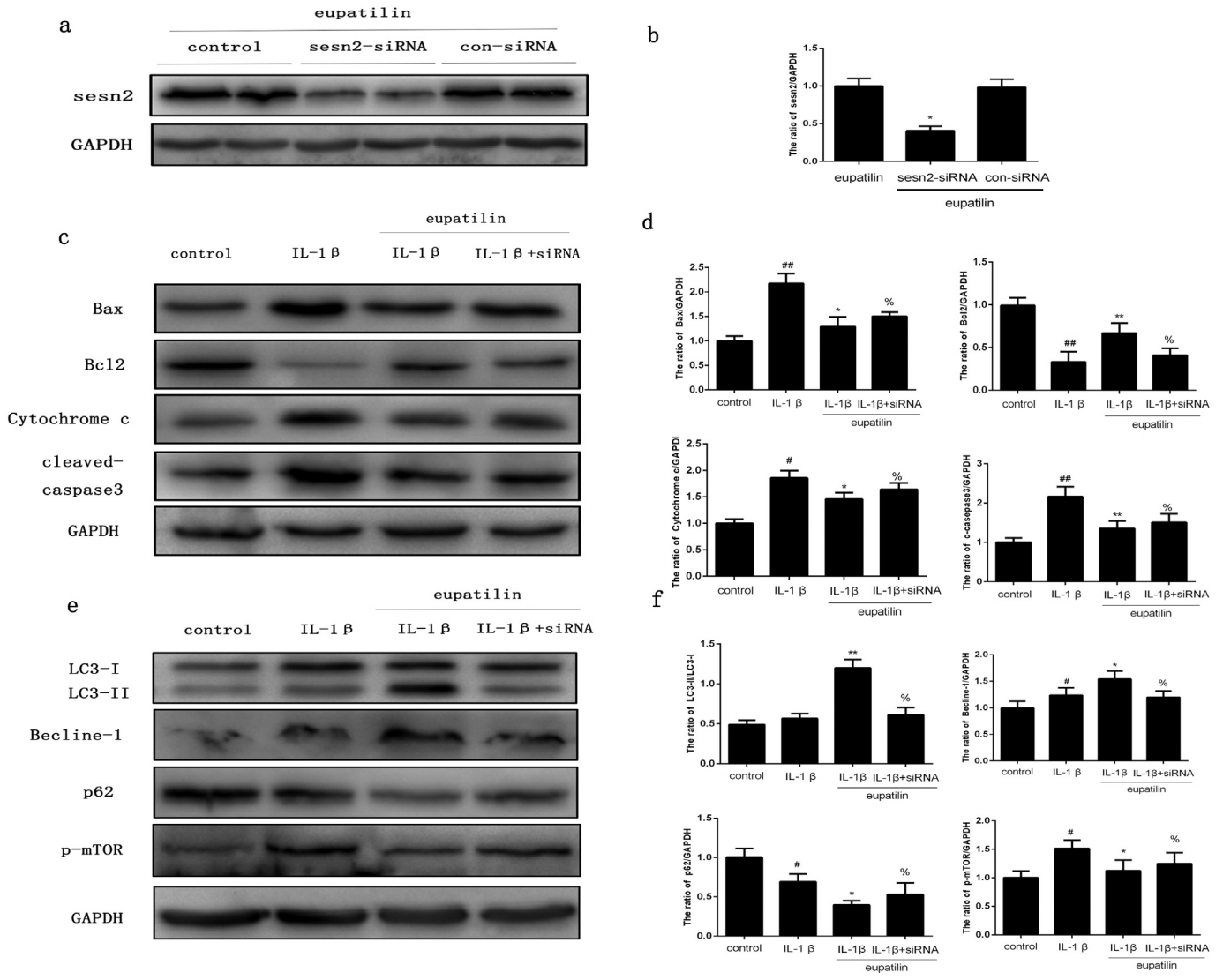

Fig. 6. Inhibition of Sestrin2 by siRNA decreased eupatilin-induced autophagy and promoted apoptosis in chondrocytes. (a–b) Chondrocytes were transfected with Sestrin2 siRNA (si- Sestrin2) or siRNA control (si-Control), and incubated with 100 μM eupatilin 24 h. The protein expression of Sestrin2 was assessed by Western blot and quantification analysis. (c–d) Chondrocytes were treated with IL-1β alone, or treated with eupatilin (100 μM) and IL-1β in the presence or absence of Sestrin2 siRNA. The protein expression of cleaved caspase 3, Bax, Bcl-2 and cytochrome *c* were assessed by Western blot and quantification analysis. (e–f) Chondrocytes was treated as above, the protein expressions of LC3, Beclin-1 and p62 of chondrocytes were determined by Western blot and quantification analysis. Data are expressed as mean ± SD. Significant differences are indicated as $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the control group, $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the IL-1β group, $^{\%}P < 0.05$ compared with the IL-1β + eupatilin group.

many chronic diseases, such as OA. Recent studies suggest that induction of autophagy can protect chondrocytes from mitochondrial dysfunction and apoptosis [33,34]. Here, we report that autophagy could be activated by eupatilin and we used a classical autophagy inhibitor (CQ) and learned that CQ inhibited autophagy induced by eupatilin. Meanwhile, anti-apoptotic effects of eupatilin were attenuated.

m-TOR, a serine/threonine protein kinase, negatively regulated autophagy and is the key to the regulation of cellular processes, such as metabolism and cell growth. mTOR has been shown to be important to articular cartilage homeostasis [35,36] and the inhibition of mTOR was confirmed to ameliorate the severity of OA. Sestrins are considered stress-inducible proteins that can maintain homeostasis and eliminate harmful metabolites. Sestrin2 is a vital sestrin family member that helps cells respond to various stresses. Moreover, sestrin2 can activate autophagy via the inhibition of mTOR. Hou's group reported that sestrin2 has a protective effect on dopaminergic cells via activation of autophagy [37]. We studied expression of mTOR and senstrin2 and noted that eupatilin increased senstrin2 and reduced mTOR in a dose-dependent manner; these data agreed with a previous study [19]. When we pretreated chondrocytes with eupatilin, upregulation of apoptosis was weakened but sestrin2-siRNA significantly reversed anti-apoptotic effects of eupatilin. Therefore, eupatilin may protect against IL-1β-induced apoptosis by regulating autophagy. Activation of autophagy induced by eupatilin was mediated by the upregulation of senstrin2-dependent autophagy. Therefore, eupatilin treatment induces autophagy in a sestrin2-dependent manner in chondrocytes, and this blocks apoptosis- and degeneration-related protein expression induced by IL-1β.

5. Conclusions

We suggest that eupatilin treatment significantly inhibits apoptosis and degeneration of chondrocytes induced by IL-1β and this may be due to senstrin2-dependent autophagy.

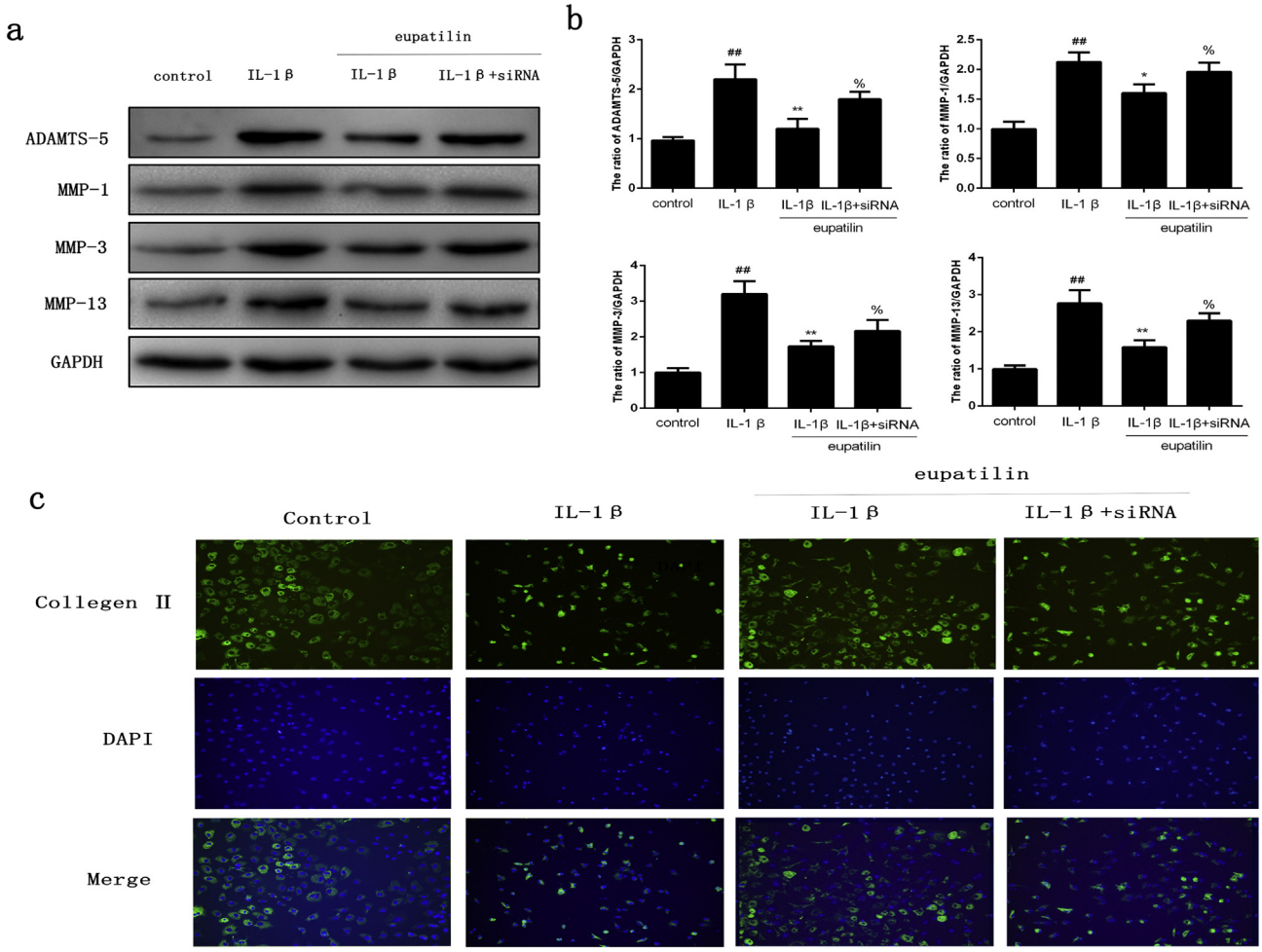

Fig. 7. Effect of eupatilin to the degeneration-related protein expression in chondrocytes. Chondrocytes were treated with IL-1β alone, or treated with eupatilin (100 μM) and IL-1β in the presence or absence of Sestrin2 siRNA. (a–b) The protein expression of MMP-1, MMP-3, MMP-13 and ADAMTS-5 were assessed by Western blot and quantification analysis. (c) Immunofluorescence staining of collagen-II were also evaluated. Data are expressed as mean ± SD. Significant differences are indicated as $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the control group, $^{*}P < 0.05$,$^{**}P < 0.01$ compared with the IL-1β group, $^{\%}P < 0.05$ compared with the IL-1β + eupatilin group.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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