Resistin Induces Chemokine and Matrix Metalloproteinase Production via CAP1 Receptor and Activation of P38-MAPK and NF- κ B Signalling Pathways in Human Chondrocytes

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Abstract

Background

Resistin is an adipokine also detected higher expression in serum and synovial fluid of patients with knee osteoarthritis (KOA). Resistin is known to be related closely to insulin resistance and inflammation. However, the pathogenic role of resistin in KOA remain unclear. Purpose of the study is to investigate whether resistin induces KOA by binding to functional receptor adenylyl cyclase-associated protein 1 (CAP1) and activating the p38 mitogen-activated protein kinase (p38-MAPK) and nuclear factor- κ B (NF- κ B) signalling pathways in human chondrocytes.

Methods

We enrolled 103 patients with radiographic KOA and 86 healthy participants as controls. The levels of resistin in serum and synovial fluid (SF) were determined via enzyme-linked immunosorbent assay (ELISA). CAP1 expression in cartilage tissues (21 samples of KOA cartilage and 10 samples of healthy hip cartilage) was measured using immunohistochemistry (IHC), quantitative real-time polymerase chain reaction (qRT-PCR), and western blotting assays. Effects of resistin on chondrocytes and CAP1 were evaluated via qRT-PCR and co-immunoprecipitation. The roles of CAP1, p38-MAPK, and NF- κ B signalling pathways in the development of KOA were evaluated via adenovirus-mediated CAP1 short hairpin RNA, qRT-PCR, western blotting, and ELISA.

Results

Expression of resistin in serum and SF was elevated in severe radiographic KOA. CAP1 levels were higher in KOA cartilage and were positively correlated with resistin expression. Resistin promoted increased expression of CCL3, CCL4, MMP13, and ADAMTS-4 through CAP1 receptor. Resistin also directly bound to CAP1 as confirmed by co-immunoprecipitation. CAP1 knockdown in chondrocytes attenuated resistin-induced expression of CCL3, CCL4, MMP13, and ADAMTS-4 and activation of the p38-MAPK and NF- κ B signalling pathways.

Conclusions

Our study shows that resistin bound to CAP1 and upregulated the expression of proinflammatory cytokines and matrix-degrading enzymes via p38-MAPK and NF- κ B signalling pathways in human chondrocytes.

Background

Knee osteoarthritis (KOA) is a joint disease involving articular cartilage, subchondral bone, ligaments, capsule and surrounding synovial tissues[1, 2]. Main symptoms of KOA are pain and restricted mobility[3-5]. Progressive deterioration of articular cartilage and loss of extracellular cartilage matrix are key features of KOA. The different KOA phenotypes are classified according to clinical, imaging, and

laboratory characteristics, and may represent different aetiopathogeneses of the disease[6-8]. Therapy is chosen based on phenotype to prevent the development of grade III/IV KOA. Despite significant advances, a substantial percentage of KOA patients are unsatisfied with their outcomes[9]. To prevent the onset and progression of KOA, it is imperative to determine the underlying mechanisms.

Although OA has traditionally been classified as a noninflammatory disease, an increasing proportion of patients present with synovial inflammation[10-12]. Numerous proinflammatory cytokines contribute to the progression of OA[13]. Although resistin is considered as proinflammatory cytokine in OA, few studies have evaluated the underlying mechanisms. Resistin, an adipokine associated with insulin resistance and inflammatory processes[14, 15], is highly expressed in synovial joints of OA patients[16-20]. Studies have shown that resistin upregulates proinflammatory cytokines and matrix-degrading enzymes in human articular chondrocytes[21, 22]. Additionally, arthritis is induced by injecting resistin into the knee joints of healthy mice[23]. Although numerous studies have examined resistin levels in sera and synovial fluid (SF) of OA patients, few have investigated the proinflammatory mechanisms of resistin. Previously, we described the proinflammatory role of resistin in osteoarthritis, but did not determine the mechanisms involved[24].

Adenylyl cyclase-associated protein 1 (CAP1) localizes to the cell membrane and cytoplasm, being a receptor for resistin and modulating the inflammatory activity in monocytes[25]. Silencing of CAP1 in fibroblast-like synoviocytes (FLSs) downregulates resistin-stimulated production of chemokines, such as CXCL8, CCL2, and IL-6[26]. CAP1 plays an important role in resistin-induced inflammation; however, its exact role in the progression of OA remains unclear.

Resistin-mediated proinflammatory activity involves activation of the nuclear factor- κ B (NF- κ B) pathway[27, 28]. In human chondrocytes, increased expression of resistin-induced inflammatory chemokines is also mediated by the NF- κ B pathway[21]. Resistin may induce the expression of pro-inflammatory cytokines and matrix catabolic enzymes in human nucleus pulposus cells via activity of the p38-MAPK and NF- κ B pathways[29, 30]. The characteristics of human articular chondrocytes are similar to those of human nucleus pulposus cells; hence, activation of p38-MAPK and NF- κ B pathways may be involved in the function of resistin in human chondrocytes.

Here, we evaluated resistin level in patients with KOA and healthy control subjects, and expression of CAP1 between cartilage of KOA patients and normal cartilage in patients with femoral neck fracture (FNF-Cartilage). We also explored the proinflammatory effects of resistin, and involvement of p38-MAPK and NF- κ B signalling in the resistin-CAP1 axis in human chondrocytes. To our knowledge, this is the first study exploring the mechanism of resistin/CAP1 in human KOA chondrocytes. in

Methods

Participants

Overall, 103 patients with KOA, diagnosed according to criteria set by the American College of Rheumatology, were recruited from May 2017 to June 2018 at the Orthopedics Department of the first hospital of Jilin University. After a 12 h fast, venous blood and SF samples were collected from patients and 86 healthy volunteers at their annual examinations conducted at our Health Evaluation Center of the first hospital of Jilin University. All collected blood and synovial fluid samples were centrifuged at 3000g for ten minutes at 4 °C. The supernatants of blood and synovial fluid were aliquoted and stored at -80 °C until assay. Clinical data of KOA patients was reviewed to exclude patients treated with intraarticular glucocorticoid or hyaluronic acid injections within 6 months. This study was approved by the Ethics Committee of the first hospital of Jilin University (reference no: 2017-429). All participants provided signed informed consent, and the study was performed in accordance with the Declaration of Helsinki. All data pertaining to study participants are presented in **Table I**.

Collection of human cartilage samples

Samples of KOA cartilage were obtained from primary total knee replacement (TKA) surgery patients. Samples of control cartilage were collected from patients with femoral neck fracture (FNF) within 12h of injury, and articular cartilage of femoral heads were macroscopically intact and showed little degeneration regardless of age (Collins score 0), as evaluated at the Orthopedics Department of the first hospital of Jilin University. Collection of all cartilage samples had the approval of the permission of the patients according to the Declaration of Helsinki. All sample details are listed in **Table II**.

Isolation and culture of human chondrocytes

Nine cartilage tissue samples were harvested randomly from patients with KOA (N=21) and divided into three groups randomly. Three cartilage tissue samples of each group were pooled prior to cell isolation and culture. Chondrocytes were isolated as previously described[31] and cultured in Dulbecco's Modified Eagle's Medium (DMEM)/F12 supplemented with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin in a CO₂ incubator at 37 °C. Then, (DMEM)/F12 media were replaced with serum-free media, and chondrocytes were starved 12 h before treatment with resistin at different concentrations and time-points. Chondrocytes at passage one and two were used in our study.

Enzyme-linked immunosorbent assay (ELISA)

Concentration of resistin in samples of serum and SF was quantitated using ELISA kits (CUSABIO, China; CSB-E06884h) according to manufacturer's instructions. The range of detection was 0.312–20 ng/mL.

Immunohistochemical assessment of cartilage tissues

A total 10 control cartilage samples from patients with FNF and 21 KOA cartilage samples were used to evaluate the expression of resistin and CAP1 via IHC. Specimens were embedded in paraffin and sectioned at 3 µm. Each section was rehydrated in ethanol (100, 90, and 80%) for 5 minutes, respectively, heated at 95°C for 10 minutes in 10 mM sodium citrate buffer solution (pH 6.0) for antigen recovery, and

then treated with 3% (v/v) H₂O₂ for 15 min to block endogenous peroxidase activity. After several washes with PBS, sections were incubated with serum-free protein block (Agilent Technologies) for 30 min to block non-specific binding. Sections were then incubated at 4°C overnight with mouse anti-resistin monoclonal antibody (concentration at 10 µg/ml, ab136877; Abcam), rabbit anti-CAP1 monoclonal antibody (diluted at 1:200, EPR8339B; Abcam), or isotype control (Agilent Technologies). Expression level was detected using a Mouse and Rabbit Specific HRP/AEC (ABC) Detection IHC Kit (Abcam), after which the sections were counterstained with hematoxylin.

An H-Score was calculated for each case using the formula: H-Score = P × I, where percentage (P) of positively stained cells (0–100) was multiplied by staining intensity (I) score (0, negative; 1, weak; 2, moderate; 3, strong staining). The H-Scores ranged from 0 to 300. Expression of CAP1 and resistin was analysed based on H-Score using ASI digital systems (powered by GenASIs™, GenASIs Client:8.1.0.47741, Israel).

Stimulatory effects of resistin on chondrocytes

Chondrocytes were seeded in 6-well plates at 1×10^4 cells/cm² and cultured as described above. Then, chondrocytes were incubated for 0, 24, 48, or 72 hours in DMEM/F12 containing human recombinant resistin (500 ng/mL) (PeproTech, USA) with 1% FBS to evaluate time-response effects, or with resistin (0, 250, 500, or 1000 ng/mL) for 48 hours to evaluate dose-response effects or 24, 48 and 72 hours to evaluate CPA1 expression. Resistin-induced mRNA expression of CCL3, CCL4, MMP13, ADAMTS-4 and CAP1 was evaluated by qRT-PCR.

Co-immunoprecipitation assays (Co-IP)

Chondrocytes were cultured in 6-well plates (at 2×10^5 cells/well) as described previously with and without resistin (at 500 ng/ml) for 48 h. Cells were then washed with ice-cold phosphate buffered saline (PBS), and protein was harvested using a cell lysis buffer (Beyotime, China). CAP1 co-immunoprecipitation was performed using a Pierce™ Co-Immunoprecipitation Kit (Thermo Scientific) according to manufacturer's instructions. For immunoprecipitation, cell lysates were incubated overnight on ice with CAP1 antibody labelled beads to examine binding of resistin and CAP1. Rabbit IgG was used as negative control. After overnight incubation, the eluted protein was detected by western blotting using an antibody against resistin (Abcam, 1:1000).

Knockdown of CAP1 via adenovirus (Adv)-mediated transfection in chondrocytes

A recombinant Adv vector was generated by cloning shRNA fragments into an Adv vector GV119 (Shanghai Genechem Co., Ltd, China) via enzymatic digestion and ligation. Three shRNA sequences were designed to silence the expression of CAP1 in human chondrocytes in vitro. One of the CAP1-shRNA-targeting sequences was 5'- CCTGGCCCTTATGTGAAAGAA -3', whereas the negative control shRNA sequence was 5'-TTCTCCGAACGTGTCACGT-3'.

Chondrocytes were transfected with CAP1-shRNA or control-shRNA for 48 h, and treated with resistin (500 ng/mL) for another 48 and 72 h. All experiments were performed at a multiplicity of infection (MOI) of 40 plaque forming units (PFU). The efficiency of CAP1 knockdown was confirmed by qRT-PCR and western blotting. Expression of CCL3, CCL4, MMP13, and ADAMTS-4 mRNA was assessed via qRT-PCR. The level of secreted proteins in culture supernatants was determined using ELISA (RayBiotech, USA) according to manufacturer's instructions.

Total RNA extraction and quantitative real-time PCR

Total RNA was extracted from chondrocytes and human cartilage and reverse-transcribed into cDNA. The qRT-PCR was conducted as described previously[32]. Primers used for qRT-PCR are shown in **Supplementary materials I**. The Ct values for target genes were calculated as described previously[22].

Western blot analysis

Preparations for western blotting, and procedures involved in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) were performed as described previously[32]. The following antibodies were used: anti-CAP1 (ab155079, 1:4000), anti-p65 (ab32536, 1:10000), antiphospho-p65 (ab76302, 1:15000), anti-p38 (ab170099, 1:3000), anti-phospho-p38 (ab195049, 1:1000), anti- β -actin (ab8226, 1:4000), anti-GAPDH (#5174, CST, 1:10000), goat anti-rabbit IgG (ab6721, 1:10000), and goat anti-mouse IgG (ab205719, 1:4000).

Signalling pathway of resistin via CAP1

Chondrocytes were seeded into 6-well plates (at 2×10^5 cells /well), cultured for 24 h, as described previously, and incubated with resistin (500 ng/mL) for 30 min, 60 min, 6 h, or 24 h. Chondrocytes incubated without resistin were used as a control group. The four groups of chondrocytes were as follows: Group 1/Group 2, transfected with CAP1-shRNA and incubated without/with resistin (500 ng/mL, 24 h); Group 3/Group 4, transfected with Control-shRNA and incubated without/with resistin (500 ng/mL, 24 h), were designed to detect resistin-CAP1 signalling pathways. Western blotting was used to examine the expression of proteins in the NF- κ B (p65 and phospho-p65) and p38-MAPK (p38 and phospho-p38) pathways.

Statistical analysis

Results are presented as mean $\pm$ standard deviation. Independent t-test and Wilcoxon Rank Sum test were used to compare values of KOA and HC groups. Levels of serum resistin in the two groups were evaluated by analysis of covariance after adjustment for age, body mass index (BMI), fasting blood glucose levels (FBG), and triglyceride levels (TG). Pearson correlation coefficient was used to analyse correlations between resistin and CAP1 expression for all IHC results. Paired t-test was used to compare expression of CCL3, CCL4, MMP13, and ADAMTS-4 between control-shRNA and CAP1-shRNA groups. For testing differences among more than two groups, one-way ANOVA was first used to test the overall

difference and followed by Bonferroni post-hoc test in various subgroups. $p < 0.05$ was considered statistically significant, and all statistical analyses were performed using SPSS 22.0 (IBM).

Results

Resistin levels in the serum and SF of patients with KOA and HCs

Characteristics of study participants are presented in **Table I**. Serum resistin levels in patients with KOA (8.26 ± 0.29 , ng/mL) were slightly higher than those in healthy controls (7.45 ± 0.30 , ng/mL); however, no significant difference was detected after adjusting for age, BMI, FBG, and TG ($P_{\text{adj}} = 0.688$) (**Fig. 1A**). Higher serum resistin levels were found in female KOA patients than female control groups ($P = 0.044$), whereas there was no significant difference in male subjects between the two groups (**Fig. 1B**). Compared with resistin levels in the SF, serum resistin levels in patients with KOA were higher regardless of gender differences ($P < 0.0001$) (**Fig. 1C-D**). The serum resistin levels of grade III patients did not differ from grade II and IV patients ($P = 0.321$ and $P = 0.096$, respectively); however, the levels of grade II and IV patients differed significantly ($P = 0.021$) (**Fig. 1E**). Female KOA patients with grade III had significantly higher serum resistin level than grade II patients ($P = 0.044$), however no significant difference between different grades of male KOA patients (**Fig. 1F**). The SF resistin levels in grade IV patients were higher than those in grade III and II patients ($P < 0.0001$ and $P < 0.0001$, respectively) (**Fig. 1G**). No matter KOA patients were male or female, patients with grade IV had higher SF resistin levels than both grade III and II patients (male: IV versus III, $P = 0.002$ and IV versus II, $P = 0.009$; female: IV versus III, $P < 0.0001$ and IV versus II, $P < 0.0001$, respectively) (**Fig. 1H**). Results indicate that higher resistin levels are associated with severe KOA.

Expression of resistin and CAP1 in cartilage tissues

Expression of CAP1 (**Fig. 2E, F, and M**) and resistin (**Fig. 2K, L, and N**) was higher in KOA cartilage than that in control cartilage, based on IHC. In 21 KOA samples, CAP1 and resistin expressions showed a significant positive correlation ($r=0.631$, $p=0.002$) (**Fig. 2O**). Compared with control cartilage, the KOA cartilage mRNA expression of CAP1 changed 2.9-fold relative to GAPDH ($p<0.001$) (**Fig. 3A**), and the expression of CAP1 also increased ($p<0.001$) (**Fig. 3B and C**). These findings show that CAP1 expression was elevated in KOA cartilage.

Effect of resistin on chondrocytes and CAP1

As shown in **Fig. 4A-D**, in chondrocytes treated with resistin (500 ng/mL), expressions of CCL3 and MMP13 peaked at 48 h, whereas expressions of CCL4 and ADAMTS-4 peaked at 24 h and gradually declined from there on. Treatment with resistin also induced CCL3, CCL4, MMP13, and ADAMTS-4 expression in a dose-dependent manner; however, MMP13 levels decreased slightly after resistin was administered at 1000 ng/mL, and levels of CCL3 and ADAMTS-4 increased slowly after resistin was administered at 500 ng/mL (**Fig. 4E-H**). Expression of CAP1 was increased when chondrocytes were

stimulated by resistin with dose of 1000 ng/ml at 48 h and 72 h, but no changes were observed at lower dose (**Fig. 4I**).

A Co-IP assay was performed to detect resistin and CAP1 binding. Results indicate that resistin directly interacted with CAP1, and binding was significantly increased in chondrocytes treated with resistin (**Fig. 4J-K**).

Adv-mediated knockdown of CAP1 reduces stimulatory effects of resistin in chondrocytes

Infection efficiency of control-shRNA and CAP1-shRNA (MOI=40) in chondrocytes was greater than 80% as assessed using fluorescence microscopy at 48 h post-transfection (**Fig. 5A-F**). As shown by qRT-PCR and western blotting, CAP1 expression decreased significantly in the CAP1-shRNA group compared to control-shRNA group (**Fig. 5G-I**). Compared with the expression profile of the control-shRNA group, mRNA expression of CCL3, CCL4, MMP13, and ADAMTS-4 was downregulated in the CAP1-shRNA group when cultured with resistin (500 ng/ml) for 48 and 72 h (**Fig. 5J-M**). As shown by ELISA, CAP1 knockdown decreased expression of CCL4 (at 48 h after treatment with resistin) (**Fig. 5O**) and MMP13 (at 72 h after treatment with resistin) (**Fig. 5P**), whereas significant differences were not observed in the expression of CCL3 or ADAMTS-4 (**Fig. 5N, Q**). These results show that CAP1 plays a key role in resistin-induced production of CCL3, CCL4, MMP13, and ADAMTS-4 on mRNA level and CCL4 and MMP13 on protein level in chondrocytes.

Resistin activates p38-MAPK and NF- κ B signalling pathways in chondrocytes

To determine whether p38-MAPK and NF- κ B pathways participate in the proinflammatory response in chondrocytes, we examined untreated chondrocytes and those treated with resistin. After treatment, a significant increase of phospho-p38 and phospho-p65 was found. Resistin-activated protein expression of phospho-p38 peaked 6 h post-treatment and remained level until 24 h post-treatment, whereas activation of phospho-p65 occurred 60 min post-treatment (**Fig. 6A-B**). This indicates that resistin exerts its proinflammatory function by activation of p38-MAPK and NF- κ B pathways in chondrocytes.

Knockdown of CAP1 inhibits p38-MAPK and NF- κ B signalling pathways

A loss-of-function study was performed to examine whether CAP1 regulates activation of p38-MAPK and NF- κ B pathways in response to resistin. Western blot assay showed that CAP1 expression in chondrocytes transfected with CAP1-shRNA was abolished by 90% compared with the control-shRNA group. Expression of phospho-p38 (p-p38) and phospho-p65 (p-p65) was significantly inhibited in the CAP1-knockdown group compared with the control group with or without resistin treatment. Consistent with the above findings, the CAP1-knockdown group showed decreased levels of non-phospho-p38(p-38).

However, non-phospho-p65 (p65) expression was not significantly reduced in CAP1-knockdown group untreated with resistin compared with those treated with resistin and the control-shRNA groups (**Fig. 6C-D**). Our results suggest that resistin activates the p38-MAPK and NF- κ B signalling pathway by binding CAP1 in human chondrocytes.

Discussion

Here, higher levels of resistin were found in the sera and SF of patients with advanced grade (K/L) KOA. The higher CAP1 expression detected in KOA cartilage tissues correlated positively with higher resistin expression. We demonstrated that CCL3, CCL4, MMP13, and ADAMTS-4 were produced by chondrocytes stimulated with resistin. Results of the Co-IP assay show that resistin directly bound CAP1, and resistin treatment increased binding. We investigated the function of CAP1 using a loss-of-function approach and culturing chondrocytes in the presence of resistin. Knockdown of CAP1 downregulated CCL3, CCL4, MMP13, and ADAMTS-4 at the mRNA level, and CCL4 and MMP13 at the protein level. The p38-MAPK and NF- κ B pathways were activated by resistin in chondrocytes; however, this was inhibited by knockdown of CAP1.

Resistin may act in a proinflammatory manner, and has been linked to the development of OA[16, 17, 19, 23]. Resistin levels are associated with the severity of symptoms and radiological cartilage degeneration in KOA patients[33-35]. Consistent with previous studies, serum levels of resistin were higher in KOA patients than in healthy controls, although no significant difference between the two was found after adjustment. This indicates that serum resistin is not a reliable biomarker for the prediction of OA because it may be affected by other factors[36-40]. The levels of SF resistin were far more related than serum levels to the severity of radiographic damage in KOA in agreement with Koskinen, who showed that SF resistin is associated with inflammatory and catabolic factors, and plays a role in KOA pathogenesis[41]. Similar to previous study, in our current study, the levels of resistin also differ regarding gender[42]. Gender differences in serum resistin level between KOA patients and healthy controls may be due to a greater abundance of monocytes in adipose tissue which was more in female patients or the physiological role of hormone between genders, however further studies must be performed to explore these. Additionally, a separate analysis for male and female are also conducted to confirm the above results that serum and SF resistin levels were close correlation with KOA. Thus, these findings indicated us to furtherly explore the mechanism of resistin in the KOA pathogenesis.

Previous studies by Lee et al. and Munjas et al. have shown that in monocytes, CAP1 acts as a functional receptor directly binding resistin and is involved in modulating the proinflammatory activity of resistin[25, 43]. Sato et al. found that CAP1 expression is higher in the synovial tissue of patients with RA compared with those with OA, and resistin increases production of chemokines by binding to CAP1[26]. We demonstrated that CAP1 expression is increased in KOA cartilage compared with control cartilage. Additionally, resistin was highly expressed in KOA cartilage but not in control cartilage. Assessment of KOA cartilage tissues via H-Score and IHC indicated that CAP1 expression was positively associated with resistin. Consistently, in vitro chondrocytes experiment, the upregulation of CAP1 expression also could be induced by higher concentration of resistin. Besides, our Co-IP assay showed that resistin directly bound to CAP1, and binding was increased after treatment with resistin. These findings indicate that resistin may induce KOA via binding to CAP1 receptor.

Consistent with Zhang et al[21, 22], we found that resistin upregulated the CCL3, CCL4, MMP13, and ADAMTS-4. This elevated pattern of expression is implicated in the pathogenesis of OA[44-47]. Knockdown of CAP1 inhibited resistin-induced expression of CCL3, CCL4, MMP13, and ADAMTS-4 at the mRNA level, and CCL4 and MMP13 at the protein level. Conversely, resistin-induced effects on protein and mRNA expression were inconsistent after the silencing of CAP1 in KOA chondrocytes. This may have occurred because CAP1 is not a unique receptor for resistin in human chondrocytes. Current studies show that resistin has another receptor, TLR-4. Therefore, silencing individual receptors only partially abolishes the effects of resistin, although it was only reported in human nucleus pulposus cells and macrophages[30, 48]. We also cannot rule out additional receptors, such as decorin and ROR1, which were not yet found in humans[49, 50]. Although decorin and ROR1 were only reported to play a role in resistin-induced inflammation for mouse, potential role could not be ignored in human chondrocytes. Hence, more future investigations were needed to uncover role of TLR4, decorin and ROR1 in human chondrocytes. But, for now at least, our present study identified that CAP1 acts as a receptor of resistin in chondrocytes, and regulates the resistin-induced pathological progression of KOA.

Activation of signalling pathways is key in the resistin-CAP1 axis. Binding to CAP1 receptor occurs first followed by signal transduction from cytoplasm to the nucleus, causing transcription of genes. The p38-MAPK and NF- κ B pathways are crucial in phenotype loss, inflammatory response, catabolism, and extracellular matrix degradation[51-54]. Resistin increases CCL3 and CCL4 production in human chondrocytes by activating the NF- κ B signalling pathway[21]. In chondrocyte-like human nucleus pulposus cells, p38-MAPK is activated by resistin to upregulate the expression CCL4 and ADAMTS-5[29, 30]. We found that resistin activated the p38-MAPK and NF- κ B pathways via phosphorylation of p38 and p65 in chondrocytes. Furthermore, CAP1 knockdown inhibited the resistin mediated increase of p38-MAPK and NF- κ B activity. We showed that p38-MAPK and NF- κ B pathways participate in resistin-CAP1 activity in human chondrocytes.

In the present study, we firstly discovered that CAP1 expression was significantly increased in KOA cartilage. Furthermore, in human KOA chondrocytes, we also proved that CAP1 was a resistin-binding receptor, and knockdown of CAP1 inhibited resistin-induced products of proinflammatory cytokines and matrix-degrading enzymes. Meanwhile, the p38-MAPK and NF- κ B signalling pathways were found to be activated by resistin-CAP1 axis, and modulate human chondrocytes to lead chronic inflammation and matrix degradation.

However, there were some potential limitations in our study. Firstly, control cartilages were derived from femoral head of FNF patients in our study, since it was hard to obtain healthy knee cartilages. However, previous studies had shown that FNF-cartilage was very similar to that of normal mature articular cartilage as previous literature described[55] and were used as control in many researches[56-60]. Secondly, overexpression of CAP1 in human chondrocytes should be examined to better identify role of CAP1 in resistin-induced pathologic progression of KOA. Thirdly, there were no in vivo experiments in our study. Further research is needed to involve in vivo animal experiment in order to verify function of resistin/CAP1 in KOA.

Conclusion

In conclusion, we showed that resistin induced the expression of proinflammatory cytokines and matrix-degrading enzymes by binding to CAP1 and activating the p38-MAPK and NF- κ B signalling pathways in human KOA chondrocytes (**Fig. 7**). These findings may serve as foundation for the development of novel resistin-targeted therapies for the treatment of patients with KOA.

Declarations

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the conception and design of the study, the drafting and revision of the manuscript. And all authors approved the final version to be submitted. Dr Xin Qi and Dr Cheng-Wu Zhao have full access to all of the data in the research and take responsibility for the integrity.

Ethics approval and consent to participate

The study has been approved in advance by the ethical committee of the First Hospital of Jilin University (reference no: 2017-429). Informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

All data relevant to the study are available from the corresponding author on reasonable request.

References

1. Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, Goldring MB, Goldring SR, Jones G, Teichtahl AJ, Pelletier JP: Osteoarthritis. *Nature reviews Disease primers* 2016, 2:16072.
2. Hunter DJ, Bierma-Zeinstra S: Osteoarthritis. *The Lancet* 2019, 393(10182):1745-1759.

3. Litwic A, Edwards MH, Dennison EM, Cooper C: Epidemiology and burden of osteoarthritis. *British medical bulletin* 2013, 105:185-199.
4. Nicolson PJA, Bennell KL, Dobson FL, Van Ginckel A, Holden MA, Hinman RS: Interventions to increase adherence to therapeutic exercise in older adults with low back pain and/or hip/knee osteoarthritis: a systematic review and meta-analysis. *British journal of sports medicine* 2017, 51(10):791-799.
5. Cross M, Smith E, Hoy D, Nolte S, Ackerman I, Fransen M, Bridgett L, Williams S, Guillemin F, Hill CL *et al*: The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. *Annals of the rheumatic diseases* 2014, 73(7):1323-1330.
6. Vina ER, Kwok CK: Epidemiology of osteoarthritis: literature update. *Current opinion in rheumatology* 2018, 30(2):160-167.
7. Lane NE, Shidara K, Wise BL: Osteoarthritis year in review 2016: clinical. *Osteoarthritis and cartilage* 2017, 25(2):209-215.
8. LA D: phenotypes of osteoarthritis:current state and future implications. *Clinical and experimental rheumatology* 2019.
9. Halawi MJ, Jongbloed W, Baron S, Savoy L, Williams VJ, Cote MP: Patient Dissatisfaction After Primary Total Joint Arthroplasty: The Patient Perspective. *The Journal of arthroplasty* 2019, 34(6):1093-1096.
10. Sokolove J, Lepus CM: Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis* 2013, 5(2):77-94.
11. Rahmati M, Mobasheri A, Mozafari M: Inflammatory mediators in osteoarthritis: A critical review of the state-of-the-art, current prospects, and future challenges. *Bone* 2016, 85:81-90.
12. Mathiessen A, Conaghan PG: Synovitis in osteoarthritis: current understanding with therapeutic implications. *Arthritis research & therapy* 2017, 19(1):18.
13. de Lange-Brokaar BJ, Ioan-Facsinay A, van Osch GJ, Zuurmond AM, Schoones J, Toes RE, Huizinga TW, Kloppenburg M: Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review. *Osteoarthritis and cartilage* 2012, 20(12):1484-1499.
14. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA: The hormone resistin links obesity to diabetes. *Nature* 2001, 409(6818):307-312.
15. Pine GM, Batugedara HM, Nair MG: Here, there and everywhere: Resistin-like molecules in infection, inflammation, and metabolic disorders. *Cytokine* 2018, 110:442-451.
16. de Boer TN, van Spil WE, Huisman AM, Polak AA, Bijlsma JW, Lafeber FP, Mastbergen SC: Serum adipokines in osteoarthritis; comparison with controls and relationship with local parameters of synovial inflammation and cartilage damage. *Osteoarthritis and cartilage* 2012, 20(8):846-853.
17. Choe JY, Bae J, Jung HY, Park SH, Lee HJ, Kim SK: Serum resistin level is associated with radiographic changes in hand osteoarthritis: cross-sectional study. *Joint, bone, spine : revue du rhumatisme* 2012, 79(2):160-165.

18. Van Spil WE, Welsing PMJ, Kloppenburg M, Bierma-Zeinstra SM, Bijlsma JWJ, Mastbergen SC, Lafeber FPJG: Cross-sectional and predictive associations between plasma adipokines and radiographic signs of early-stage knee osteoarthritis: data from CHECK. *Osteoarthritis and cartilage* 2012, 20(11):1278-1285.
19. Fioravanti A, Cheleschi S, De Palma A, Addimanda O, Mancarella L, Pignotti E, Pulsatelli L, Galeazzi M, Meliconi R: Can adipokines serum levels be used as biomarkers of hand osteoarthritis? *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals* 2018, 23(3):265-270.
20. Perruccio AV, Mahomed NN, Chandran V, Gandhi R: Plasma adipokine levels and their association with overall burden of painful joints among individuals with hip and knee osteoarthritis. *The Journal of rheumatology* 2014, 41(2):334-337.
21. Zhang Z, Zhang Z, Kang Y, Hou C, Duan X, Sheng P, Sandell LJ, Liao W: Resistin stimulates expression of chemokine genes in chondrocytes via combinatorial regulation of C/EBPbeta and NF-kappaB. *International journal of molecular sciences* 2014, 15(10):17242-17255.
22. Zhang Z, Xing X, Hensley G, Chang LW, Liao W, Abu-Amer Y, Sandell LJ: Resistin induces expression of proinflammatory cytokines and chemokines in human articular chondrocytes via transcription and messenger RNA stabilization. *Arthritis and rheumatism* 2010, 62(7):1993-2003.
23. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A: Resistin, an Adipokine with Potent Proinflammatory Properties. *The Journal of Immunology* 2005, 174(9):5789-5795.
24. Zhao CW, Gao YH, Song WX, Liu B, Ding L, Dong N, Qi X: An Update on the Emerging Role of Resistin on the Pathogenesis of Osteoarthritis. *Mediators of inflammation* 2019, 2019:1532164.
25. Lee S, Lee HC, Kwon YW, Lee SE, Cho Y, Kim J, Lee S, Kim JY, Lee J, Yang HM *et al*: CAP1-Adenylyl cyclase-associated protein 1 is a receptor for human resistin and mediates inflammatory actions of human monocytes. *Cell metabolism* 2014, 19(3):484-497.
26. Sato H, Muraoka S, Kusunoki N, Masuoka S, Yamada S, Ogasawara H, Imai T, Akasaka Y, Tochigi N, Takahashi H *et al*: Resistin upregulates chemokine production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Arthritis research & therapy* 2017, 19(1):263.
27. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ: Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. *Biochemical and biophysical research communications* 2005, 334(4):1092-1101.
28. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A: Resistin, an adipokine with potent proinflammatory properties. *Journal of immunology (Baltimore, Md : 1950)* 2005, 174(9):5789-5795.
29. Liu C, Yang H, Gao F, Li X, An Y, Wang J, Jin A: Resistin Promotes Intervertebral Disc Degeneration by Upregulation of ADAMTS-5 Through p38 MAPK Signaling Pathway. *Spine* 2016, 41(18):1414-1420.
30. Li Z, Wang X, Pan H, Yang H, Li X, Zhang K, Wang H, Zheng Z, Liu H, Wang J: Resistin promotes CCL4 expression through toll-like receptor-4 and activation of the p38-MAPK and NF-kappaB signaling pathways: implications for intervertebral disc degeneration. *Osteoarthritis and cartilage* 2017, 25(2):341-350.

31. Fukui N, Zhu Y, Maloney WJ, Clohisy J, Sandell LJ: Stimulation of BMP-2 expression by pro-inflammatory cytokines IL-1 and TNF-alpha in normal and osteoarthritic chondrocytes. *The Journal of bone and joint surgery American volume* 2003, 85-A Suppl 3:59-66.
32. Liu B, Gao YH, Dong N, Zhao CW, Huang YF, Liu JG, Qi X: Differential expression of adipokines in the synovium and infrapatellar fat pad of osteoarthritis patients with and without metabolic syndrome. *Connect Tissue Res* 2019, 60(6):611-618.
33. Calvet J, Orellana C, Gratacos J, Berenguer-Llargo A, Caixas A, Chillaron JJ, Pedro-Botet J, Garcia-Manrique M, Navarro N, Larrosa M: Synovial fluid adipokines are associated with clinical severity in knee osteoarthritis: a cross-sectional study in female patients with joint effusion. *Arthritis research & therapy* 2016, 18(1):207.
34. Song YZ, Guan J, Wang HJ, Ma W, Li F, Xu F, Ding LB, Xie L, Liu B, Liu K *et al*: Possible Involvement of Serum and Synovial Fluid Resistin in Knee Osteoarthritis: Cartilage Damage, Clinical, and Radiological Links. *Journal of clinical laboratory analysis* 2016, 30(5):437-443.
35. Han W, Aitken D, Zheng S, Wang B, Wluka AE, Zhu Z, Blizzard L, Wang X, Winzenberg T, Cicuttini F *et al*: Higher Serum Levels of Resistin Are Associated With Knee Synovitis and Structural Abnormalities in Patients With Symptomatic Knee Osteoarthritis. *Journal of the American Medical Directors Association* 2019.
36. Niaz S, Latif J, Hussain S: Serum resistin: A possible link between inflammation, hypertension and coronary artery disease. *Pakistan journal of medical sciences* 2019, 35(3):641-646.
37. Sawicka K, Michalska-Jakubus M, Kowal M, Potembska E, Krasowska D: Resistin: a possible biomarker of organ involvement in systemic sclerosis patients? *Clinical and experimental rheumatology* 2017, 35 Suppl 106(4):144-150.
38. Georgiou GP, Provatopoulou X, Kalogera E, Siasos G, Menenakos E, Zografos GC, Gounaris A: Serum resistin is inversely related to breast cancer risk in premenopausal women. *Breast (Edinburgh, Scotland)* 2016, 29:163-169.
39. Dan S, Aditya P, Banerjee P, Bal C, Roy H, Banerjee I: Effect of chronic kidney disease on serum resistin level. *Nigerian journal of clinical practice* 2014, 17(6):735-738.
40. Adrych K, Smoczynski M, Sledzinski T, Dettlaff-Pokora A, Goyke E, Swierczynski J: Increased serum resistin concentration in patients with chronic pancreatitis: possible cause of pancreatic fibrosis. *Journal of clinical gastroenterology* 2009, 43(1):63-68.
41. Koskinen A, Vuolteenaho K, Moilanen T, Moilanen E: Resistin as a factor in osteoarthritis: synovial fluid resistin concentrations correlate positively with interleukin 6 and matrix metalloproteinases MMP-1 and MMP-3. *Scandinavian journal of rheumatology* 2014, 43(3):249-253.
42. Azamar-Llamas D, Hernandez-Molina G, Ramos-Avalos B, Furuzawa-Carballeda J: Adipokine Contribution to the Pathogenesis of Osteoarthritis. *Mediators of inflammation* 2017, 2017:5468023.
43. Munjas J, Sopic M, Spasojevic-Kalimanovska V, Kalimanovska-Ostic D, Andelkovic K, Jelic-Ivanovic Z: Association of adenylate cyclase-associated protein 1 with coronary artery disease. *European journal of clinical investigation* 2017, 47(9):659-666.

44. Zhao XY, Yang ZB, Zhang ZJ, Zhang ZQ, Kang Y, Huang GX, Wang SW, Huang H, Liao WM: CCL3 serves as a potential plasma biomarker in knee degeneration (osteoarthritis). *Osteoarthritis and cartilage* 2015, 23(8):1405-1411.
45. Yang DW, Qian GB, Jiang MJ, Wang P, Wang KZ: Inhibition of microRNA-495 suppresses chondrocyte apoptosis through activation of the NF-kappaB signaling pathway by regulating CCL4 in osteoarthritis. *Gene therapy* 2019, 26(6):217-229.
46. Wang M, Sampson ER, Jin H, Li J, Ke QH, Im HJ, Chen D: MMP13 is a critical target gene during the progression of osteoarthritis. *Arthritis research & therapy* 2013, 15(1):R5.
47. Tortorella MD, Malfait AM, Deccico C, Arner E: The role of ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) in a model of cartilage degradation. *Osteoarthritis and cartilage* 2001, 9(6):539-552.
48. Zuniga MC, Raghuraman G, Hitchner E, Weyand C, Robinson W, Zhou W: PKC-epsilon and TLR4 synergistically regulate resistin-mediated inflammation in human macrophages. *Atherosclerosis* 2017, 259:51-59.
49. Daquinag AC, Zhang Y, Amaya-Manzanares F, Simmons PJ, Kolonin MG: An isoform of decorin is a resistin receptor on the surface of adipose progenitor cells. *Cell stem cell* 2011, 9(1):74-86.
50. Sanchez-Solana B, Laborda J, Baladron V: Mouse resistin modulates adipogenesis and glucose uptake in 3T3-L1 preadipocytes through the ROR1 receptor. *Molecular endocrinology* 2012, 26(1):110-127.
51. Sondergaard BC, Schultz N, Madsen SH, Bay-Jensen AC, Kassem M, Karsdal MA: MAPKs are essential upstream signaling pathways in proteolytic cartilage degradation—divergence in pathways leading to aggrecanase and MMP-mediated articular cartilage degradation. *Osteoarthritis and cartilage* 2010, 18(3):279-288.
52. Rosenzweig DH, Ou SJ, Quinn TM: P38 mitogen-activated protein kinase promotes dedifferentiation of primary articular chondrocytes in monolayer culture. *Journal of cellular and molecular medicine* 2013, 17(4):508-517.
53. Roman-Blas JA, Jimenez SA: NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. *Osteoarthritis and cartilage* 2006, 14(9):839-848.
54. Marcu KB, Otero M, Olivotto E, Borzi RM, Goldring MB: NF-kappaB signaling: multiple angles to target OA. *Current drug targets* 2010, 11(5):599-613.
55. Burkhardt D: Comparison of chondroitin sulphate composition of femoral head articular cartilage from patients with femoral neck fractures and osteoarthritis and controls. 1995.
56. Kimura T, Okada A, Yatabe T, Okubo M, Toyama Y, Noda M, Okada Y: RECK is up-regulated and involved in chondrocyte cloning in human osteoarthritic cartilage. *The American journal of pathology* 2010, 176(6):2858-2867.
57. Okada A, Mochizuki S, Yatabe T, Kimura T, Shiomi T, Fujita Y, Matsumoto H, Sehara-Fujisawa A, Iwamoto Y, Okada Y: ADAM-12 (meltrin alpha) is involved in chondrocyte proliferation via cleavage

of insulin-like growth factor binding protein 5 in osteoarthritic cartilage. *Arthritis and rheumatism* 2008, 58(3):778-789.

58. Weng LH, Wang CJ, Ko JY, Sun YC, Su YS, Wang FS: Inflammation induction of Dickkopf-1 mediates chondrocyte apoptosis in osteoarthritic joint. *Osteoarthritis and cartilage* 2009, 17(7):933-943.
59. Kozawa E, Cheng XW, Urakawa H, Arai E, Yamada Y, Kitamura S, Sato K, Kuzuya M, Ishiguro N, Nishida Y: Increased expression and activation of cathepsin K in human osteoarthritic cartilage and synovial tissues. *Journal of Orthopaedic Research* 2016, 34(1):127-134.
60. Chijimatsu R, Kunugiza Y, Taniyama Y, Nakamura N, Tomita T, Yoshikawa H: Expression and pathological effects of periostin in human osteoarthritis cartilage. *BMC musculoskeletal disorders* 2015, 16(1).

Figures

Figure 1

Resistin levels in healthy control and KOA groups. (A). Serum resistin levels were higher in radiographic KOA group (KOA), but no significant difference was found after adjustment for age, BMI, FBG, and TG. (B). Higher serum resistin levels were found in female KOA patients than female control groups, whereas there was no significant difference in male subjects between the two groups. (C-D). Patients with KOA showed significantly higher expression of resistin in serum than in synovial fluid regardless of gender differences. (E). Serum resistin levels of grade I patients were higher than those of grade II patients; no significant differences were observed between grade II, grade III, and grade IV patients. (F). Female KOA patients with grade I had significantly higher serum resistin level than grade II patients, however no significant difference between different grades of male KOA patients. (G-H). Synovial fluid resistin levels of grade I patients were higher than those of patients with grade II and grade III in the KOA group and male/female KOA patient group. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, no significant statistical difference.

Figure 2

Immunohistochemical detection of CAP1 and resistin in KOA and control cartilage (obtained from healthy patients with femoral head fracture). Cartilage tissues from CON (A, B, C, G, H, and I) or KOA (D, E, F, J, K, and L) were stained with H&E (A, D, G and J), rabbit anti-CAP1 monoclonal antibody (B, C, E and F), or mouse anti-resistin monoclonal antibody (H, I, K, and L). Expression of CAP1 was significantly increased (M) in the KOA group (E and F) compared with that in HC group (B and C). Resistin expression was much stronger (N) in KOA (K and L) than in CON (H and I). CAP1 expression was positively correlated with resistin expression in KOA cartilage tissues (O) as assessed by ASI digital systems (powered by GenASIs™) using H-Score. Ten CON cartilage sections and 21 KOA cartilage sections were assessed (100

μm, ×100; 20 μm, ×400). CON, control; KOA, knee osteoarthritis; H-Score of each sample was assessed in triplicate; ***, p<0.001.

Figure 3

Expression of CAP1 in KOA and control cartilage. CAP1 expression was evaluated by qRT-PCR in KOA cartilage and control FHF cartilage groups. (A). Expression level of CAP1 was higher in KOA cartilage (N=21 patients with KOA) than in control FHF cartilage (N=10 controls) as assessed using qRT-PCR. (B, C). CAP1 showed higher expression in KOA cartilage than in control FHF cartilage as assessed via western blotting (control FHF cartilage was obtained from three controls; KOA cartilage was obtained from three patients with KOA). CON, control; KOA, knee osteoarthritis; ***, p<0.001.

Figure 4

Time-dependent and dose-response stimulation via resistin in KOA chondrocytes. Chondrocytes from patients with KOA were treated with 500 ng/ml human resistin for 0, 24, 48, or 72 hours (A-D), and exposed to human resistin at 0 ng/ml, 250 ng/ml, 500 ng/ml or 1000 ng/ml for 48 hours (E-H) or 24, 48 and 72 hours (I). mRNA levels of CCL3 peaked at 48 h post-treatment with resistin (A) and increased slowly after stimulation with 500 ng/ml resistin (E). CCL4 levels were induced rapidly at 24 h post-treatment with resistin and then decreased quickly (B). Human resistin promoted the expression of CCL4 in a dose-dependent manner (F). MMP13 levels showed a slower increase and peaked at 48 h post-treatment with resistin (C) and 500 ng/ml (G). ADAMTS-4 expression levels peaked at 24 h post-treatment with resistin (D); resistin moderately increased the expression of ADAMTS-4 mRNA in a dose-dependent manner (H). Expression of CAP1 was increased when chondrocytes were stimulated by resistin with dose of 1000 ng/ml at 48 h and 72 h (I). Co-immunoprecipitation showed that the binding of resistin to CAP1 was significantly increased in the group treated with resistin (labelled as R) compared with those treated with IgG and control (labelled as IgG and C) (J, K); quantified Co-IP results in K were normalized to CAP1. Values are shown as mean $\pm$ SD for three experiments conducted using cells from three patients with

KOA. The relative expression compared with no resistin treatment is shown. Each experiment was performed in triplicate. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Figure 5

Knockdown of CAP1 attenuates resistin-induced expression of CCL3, CCL4, MMP13, and ADAMTS-4 in human chondrocytes. The stable knockdown of CAP1 in human chondrocytes, achieved using adenoviral shRNA vector, was evaluated using fluorescence microscopy. Cell morphology images were captured by inverted microscope (A-C). Images captured by fluorescence microscopy show that transduction efficiency (percentage of GFP-positive cells) was $>80\%$ at 48 h post-transduction (E-F). Expression of CAP1 was decreased significantly when determined by qRT-PCR with β -actin as an internal control (G) and western blot, normalized to β -actin (H-I). qRT-PCR and ELISA were used to compare the expression profiles of control-shRNA and CAP-1-knockdown chondrocytes cultured with human resistin (500 ng/ml) at 48 and 72 h post-knockdown of CAP1. The results show that mRNA levels of CCL3, CCL4, MMP13, and ADAMTS-4 were decreased at both time points in the CAP-1 knockdown group compared with the levels of the control group (J-M). Protein level of CCL4 at both time points, and MMP13 at 72 h was decreased in the CAP-1 knockdown group compared with the levels of the control group (O, P). CCL3 and ADAMTS-4 did not show statistically significant differences at either time point in the CAP1-knockout group compared with the levels of the control group (N, Q). Data are presented as mean $\pm$ standard deviation (SD). shRNA: short hairpin RNA; GFP: green fluorescent protein (magnification $\times 100$); *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, no significant statistical difference.

Figure 6

Resistin activates p38-MAPK and NF- κ B (p65) signalling pathways via CAP1. Western blotting showed an elevation in the phosphorylation level of p38, which increased at 6 h and peaked at 24 h. Phosphorylation level of p65 peaked at 60 min, but declined only slightly until 24 h post-treatment with resistin (A-B) ($n=3$, compared with the Control group). Knockdown of CAP1 abolished resistin-induced increases in the expression of phospho-p38 and phospho-p65, and decreased the levels of non-phospho-p38 and non-phospho-p65 (C-D). ($n=3$, compared with CAP1-shRNA group). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, no significant statistical difference.

Figure 7

The pathogenic mechanism of resistin in KOA Resistin promoted the expression of proinflammatory cytokines (CCL3, CCL4) and matrix-degrading enzymes (MMP-13, ADAMTS-4) by binding to CAP1 and activating the p38-MAPK and NF- κ B signalling pathways in human chondrocytes. The great release of

CCL3, CCL4, MMP-13 and ADAMTS-4 by chondrocytes disrupted intraarticular homeostasis and then induced synovitis and cartilage degradation in knee joint.

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