

High C1r expression is associated with cartilage damage in post-traumatic osteoarthritis

BO DONG^{1,2}, KUI MA³, QI DONG², GUANGYOU YANG², XIAOFEI LI⁴, JIANCHAO GUI¹

¹Department of Sports Medicine and Joint Surgery, Nanjing First Hospital, Nanjing Medical University, Nanjing, 210000, China; ²Department of Joint Surgery, Ganyu District People's Hospital of Lianyungang City, Lianyungang, 222000, China; ³Department of Orthopedics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou, 215000, China; ⁴Department of Joint Surgery, The First People's Hospital of Lianyungang, Lianyungang, 222000, China.

Correspondence at: Jianchao Gui, Department of Sports Medicine and Joint Surgery, Nanjing First Hospital, Nanjing Medical University, No. 68 Changle Road, Qinhuai District, Nanjing City, Jiangsu Province, 210000, China. Tel: 86-025-52271250. Email: Gui1997@126.com.

ABSTRACT

Background: The complement system undergoes extensive activation in osteoarthritis. Nevertheless, the specific function of C1r, a constituent of this system, in post-traumatic osteoarthritis (PTOA) remains unclear.

Purpose: To explore the clinical value and function of C1r in PTOA.

Methods: Synovial fluid was obtained from 90 patients diagnosed with PTOA and 86 patients with knee joint injuries. Three specimens were randomly selected from each group for proteomic examination. The expression of C1r in all these specimens was determined via RT-qPCR, followed by ROC curve analysis and logistic regression analysis. The expression of C1r, apoptosis, and cytokine levels in chondrocytes and mouse cartilage tissues were detected through Western blotting/immunohistochemistry, flow cytometry/TUNEL, and ELISA.

Results: Proteomic analysis unveiled 51 proteins that exhibited down-regulation in PTOA and identified 309 up-regulated proteins, among which C1r was included. RT-qPCR analysis of all samples indicated that C1R was significantly up-regulated in the PTOA cohort and demonstrated a moderate discriminatory ability between PTOA patients and non-PTOA individuals (AUC = 0.777). After adjustment for other confounding factors, C1R was determined to be potentially related to PTOA occurrence. High expression of C1R was correlated with a higher Kellgren-Lawrence grade and a lower Knee Injury and Osteoarthritis Outcome Score. C1r was overexpressed in PTOA cells and mouse cartilage tissues, and its silencing led to a reduction in apoptosis, as well as the levels of inflammatory factors (IL-6/IL-8) and matrix catabolic enzymes (MMP-13/ADAMTS-4) in chondrocytes and cartilage tissues.

Conclusion: C1r serves as a potential candidate biomarker for PTOA and warrants further in-depth exploration. In experimental models, targeting C1r may contribute to the mitigation of cartilage damage.

Keywords: Fracture fixation, hardware removal, unexpected intraoperative positive cultures, low-grade infection, implant colonization.

INTRODUCTION

Osteoarthritis (OA), primarily characterized by the progressive degradation of articular cartilage, synovial inflammation, subchondral bone remodeling, and joint dysfunction, exerts a global influence on the population¹⁻³. Post-traumatic osteoarthritis (PTOA) is a significant subtype of OA that stems from acute joint injuries (e.g., meniscal tears, intra-articular fractures, and anterior cruciate ligament tears), constituting approximately 12% of all OA cases⁴. In contrast to primary OA, PTOA has an abrupt onset and a rapid progression, often affecting young and middle-aged

individuals. Currently, there are no disease-modifying medications capable of decelerating or arresting its progression, and clinical interventions are mainly limited to symptom management and joint replacement in the advanced stages^{5,6}. Therefore, a more profound understanding of the molecular mechanisms underlying PTOA and the identification of biomarkers for early diagnosis and effective intervention targets carry substantial clinical significance.

The complement system, composed of numerous plasma proteins and membrane-bound proteins, is an integral component of the innate immune system. It participates in the body's defense mechanisms and the

regulation of inflammatory responses through various pathways^{7,8}. In recent years, the role of the complement system in the pathogenesis of OA has attracted increasing attention. Research has demonstrated that the levels of complement components are significantly elevated in OA patients, and they show a positive correlation with the degree of cartilage degeneration and inflammatory markers⁹⁻¹⁴. This suggests that the abnormal activation of the complement system may be a critical factor in the pathological process of OA. C1r is one of the key molecules in the classical complement pathway and, in conjunction with C1q and C1s, forms the C1 complex. When C1q recognizes immune complexes or damage-associated molecular patterns, C1r is activated and then cleaves and activates C1s, thereby initiating the complement cascade^{15,16}. A previous proteomics analysis indicated that synovial fluid C1r is the most significantly up-regulated protein in OA patients, and there is a positive correlation between the severity of OA and C1r levels¹⁷. However, it remains unclear whether C1r is specifically dysregulated in PTOA, whether it has clinical utility as a biomarker, and whether it plays a causal role in cartilage injury.

To address these research gaps, this study conducted a systematic assessment of C1r expression, diagnostic significance, and functional mechanism in PTOA. In contrast to previous correlative findings in mixed OA cohorts, this study presents the initial evidence indicating that C1r serves as an independent risk factor and a causative agent in PTOA, thereby providing a potential target for early intervention.

MATERIALS AND METHODS

Clinical participants

Synovial fluid specimens were obtained from 86 patients with knee joint injury and 90 patients with PTOA during their surgical operations at our hospital. Three specimens were randomly selected from each of these two groups for proteomic analysis. PTOA patients were diagnosed based on relevant imaging examinations and clinical manifestations, and the severity of their condition was assessed using the Kellgren - Lawrence classification and the Knee Injury and Osteoarthritis Outcome Score (KOOS). Patients with incomplete clinical records or those suffering from other types of OA or other knee diseases were excluded. The demographic characteristics of these two groups of subjects were comparable, and no significant differences were found (Supplementary Table I). This study was approved by the Ethics

Committee of Nanjing First Hospital, Nanjing Medical University, and all subjects were informed of the research content. The study was carried out in full accordance with the relevant provisions of the Declaration of Helsinki.

Cell culture and treatment

Human chondrocytes (C-28/I2) obtained from Bluefcell Company (China) were cultured in high-glucose Dulbecco's Modified Eagle Medium (MeilunBio, China) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin (Solarbio, China) under growth conditions of 37°C and 5% CO₂. When the cells achieved a confluence of over 90%, they were digested with trypsin-ethylenediaminetetraacetic acid (EDTA) (0.25%) (TargetMol, USA) and then subcultured at a ratio of 1:2.

The C-28/I2 cells (9×10^4) were seeded into each well of a 6-well plate. Following cell attachment, the cells were subjected to a 2-hour stimulation with IL-1 β (10 ng/mL, PeproTech, China) to induce an OA cell model^{18,19}. Subsequently, the cells were transfected with C1R small interfering RNA (siC1R, Ubigene, China) or its negative control (siNC) using the Lipofectamine 3000 transfection reagent (Invitrogen, USA). After 6 hours of transfection, the cells were further cultured in fresh growth medium for 48 hours.

PTOA mouse model

A total of 24 eight-week-old male C57BL/6J mice, supplied by AnipheBio (China), were acclimated for one week under standard rearing conditions before model establishment. The mice were randomly allocated into four groups: the sham group, the PTOA group, the PTOA + siNC group, and the PTOA + siC1R group.

After anesthesia was induced through intraperitoneal injection of pentobarbital sodium (at a dosage of 35 mg/kg, mice in the PTOA group underwent surgery to transect the medial meniscus-tibial ligament of the right knee, thus inducing knee instability and subsequent PTOA. Mice in the sham group underwent open surgery without transecting the medial meniscus-tibial ligament.

Post-operation, a subcutaneous injection of buprenorphine at a dosage of 0.05 mg/kg was administered every 12 hours for pain management, and this treatment was continued for three days. In the PTOA + siNC and PTOA + siC1R groups, the mice received intra-articular injection of siNC or siC1R (20 μ L, 20 μ g) one week after the operation, and the injection was repeated two weeks after the operation.

Eight weeks later, the mice were euthanized via intraperitoneal injection of pentobarbital sodium at a dosage of 150 mg/kg, and their knee cartilage tissue was collected for analysis. All animal-related procedures were conducted in accordance with the “Regulations on the Administration of Laboratory Animals” and were approved by the Animal Ethics Committee of Nanjing First Hospital, Nanjing Medical University.

RT-qPCR

Total ribonucleic acid (RNA) was extracted from synovial fluid samples using the cRNA Easy Kit (Starvio, China). Meanwhile, RNA from C-28/I2 cells or mouse cartilage tissues was extracted by the TRIzol method. After reverse-transcribing the extracted RNA into cDNA with the AdvanceFast 1st Strand cDNA Synthesis Kit (YEASEN, China), gene amplification was performed using the Maxima SYBR Green qPCR premixed solution (Thermo Fisher Scientific). The relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method, with GAPDH as the internal reference.

Western blotting

Subsequent to cell processing, RIPA lysis buffer (Beyotime, China) supplemented with 1% phenylmethylsulfonyl fluoride (MedChemExpress, USA) was introduced to the cells and incubated on ice for 30 minutes to facilitate protein extraction. After centrifugation at 12,000 g for 20 minutes at 4°C, the supernatant (total protein solution) was collected, and its concentration was determined via a BCA assay kit (Solarbio). Forty micrograms of protein samples were subjected to SDS-PAGE. After the proteins were transferred onto the PVDF membrane, the membrane was blocked with 5% skimmed milk for 2 hours. Subsequently, it was incubated with the primary antibody against C1r (1:1000, Proteintech, China) or GAPDH (1:1500, Proteintech) at 4°C overnight. On the next day, following a 1.5-hour incubation of the membrane with the secondary antibody (1:10000, Proteintech, China), the protein signals were detected using ECL luminescent solution (Beyotime) under a chemiluminescence instrument, and grayscale analysis was performed using ImageJ software.

Cell apoptosis assay

An Annexin V-FITC/PI apoptosis detection kit (Elabscience, China) was utilized for the detection of cell apoptosis. The experimental procedure is as follows: After treatment, the cells were collected

subsequent to digestion with EDTA-free trypsin. Subsequently, they were resuspended in $1 \times$ binding buffer (at a cell density of 1×10^6 cells/mL. A 100- μ L sample of the cell suspension was transferred to a flow cytometry tube. Then, 5 μ L of Annexin V-FITC and 10 μ L of propidium iodide were added, followed by a 15-minute incubation period. Next, the fluorescence signals were detected by means of flow cytometry within 1 hour. The data were analyzed using FlowJo software.

ELISA assay

The cell culture supernatant was acquired after centrifuging the cell medium at 300 g for 10 minutes. After the cartilage tissue was thoroughly pulverized in liquid nitrogen, it was homogenized with lysis buffer, and the supernatant was obtained after complete centrifugation (at 12000 g for 20 minutes. The levels of cytokines in the aforementioned samples were detected using commercial sandwich ELISA kits as per the instructions: human/mouse IL-6/IL-8 ELISA kit (Abcam, UK), human/mouse MMP-13 ELISA kit (Elabscience), human/mouse ADAMTS-4 ELISA kit (CUSABIO, China).

Immunohistochemistry

The murine cartilage tissue was fixed in 4% paraformaldehyde (Beyotime) for 24 hours. Subsequently, it was decalcified with 20% EDTA (MedChemExpress) for a period of two weeks. Thereafter, the tissue was embedded in paraffin and sectioned. Following the procedures of baking, dewaxing, and rehydration, the sections were incubated with the C1r primary antibody (1:100, Proteintech) overnight at 4°C. Subsequently, the sections were incubated with a secondary antibody (OriGene, USA) for one hour. After the sections were stained with 3,3'-diaminobenzidine (Sigma-Aldrich, USA) and counterstained with hematoxylin (Beyotime) to highlight the cell nucleus, they were dehydrated with ethanol, cleared with xylene, and mounted with neutral resin. Finally, images were captured using an optical microscope and analyzed using ImageJ software.

TUNEL assay

The aforementioned wax-embedded section was affixed to a glass slide pre-coated with polylysine. The TUNEL assay was conducted in accordance with the instructions of the commercially available TUNEL detection kit (Epizyme, China). After deparaffinizing the sections with xylene and rehydrating them using

a gradient of ethanol, the sections were incubated with proteinase K (20 µg/mL) at 37°C for 20 minutes. After being washed three times with PBS, 50 µL of the TUNEL reaction mixture was added and incubated at 37°C for 60 minutes. Subsequently, the sections were counterstained with DAPI at room temperature for 5 minutes to label the nucleus. After mounting the sections with an anti-fade mounting medium (Beyotime), the sections were examined under a fluorescence microscope. The apoptosis rate was calculated using ImageJ software.

Statistical analysis

Data analysis and statistical graph generation were carried out using GraphPad 8.0 or SPSS software. Continuous measurement data were described by the mean $\pm$ standard deviation and analyzed through the Wilcoxon test, Student's t test, and one-way/two-way analysis of variance. In contrast, categorical data were presented as the number (%), and the chi-square test was employed for analysis. A p-value less than 0.05 was considered statistically significant.

RESULTS

Proteomics analysis

Principal component analysis (PCA) demonstrated that the two sample groups could be clearly discriminated by the first principal component (PC1) (Figure 1A), indicating that inter-group disparities serve as the primary determinant of the total data variance. This validates the experimental grouping and exhibits favorable biological reproducibility. In the PTOA and CONT groups, 360 differentially expressed proteins were identified. Among these, in the PTOA cohort, 309 proteins were highly expressed and 51 were lowly expressed (Figure 1B), and C1r was one of the highly expressed proteins (Figure 1C). GO analysis (Figure 1D) of these differentially expressed proteins revealed their involvement in biological processes such as protein stabilization/folding, complement activation, and glycolysis. They are associated with cellular components, including extracellular exosomes, cytosol, and extracellular region/space, and execute molecular functions such as identical protein binding, RNA binding, and cadherin

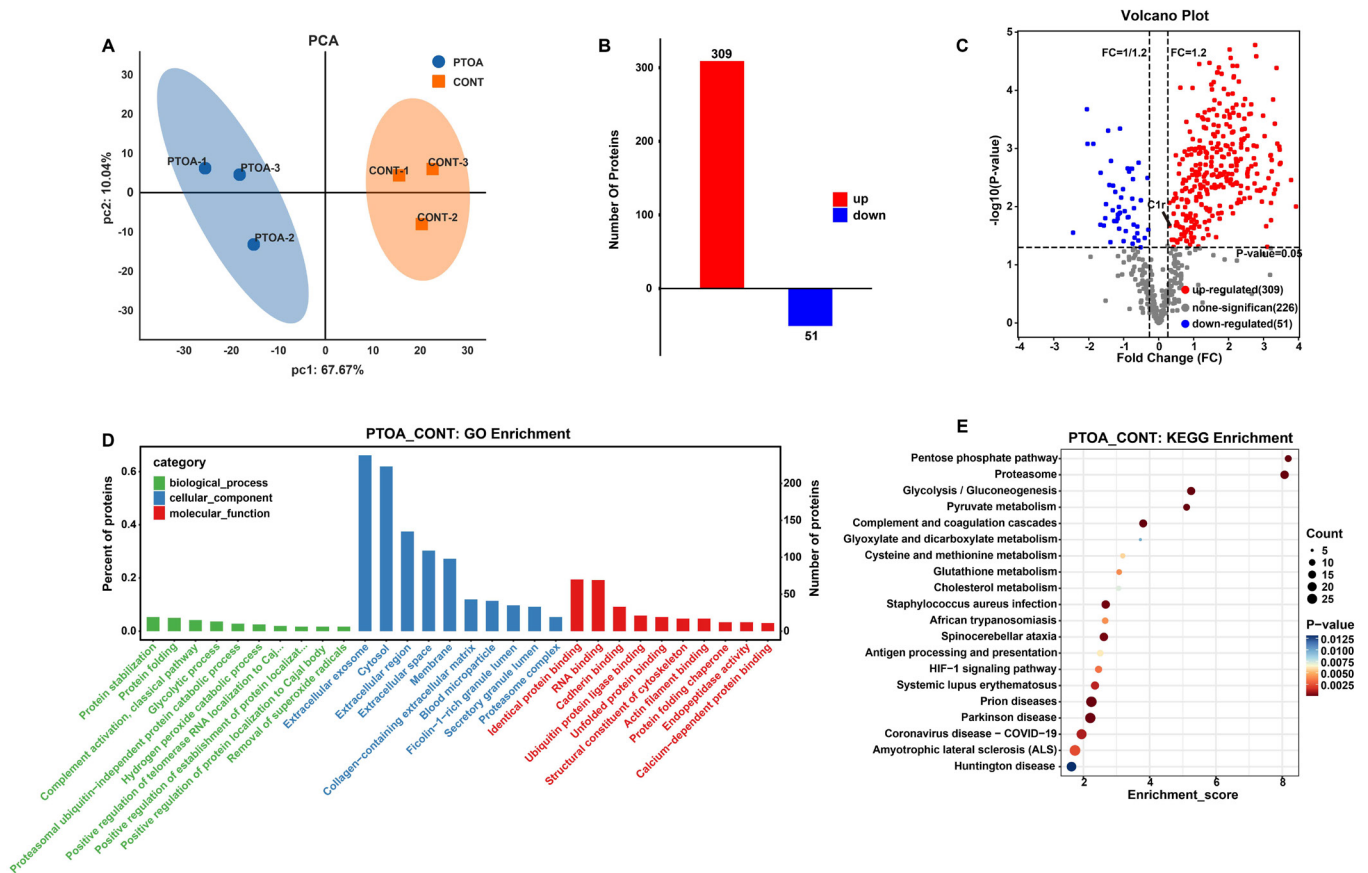


Fig. 1 — Proteomic analysis based on the synovial fluid of patients with knee joint injury (CONT) and PTOA patients. (A) Evaluation of sample grouping effectiveness. (B-C) Differentially expressed proteins between the two sample groups. GO (D) and KEGG (E) analysis of differentially expressed proteins.

binding, etc. KEGG analysis (Figure 1E) showed that these proteins are enriched in signaling pathways such as the pentose phosphate pathway, proteasome, glycolysis/gluconeogenesis, pyruvate metabolism, complement and coagulation cascades.

High expression of C1R may be associated with PTOA occurrence

In comparison to non-PTOA individuals, the expression of C1R was significantly upregulated in the synovial fluid of PTOA patients (Figure 2A). Receiver operating characteristic (ROC) analysis based on this expression data indicated that C1R exhibited a moderate discriminatory ability between non-PTOA and PTOA individuals (AUC = 0.777, sensitivity = 70.0%, specificity = 75.6%, Figure 2B). Further multivariate logistic regression analysis demonstrated that C1R may be associated with increased risk of PTOA occurrence after adjusting for other clinical characteristics and confounding factors (odds ratio = 1.966, $p = 0.007$, Table I), suggesting that each unit increase in C1R

expression was associated with an approximate 96.6% increase in the odds of having PTOA.

Based on the mean of C1R expression level, PTOA patients were classified into low- and high-expression groups for further analysis. The chi-square test indicated that a higher proportion of patients with a high Kellgren-Lawrence grade was observed in the C1R high-expression group (Table II). The Wilcoxon test revealed that patients with high C1R expression had lower KOOS scores and higher levels of erythrocyte sedimentation rate (ESR) and high-sensitivity C-reactive protein (hs-CRP) in the synovial fluid (Table II). These findings suggest that elevated C1R expression may be related to more severe PTOA degree and an inflammatory status.

Silencing C1R may alleviate cartilage damage

In an IL-1 β -induced OA cell model, the expression of C1r was significantly upregulated. Furthermore, its expression was markedly inhibited subsequent to

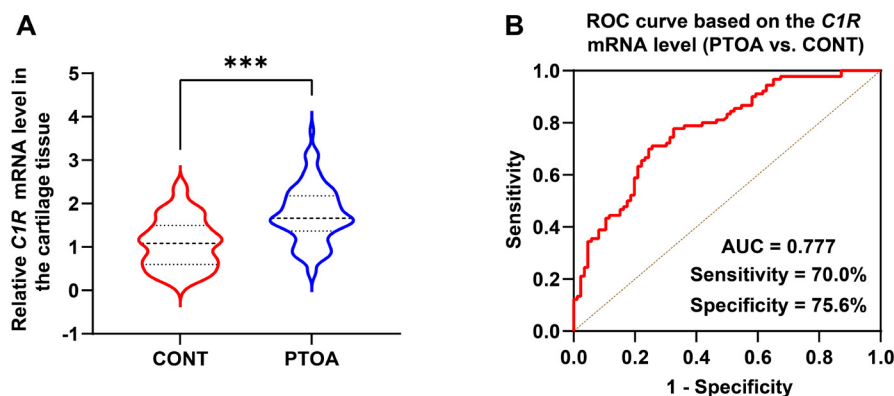


Fig. 2 — Clinical value assessment of C1R expression. (A) Expression of C1R in synovial fluid of patients with knee joint injury or PTOA. (B) ROC curve distinguishing knee joint injury and PTOA patients based on C1R expression levels in cartilage. *** $p < 0.001$.

Table I. — Multiple logistic regression analysis (PTOA vs. CONT).

Characteristics	Odds ratio	95% confidence interval		p-value
		Lower	Higher	
Age	0.999	0.964	1.035	0.940
Gender	0.647	0.336	1.247	0.194
BMI	1.072	0.976	1.177	0.145
Smoking	1.056	0.547	2.041	0.870
Drinking	1.151	0.597	2.221	0.674
ESR	1.087	1.018	1.161	0.013
hs-CRP	2.857	1.286	6.348	0.010
C1R	1.966	1.206	3.206	0.007

PTOA, post-traumatic osteoarthritis; CONT, patients with knee joint trauma and without osteoarthritis; BMI, body mass index; ESR, erythrocyte sedimentation rate; hs-CRP, high-sensitivity C-reactive protein.

Table II. — The correlation between the expression level of C1r and various clinical characteristics of PTOA patients.

Characteristics		Total	C1R expression		p-value
			Low (n = 48)	High (n = 42)	
Age	Years	45.6 ± 8.76	46.0 ± 8.43	45.2 ± 9.11	0.692
Gender	Female	39 (43.3)	20 (22.2)	19 (21.1)	0.733
	Male	51 (56.7)	28 (31.1)	23 (25.6)	
BMI	kg/m ²	21.3 ± 3.61	21.7 ± 3.40	20.9 ± 3.78	0.293
Smoking	No	50 (55.6)	27 (30.0)	23 (25.6)	0.887
	Yes	40 (44.4)	21 (23.3)	19 (21.1)	
Drinking	No	38 (42.2)	21 (23.3)	17 (18.9)	0.754
	Yes	52 (57.8)	27 (30.0)	25 (25.8)	
Kellgren-Lawrence	III	47 (52.2)	33 (36.7)	14 (15.6)	0.001
	IV	43 (47.8)	15 (16.7)	28 (31.1)	
KOOS	Score	45.9 ± 15.72	50.2 ± 16.01	41.1 ± 13.90	0.006
ESR	mm/h	23.3 ± 5.06	21.0 ± 4.66	26.0 ± 4.06	< 0.001
hs-CRP	mg/L	0.82 ± 0.43	0.65 ± 0.35	1.02 ± 0.43	< 0.001

The continuous data were presented as the mean ± standard deviation, and were analyzed using the Wilcoxon test. The categorical data were presented in terms of number (%), and were analyzed using the chi-square test.
PTOA, post-traumatic osteoarthritis; BMI, body mass index; KOOS, knee injury and osteoarthritis outcome score; ESR, erythrocyte sedimentation rate; hs-CRP, high-sensitivity C-reactive protein.

transfection with small interfering RNA targeting C1r (siC1R) (Figure 3A). As the expression of C1r increased, the apoptosis rate in the OA cell model and the secretion levels of IL-6, IL-8, MMP-13, and ADAMTS-4 also increased significantly. These alterations were concurrently reversed by siC1R transfection (Figure 3B-C). Similarly, the expression of C1r was elevated in the cartilage tissues of PTOA mice and was suppressed following treatment with siC1R injection (Figure 3D). Higher apoptosis rates and elevated levels of IL-6, IL-8, MMP-13, and ADAMTS-4 were observed in the cartilage tissues of PTOA mice. All of these were reduced after siC1R treatment as a result of the downregulation of C1r (Figure 3E-F).

DISCUSSION

Over the past ten years, an increasing amount of evidence has confirmed that the complement system actively participates in the pathogenesis of OA. Elevated levels of complement activation products, including C4d, C3bBbP, and sTCC, have been detected in the synovial fluid of patients with knee injury and OA, with C4d remaining at an elevated level for years after the injury¹¹. Animal studies simulating anterior cruciate ligament injury and meniscal tear have revealed an increased expression of C3aR1 and C5aR1 in synovial cells⁹, and the genetic deficiency of C3aR1 or C5aR1 protects mice from OA induction²⁰. Moreover, the levels of C3a and C5b-9 in synovial fluid

are correlated with the degree of cartilage degeneration, and C5 deficiency exerts joint-protective effects in animal models¹². Inhibition of complement C3 or factor D also decelerates the progression of OA^{21,22}.

Despite these advancements, the role of the highly upstream complement component C1r, which initiates the classical pathway, has remained largely uninvestigated in PTOA. Specifically, it is unclear whether C1r is dysregulated in PTOA, whether it has clinical diagnostic or risk assessment value, and whether it directly contributes to cartilage damage. In this study, we systematically address these research gaps. Through proteomic screening followed by validation in a larger cohort, we demonstrate for the first time that synovial fluid C1r is significantly over-expressed in PTOA patients and is closely associated with disease onset. Clinically, C1r can effectively distinguish PTOA patients from non-PTOA individuals (ROC analysis) and may be associated with an increased risk of PTOA in multivariate regression. Thus, beyond confirming the general involvement of the complement system in OA, this study expands the existing literature by identifying C1r as a previously unrecognized key participant specifically in PTOA and as a potential intervention target.

The pathogenesis of PTOA involves the overexpression of catabolic enzymes, the release of damage-associated molecular patterns, and synovial inflammation, which is characterized by increased infiltration of immune cells and elevated levels of

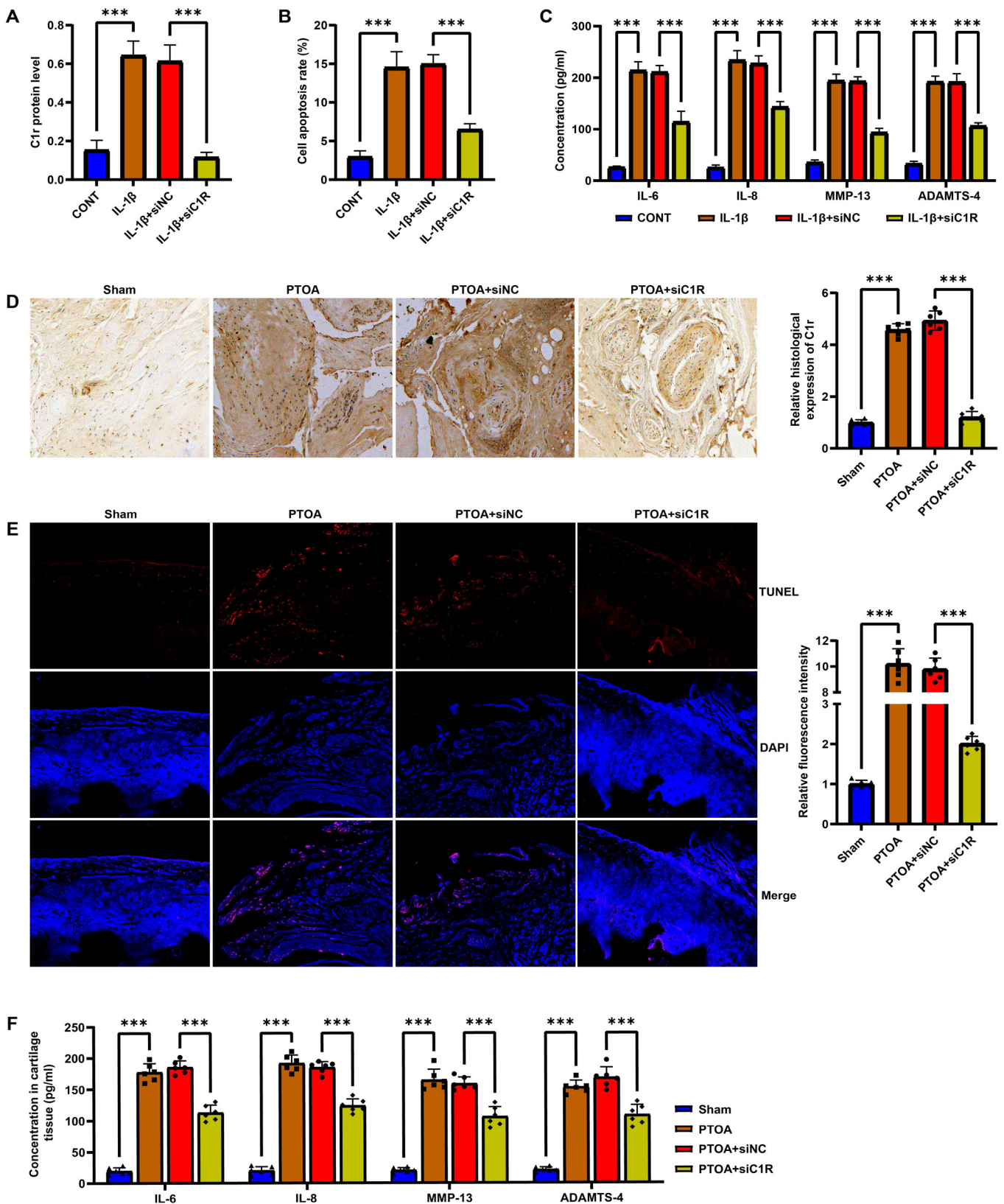


Fig. 3 — The role of C1r in PTOA-related cartilage damage. (A) The western blotting experiment was used to detect the expression of C1r in IL-1 β -treated C-28/I2 cells and transfection efficiency of siC1R. (B) The Annexin V-FITC/PI method was used to detect C-28/I2 cell apoptosis rate following IL-1 β treatment and siC1R transfection. (C) ELISA assay was for concentration detection of IL-6, IL-8, MMP-13, and ADAMTS-4 in the culture medium of C-28/I2 cells treated with IL-1 β and transfected with siC1R. (D) Immunohistochemistry was used to detect the expression of C1r in mouse cartilage tissue. (E) The TUNEL method was for apoptosis detection in mouse cartilage tissue. (F) The levels of IL-6, IL-8, MMP-13, and ADAMTS-4 in mouse cartilage tissue detected via ELISA assay. *** $p < 0.001$.

pro-inflammatory cytokines²³⁻²⁵. It is worth noting that patients with high C1r expression presented with higher Kellgren-Lawrence grades and lower KOOS scores, along with significantly elevated ESR and hs-CRP levels. This suggests that C1r may be closely associated with disease severity and joint inflammation. In an IL-1 β -induced chondrocyte inflammation model, the expression of C1r was significantly upregulated. When C1r was silenced, the expression of pro-inflammatory factors (such as IL-6 and IL-8) and matrix catabolic enzymes (such as MMP-13 and ADAMTS-4) decreased significantly, and cell apoptosis was reduced. This implies that C1r may participate in the pathological process of PTOA by modulating inflammatory responses and matrix degradation. In animal models, an elevated expression of C1r was also observed in the cartilage tissue of PTOA mice. Local injection of siC1r effectively reduced its expression and significantly decreased the levels of apoptosis and inflammation in the cartilage tissue. These results are highly consistent with those obtained from in vitro experiments. During the progression of OA, the excessive release of IL-6, IL-8, TNF- α , matrix metalloproteinases (MMPs), and ADAMTS4/5 exacerbates synovial inflammation, cartilage degeneration, and even leads to the structural deterioration of the joint^{26,27}. The activation of the complement system in joint tissues may form a vicious cycle by enhancing the local inflammatory response, inducing chondrocyte damage, and promoting the expression of matrix catabolic enzymes, thereby accelerating cartilage destruction²⁸⁻³⁰. As a key initiator of the classical complement pathway, the upregulation of C1r may amplify the complement cascade, thus exacerbating joint damage.

Although this study has verified the role of C1r in PTOA at multiple levels, certain limitations must be recognized. First, the proteomic discovery cohort was based on a relatively small number of synovial fluid samples, which may have introduced selection bias and increased the risk of false-positive findings. While this limitation was partly mitigated by subsequent validation in a larger cohort, the exploratory nature of the discovery phase should still be considered. Second, the animal model used in this study involved only a relatively short-term observation period following PTOA induction, which limits our ability to assess the long-term effects or chronic progression of C1r-related pathological changes. Third, the upstream regulatory mechanisms of C1r remain unclear, including whether it is regulated by mechanical stress, inflammatory factors, or feedback within the complement system. Fourth, causal evidence is lacking. This study did not

use C1r knockout animals or specific inhibitors, so we cannot definitively establish that C1r plays a causal role in PTOA, nor can we conclude that it is a validated therapeutic target. Fifth, the mechanism of action of C1r - whether through the complement-dependent pathway or the non-complement pathway (such as the Wnt/ β -Catenin pathway³¹) - requires further investigation. Fifth, the clinical significance of C1r needs to be further confirmed. The clinical value of C1r should be validated in an independent prospective cohort before any potential clinical application.

CONCLUSION

In conclusion, C1r exhibits a significant up-regulation in PTOA. Silencing C1r could effectively mitigate chondrocyte apoptosis and inflammatory responses, and alleviate cartilage tissue damage both in vitro and in vivo. C1r may serve as a potential candidate biomarker for the early detection of PTOA, subject to validation in larger, prospective cohorts. This study offers preliminary experimental evidence to support the further exploration of complement-targeting strategies, including those targeting C1r, for PTOA intervention.

Ethics approval and consent to participate: The Ethics Committee of Nanjing First Hospital, Nanjing Medical University approved this study, and all subjects were informed of the content of this research. The study was conducted in full compliance with the relevant provisions of the Declaration of Helsinki.

All animal processes were conducted in accordance with the “Regulations on the Administration of Laboratory Animals” and have been approved by the Animal Ethics Committee of Nanjing First Hospital, Nanjing Medical University.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: BD and JCG conceptualized and designed the study. BD, KM, QD, XFL and GYY collected, organized, and drafted the information. BD and KM analyzed the data. BD wrote the manuscript. JCG performed manuscript revision. All the authors have read and approved the manuscript.

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Supplementary Table I. — The basic characteristics of the subjects.

Characteristics		CONT (n = 86)	PTOA (n = 90)	p-value
Age	Years	46.2 ± 9.64	45.6 ± 8.76	0.699
Gender	Female	46 (26.1)	39 (22.2)	0.178
	Male	40 (22.7)	51 (29.0)	
BMI	kg/m ²	20.4 ± 3.39	21.3 ± 3.61	0.081
Smoking	No	48 (27.3)	50 (28.4)	0.972
	Yes	38 (21.6)	40 (22.7)	
Drinking	No	41 (23.3)	38 (21.6)	0.467
	Yes	45 (25.6)	52 (29.5)	
Kellgren-Lawrence	III	-	47 (52.2)	-
	IV	-	43 (47.8)	
KOOS	Score	-	45.9 ± 15.72	-
ESR	mm/h	19.1 ± 4.68	23.3 ± 5.06	< 0.001
hs-CRP	mg/L	0.53 ± 0.36	0.82 ± 0.43	< 0.001
The continuous data were presented as the mean ± standard deviation, and were analyzed using the Wilcoxon test. The categorical data were presented in terms of number (%), and were analyzed using the chi-square test. CONT, patients with knee joint trauma and without osteoarthritis; PTOA, post-traumatic osteoarthritis; BMI, body mass index; KOOS, knee injury and osteoarthritis outcome score; ESR, erythrocyte sedimentation rate; hs-CRP, high-sensitivity C-reactive protein.				

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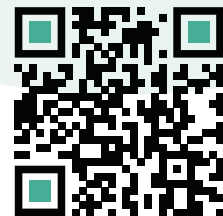
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