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Research Article

Research Article

CD5L aggravates rheumatoid arthritis progression via promoting synovial fibroblasts proliferation and activity

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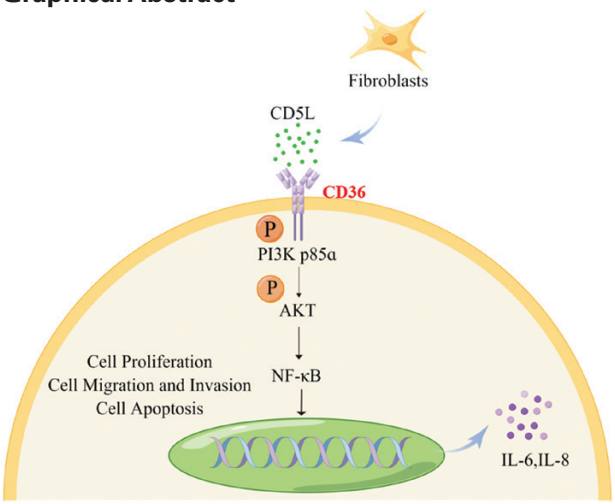
Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease with progressive cartilage erosion and joint destruction. Synovial fibroblasts (SFs) play a crucial role in the pathogenesis of RA. This study aims to explore the function and mechanism of CD5L during RA progression. We examined the levels of CD5L in synovial tissues and SFs. The collagen-induced arthritis (CIA) rat models were used to investigate the effect of CD5L on RA progression. We also investigated the effects of exogenous CD5L on the behavior and activity of RA synovial fibroblasts (RASFs). Our results showed that CD5L expression was significantly upregulated in synovium of RA patients and CIA-rats. Histology and Micro-CT analysis showed that synovial inflammation and bone destruction were more severe in CD5L-treated CIA rats compared with control rats. Correspondingly, CD5L blockade alleviated bone damage and synovial inflammation in CIA-rats. The exogenous CD5L treatment promoted RASFs proliferation invasion and proinflammatory cytokine production. Knockdown of CD5L receptor by siRNA significantly reversed the effect of CD5L treatment on RASFs. Moreover, we observed that CD5L treatment potentiated PI3K/Akt signaling in the RASFs. The promoted effects of CD5L on IL-6 and IL-8 expression were significantly reversed by PI3K/Akt signaling inhibitor. In conclusion, CD5L promote RA disease progression via activating RASFs. CD5L blocking is a potential therapeutic approach for RA patients.

Graphical Abstract



Keywords: rheumatoid arthritis, synovial fibroblasts, CD5L, PI3K/Akt, cytokine

Abbreviations: BMD: bone mineral density; CIA: collagen-induced arthritis; ELISA: enzyme-linked immunosorbant assay; HRP: horseradish peroxidase; IF: immunofluorescence; IHC: immunohistochemistry; IL-6: interleukin 6; IL-8: interleukin 8; OA: osteoarthritis; OASFs: osteoarthritis synovial fibroblasts; PBMC: peripheral blood mononuclear cells; RA: rheumatoid arthritis; RASFs: rheumatoid arthritis synovial fibroblasts; SFs: synovial fibroblast; SRCR: scavenger receptor cysteine-rich; TNF-α: tumor necrosis factor α; WB: Western blot.

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Introduction

Rheumatoid arthritis (RA) is a common chronic autoimmune disease, characterized by synovial inflammation, hyperplasia, and pannus formation, which can lead to irreversible joint destruction and permanent disability [1–3]. The etiology of RA was complex and remains unclear. Synovial fibroblasts (SFs) are critical in driving synovial inflammation and cartilage damage during RA development [4]. Previous studies suggested that the numbers and activity of synovial fibroblasts are often increased in the hyperplastic synovium of RA patients [5, 6]. The activated SFs of RA patients (RASFs) showed aggressive tumor-cell-like properties, exhibiting high proliferative activity and apoptosis inhibition. Moreover, RASFs also secrete a variety of inflammatory cytokines and matrix proteolytic enzyme, which are involved in the inflammatory response during RA progression [7, 8]. Thus, the molecule which could promote proliferation or activation of RASFs might play important roles in RA development and could be a potential therapeutic target for RA.

CD5L, also known as apoptosis inhibitory factor of macrophage (AIM), belongs to the cysteine scavenger receptor (SRCR) superfamily and is mainly expressed by macrophages, lymphocytes, and inflammatory tissues [9, 10]. CD5L acts as a regulator of lipid synthesis and plays an important role in regulating inflammatory responses. CD36, the major cell surface receptor of CD5L, is widely expressed on a variety of cells, including adipocytes, endothelial cells, and fibroblasts [11]. After binding to CD36, CD5L activates intracellular signaling pathways and regulates cell biological behavior and expression profile. Accumulating evidences have indicated that CD5L levels were significantly changed in a variety of diseases caused by acute or chronic inflammation, including infectious, metabolic, and autoimmune diseases [12–14]. Our previous study have showed that CD5L levels in serum and synovial fluid from RA patients were elevated and positively correlated with disease activity [15]. However, the potential function of CD5L during RA progress remains unclear. In this study, we investigated the effect of CD5L on RA disease progression and explored the underlying mechanisms.

Material and methods

Peripheral Blood and Synovial Tissues Collection

The peripheral blood of healthy controls (HC), osteoarthritis (OA), and RA patients was collected from the Department of Laboratory, Tangdu Hospital, Airforce Medical University (Xian, Shaanxi, China). Then, Peripheral Blood Mononuclear Cells (PBMC) were separated using Ficoll-Paque (Haoyang, Tianjin, China) density gradient centrifugation according to the operation manual.

Synovial tissues were obtained from patients with RA (n = 5) and OA (n = 5) who undergoing joint replacement. All RA and OA patients satisfied the clinical and radiographic criteria of the American College of Rheumatology [16, 17]. The present study was approved by the Medical Ethics Committee of the Tangdu hospital, Fourth Military Medical University (No. 202203-046).

Immunohistochemistry (IHC) analyses

IHC analysis were used to detect the CD5L protein levels in RASFs and OASFs. The primary antibodies targeted against human CD5L (Abcam, ab45408) with a ultrasensitive polymer method detection systems according to the instructions of the manufacturer (ZSGB-Bio, Beijing,China).

Immunofluorescence (IF) analyses

The rats were killed by isoflurane anesthesia at 35 days, and the ankle joints were decalcified, embedded, and sectioned. The tissue was fixed with 4% paraformaldehyde at room temperature for 20 min and incubated with anti-rat CD5L (Abcam, ab45408) and CD36 (Servicebio, GB112562) antibody at 4°C overnight. The tissue slides were then incubated with FITC-conjugated and PE-conjugated secondary antibody for 1 h and with DAPI (Beyotime, Shanghai, China). The slides were scanned using Pannoramic scanner (3D Histech Ltd, Hungary).

Collagen-Induced Arthritis (CIA) Rat Model

The CIA model was established as described previously [18]. Female Sprague-Dawley (SD) rats (6–8 weeks; 180–220 g) were purchased from Animal Experiment Center, Fourth Military Medical University, and handled in accordance with approved institutional Animal Care and Use Committee protocols (No. 20220652). Rats were immunized with chicken type II collagen (Chondrex, MA, USA) emulsified in complete Freund's adjuvant (Chondrex, MA, USA) at 3–4 points from the tail root to the back, followed by a booster immunization 7 days later. The control group rats were injected with an equal volume of complete Freund's adjuvant emulsion. The development of CIA was monitored every 3 days from 14 days by visually assessing and scoring the paws of these rats. Each paw was graded from 0 to 4 as follows: 0 = normal; 1 = erythema and mild swelling; 2 = erythema and swelling extending to ankle joints and one or two toes; 3 = erythema and swelling extending to metatarsal joints and more than two toes; and 4 = ankylosing deformity with joint swelling. The arthritic index of four paws were summed, and rats with a score greater than 2 were selected as the CIA model.

The recombinant CD5L (Cat: 50020-M08H, SinoBiological Inc., China) was administered via intraperitoneally (i.p.) injection (25 μg/kg) once every 3 days in CIA rats, PBS i.p. as a control group. All rats were sacrificed on day 35 after the first collagen immunization. The left ankle joints were fixed with 4% paraformaldehyde solution. Hematoxylin and eosin (H&E) staining and Safranin O/Fast Green staining were used to evaluate synovial tissue inflammation and articular cartilage destruction.

Micro-CT Scanning

To further evaluate bone damage, the right ankle joints of rats sacrificed at day 35 were scanned using the Perkin Elmer Quantum GX microCT Imaging System (Waltham, MA, USA). Three-dimensional reconstruction was performed using the volume rendering method and calculated the bone mineral density (BMD).

Synovial Fibroblasts (SFs) Isolation and Culture

SFs were isolated from synovial tissues of patients as previously described [19]. In short, the synovial tissues were minced and digested with 4 mg/ml type I collagenase (Shanghai Sangon Biotech, China) in Dulbecco's modifed Eagle's medium (DMEM) at 37°C for 2 h. The suspensions were filtering using a disposable nylon mesh (75 μm). Then, SFs were harvested and cultured in DMEM supplemented

with 10% fetal bovine serum (FBS, ExCellBio, Shanghai, China), and 1% penicillin and streptomycin (Hyclone, Logan, USA).

Flow cytometry (FCM) was used to detected the purity of isolated rheumatoid arthritis synovial fibroblasts (RASFs), by using FITC-CD68 (cat: FHF068, 4A Biotech Co., Ltd, Beijing, China) and PE-CD90 (cat: FHP090, 4A Biotech Co., Ltd, Beijing, China) antibody according to the operation manual. RASFs at passages 3–5 were selected for subsequent experiments.

Cell Transfection

The small interfering RNA (siRNA) targeting human CD36 (siCD36) were designed and synthesized by Sangon Biotech (Shanghai, China). The transfection was performed in RASFs when cells confluency was reached approximately 60%–70%. Transfection efficiency was validated by qRT-PCR. The siRNA is described in Table 1.

Cell Viability Assay

RASFs cells were seeded in 96-well plate with a density of 2 × 10^3 cells/well and incubation 12 h to allow cells adherence. RASFs were treated with gradient concentration of CD5L protein (Cat: 10791-H08H, SinoBiological Inc., China), including 0.5 , 1.0, and 2.0 μg/ml. Cell viability was evaluated by measuring cell number using the Cell Imaging System (Cytation1, Biotek, VT, USA).

Cell Invasion and Migration Assays

Cell invasion and migration assay were performed using transwell chamber (Corning, NY, USA). For invasion assay, cells (1 × 10^5 cells/well) were added to the upper matrigel-coated chamber and inserted into 24-well plates in DMEM containing 20% FBS. After incubation at 37°C for 48 h, the filters were fixed with 75% ethanol for 10 min and stained with 0.1% crystal violet for 30 min. A cotton swab was used to gently remove cells from the upper surface of the membrane. Then, 5 randomly selected fields in each well were photographed at the magnification of 100× and counted. The migration assay was carried out similarly to the invasion experiment except for the matrigel coating.

Measurement of Inflammatory Cytokines

RASFs were seeded into 6-well plates (1 × 10^5 cells/ml) with DMEM containing 10% FBS and incubated overnight. Cells were cultured with or without CD5L at various concentration for 48 h. Cell supernatant was collected. Then, IL-6 levels in supernatant were detected by electrochemiluminescence immunoassay (Cobas e801, Roche,Switzerland). IL-8 and TNF-α levels were detected by ELISA kits (R&D Systems, MN, USA). For the cytokines detection in synovial tissue from CIA Rat model, the levels of the IL-6 (ExCellBio, Beijing, China), TNF-α (ExCellBio, Beijing, China), and IL-8 (A&E Bio, Guangzhou, China) were determined using ELISA kits. The experimental operation is carried out according to the instructions.

Real-Time Quantitative PCR (qRT-qPCR)

Total RNA was isolated with Trizol Reagent (Takara,Kyoto, Japan). cDNA was then prepared by reverse transcription kit (Cat: AG21102, Accurate Biotechnology, Hunan, China) according to the manufacturer's instructions. RT-qPCR was performed with a SYBR Green PCR kit (Abm, Jiangsu, China). All primers were synthesized by Qingke Biotechnology (Xian, China). The primer sequences were listed in Table 2. The relative expression was expressed as $2^{-\Delta\Delta Ct}$ normalized to housekeeping gene GAPDH.

Western Blotting (WB)

Total proteins were extracted with RIPA buffer (Sigma-Aldrich, Darmstadt, Germany) containing protease inhibitors. Then, cell protein was separated by 12% sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membrane. Membranes were blocked with 5% skim milk for 2 h and incubated with primary antibody overnight [primary antibodies: PI3K p38 (Abcam, ab191606), p-PI3K p38 (Abcam, phospho Y607, ab182651), Akt (Abcam, ab179463), p-Akt (Abcam, phospho

Table 1. Oligonucleotides sequences of siCD36

Gene	Sense (5′–3′)	Antisense (5′–3′)
siCD36-1	CCGACGUUAAUCUGAAAGGAATT	UUCCUUUCAGAUUAACGUCGGTT
siCD36-2	CCUGCUUAUCCAGAAGACAAUTT	AUUGUCUUCUGGAUAAGCAGGTT
siCD36-3	CCAUUGGUGAUGAGAAGGCAATT	UUGCCUUCUCAUCACCAAUGGTT

Table 2. qRT-PCR primer sequences

Gene	Forward (5′–3′)	Reverse (5′–3′)
hGAPDH	TCCTTGGAGGCCATGTGGGCCAT	TGATGACATCAAGAAGGTGGTGAAG
hIL-6	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
hIL-8	ACTGAGAGTGATTGAGAGTGGAC	AACCCTCTGCACCCAGTTTTC
hCD5L	GTGGGTCGAATGTGAAGATCC	CATAGCATTTCCGGTCTCTGAAG
hCD36	CTTTGGCTTAATGAGACTGGGAC	GCAACAAACATCACCACACCA

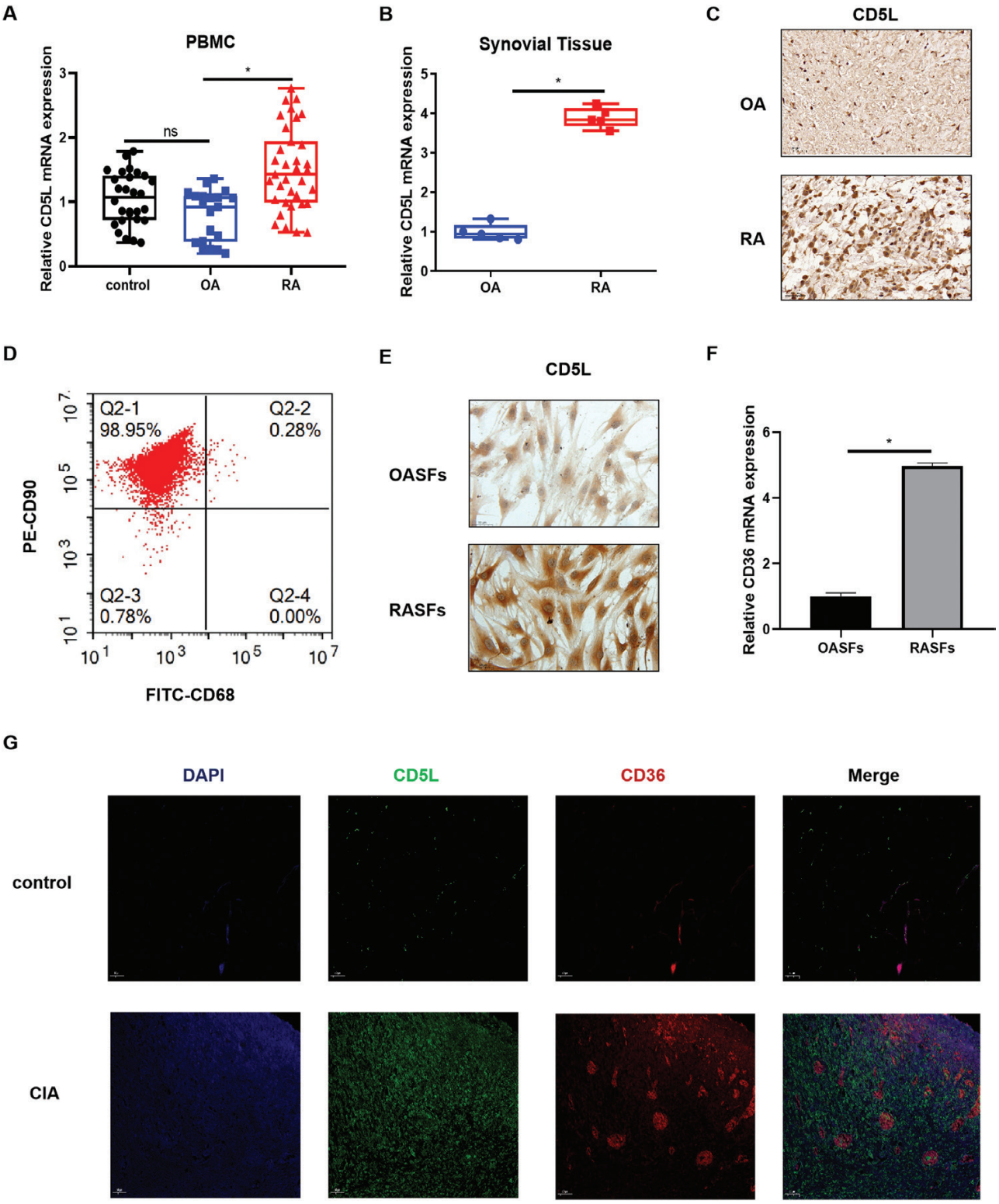

Figure 1. CD5L was highly expressed in RA. (A) CD5L expression in peripheral blood mononuclear cells (PBMC) of control (n = 28) and patients with osteoarthritis (OA, n = 21) and RA (n = 35). (B, C) CD5L mRNA and protein expression in synovial tissue of patients with OA (n = 5) and RA (n = 5). Scale bar = 50 μm. (D) Identification of primary synovial fibroblasts by flow cytometry. (E) Immunohistochemical analysis of CD5L expression in synovial fibroblasts from osteoarthritis (OASFs) and rheumatoid arthritis (RASFs). Scale bar = 50 μm. (F) The expression of CD36 in RASFs and OASFs was detected by qRT-PCR. (G) Immunofluorescence analysis of CD5L and CD36 expression in synovium of rats with collagen-induced arthritis (CIA). Scale bar = 50 μm

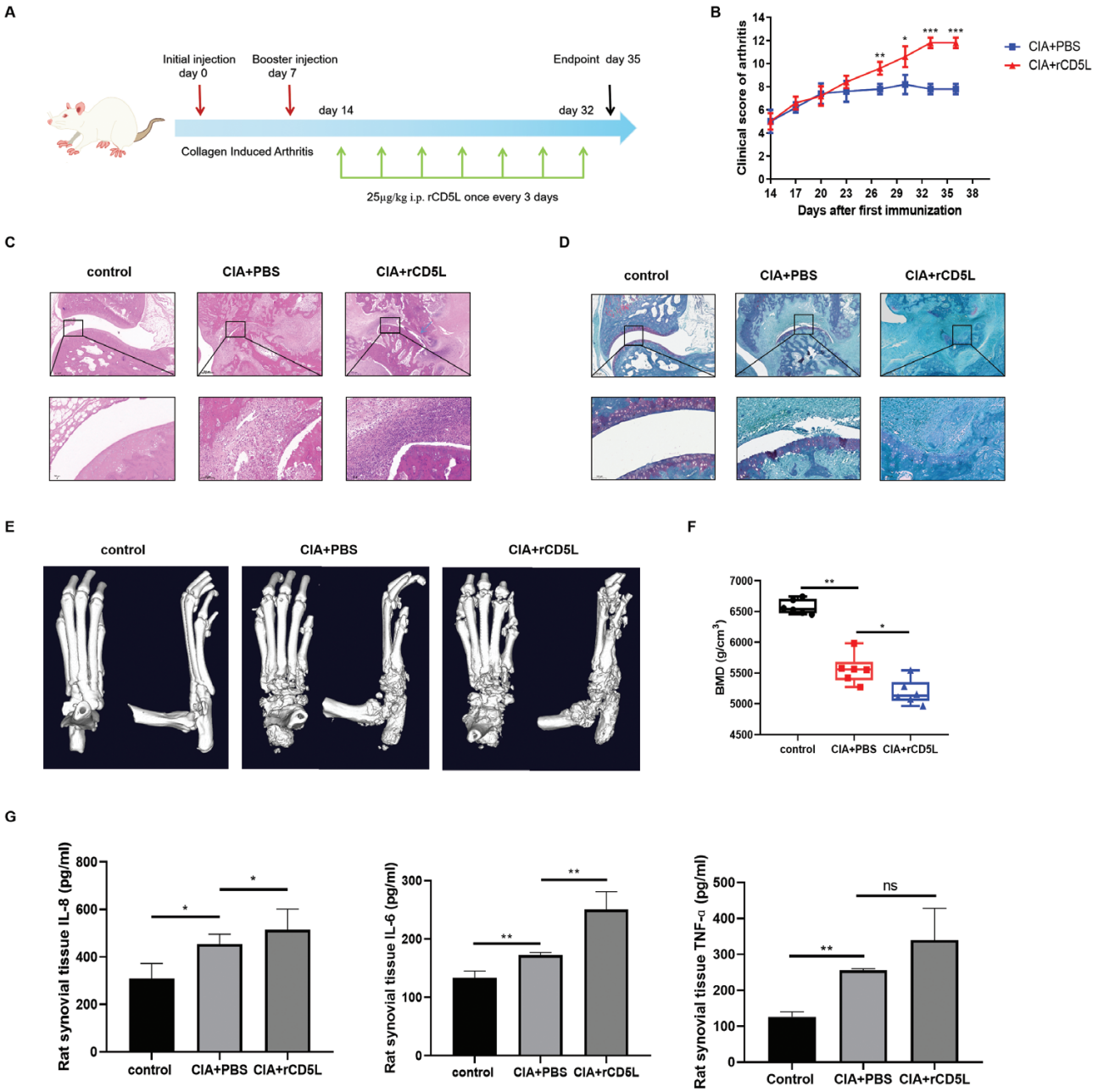

Figure 2. CD5L aggravates collagen-induced arthritis (CIA) progression. (A) Schematic illustration of rat model of CIA and rCD5L dosing. (B) The severity of arthritis was assessed in the CD5L-treated group (CIA+rCD5L) compared with the CIA group (CIA+PBS) rats. (C) Hemoxylin-eosin (HE) staining was assessed synovial inflammation in each group rats. The arrow shows pannus formation. Scale bar = 500 μm; scale bar = 100 μm. (D) Safranin O/solid green staining was assessed cartilage damage in each group rats. Scale bar = 500 μm; scale bar = 100 μm. (E, F) Bone destruction was performed by Micro-CT. (G) The expression of IL-6, IL-8, and TNF-α in rat synovial tissue was determined by ELISA

T308, ab38449)]. Then, membranes were incubated with secondary antibody for 1 h and visualized with an ECL kit (4A Biotech Co., Ltd, Beijing, China).

Statistical analysis

Normally distributed continuous variables are presented as mean ± standard deviation (SD) and analyzed by Student *t* test. Skewed data as median (interquartile range, IQR)and analyzed by the Mann-Whitney *U* test. Statistical analysis was performed using GraphPad Prism 8.0 software. $P < 0.05$ was considered to be statistically significant.

Results

CD5L Was Highly Expressed in RA Synovial Tissue

We have showed the increased CD5L levels in serum and synovial fluid from RA patients than that from OA [15]. Here, we investigated the expression of CD5L in peripheral blood mononuclear cells (PBMC) and synovial tissue of patients with OA and RA. CD5L mRNA expression was significant higher in PBMC from RA patients than that from OA patients (**Figure 1A**). Consistently, compared with OA, qRT-PCR, and IHC results showed that CD5L proteins was increased in RA synovial tissues. (**Figure 1B,C**). FCM was used to identify the

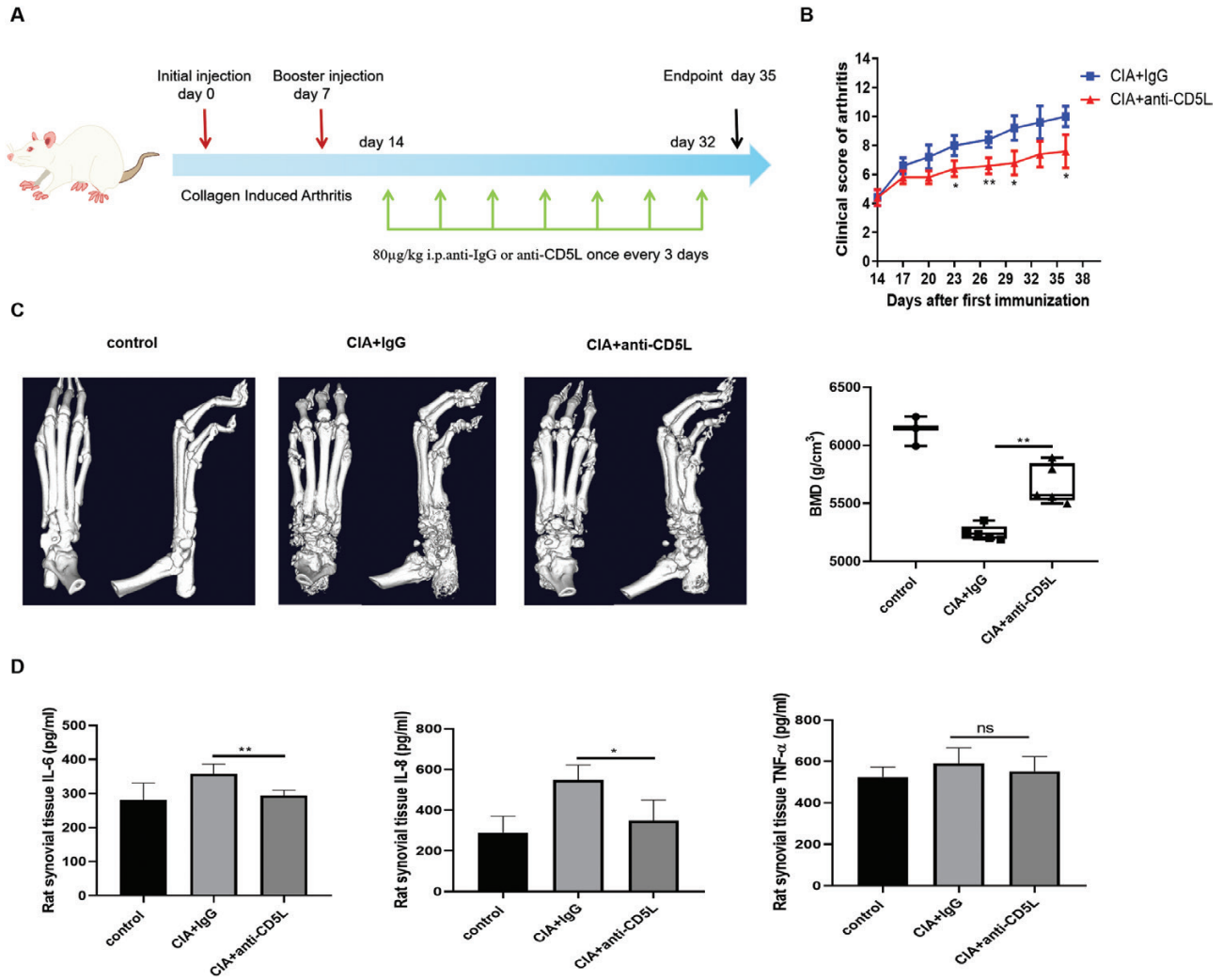

Figure 3. Anti-CD5L alleviated collagen-induced arthritis (CIA) rats disease activity. (A) Schematic illustration of rat model of CIA and anti-CD5L dosing. (B) The severity of arthritis was assessed in the anti-CD5L treated group (CIA+anti-CD5L) compared with the IgG group (CIA+IgG) rats. (C) Bone destruction was performed by Micro-CT. (D) The expression of IL-6, IL-8, and TNF-α in rat synovial tissue was determined by ELISA

purity of the RASFs, and the results showed that $CD90^{+}CD68^{-}$ RASFs cells accounted for 98.95% (**Figure 1D**). IHC results indicated that CD5L proteins was increased in RASFs (**Figure 1E**). Moreover, qRT-PCR results showed that the expression of CD36 was upregulated in RASFs compared with OASFs (**Figure 1F**). Furthermore, IF results revealed that CD5L and CD36 protein was higher in CIA models compared with control group (**Figure 1G**).

CD5L Aggravates Collagen-Induced Arthritis (CIA) Progression

To further investigate the role of CD5L in RA progression, CIA rats were treated with recombinant CD5L protein (**Figure 2A**). CIA rats exhibited swelling and deformity in both hind paws, and the joint swelling of some rats in the CD5L-treated group could spread to both forepaws after 14 days. The severity of arthritis was significantly increased in the CD5L-treated CIA rats compared with the CIA rats (**Figure 2B**). Furthermore, we assessed synovial inflammation and cartilage damage by H&E and safranin O staining. Synovial inflammation and cartilage erosions were much more severe in the CD5L-treated CIA rats (**Figure 2C, D**). Micro-CT showed that more severe bone destruction was observed in the CD5L-treated CIA rats compared with the control rats (**Figure 2E**). Moreover, BMD was also significantly downregulated in CD5L-treated group (**Figure 2F**). Furthermore, we found that the expression of IL-6 and IL-8 was markedly upregulated in the synovial tissues of CD5L-treated CIA rats. However, there was no significant difference in the TNF levels (**Figure 2G**). Notably, intraperitoneal injection with CD5L did not induce RA in rats (Supplementary Figure 1).

Anti-CD5L Alleviates Collagen-Induced Arthritis (CIA) Progression

To further verify the function of CD5L in RA, we investigated the effect of CD5L blockage (by using anti-CD5L antibody) on RA disease. The schematic was shown in **Figure 3A**. The severity of arthritis was significantly decreased in the anti-CD5L group compared with control group (**Figure 3B**). Micro-CT images showed that the anti-CD5L treated group had significantly improved bone damage and upregulated BMD compared with control group (**Figure 3C**). Moreover, anti-CD5L treatment inhibited the expression levels of IL-6 and IL-8 in the synovial tissue (**Figure 3D**).

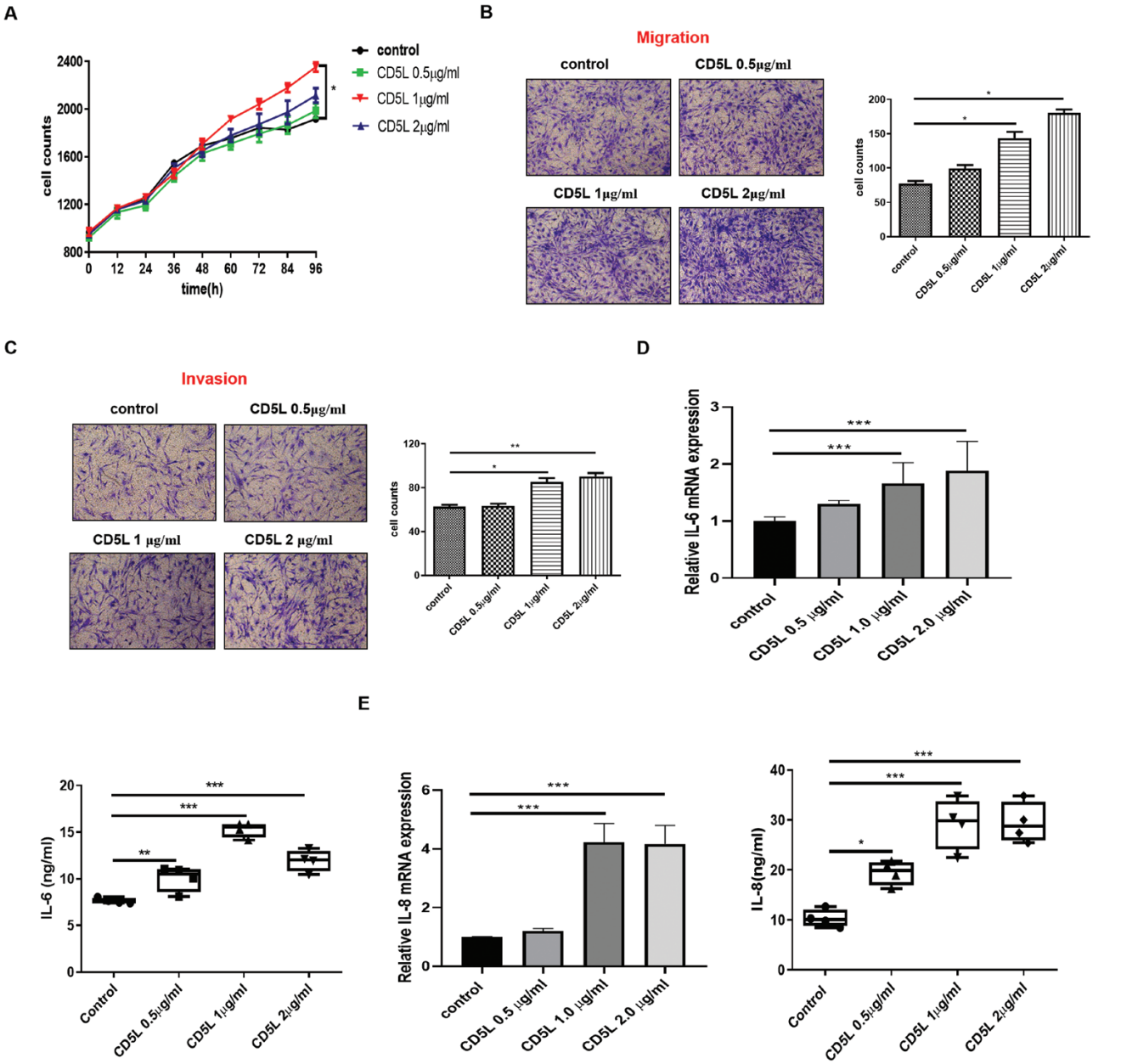

Figure 4. CD5L promoted cell proliferation, migration, invasion, and inflammatory cytokine expression in RA. (A) Cell proliferation was evaluated by measuring cell number using the cell imaging system. (B, C) Cell migration and invation were evaluated by Transwell assays. Scale bar = 100 μm. (D, E) The expression of inflammatory cytokines (IL6 and IL8) at the transcription and protein levels in RASFs

CD5L Promoted Cell Proliferation, Migration, Invasion, and Inflammatory Cytokine Expression of RASFs

The proliferation rate of RASFs was significantly promoted by CD5L treatment (1 μg/ml). However, there were no effect of 0.5 and 2 μg/ml CD5L treatment on RASFs proliferation (Figure 4A). Moreover, the RASFs treated with CD5L (1 and 2 μg/ml) had accelerated migration and invasion ability than control cells (Figure 4B,C). Furthermore, our results showed that incubating RASFs with CD5L (1 and 2 μg/ml) significantly promoted the levels of IL-6 and IL-8 (Figure 4D,E).

CD36 knockdown reversed the effects of CD5L on RASFs

CD36 is the major cell surface receptor of CD5L. CD36 knockdown experiment were used to explore whether the effects of CD5L on RASFs dependent on CD36. The results showed that the RASFs proliferation was significantly decreased in CD5L+siCD36 treatment group compared with CD5L treatment group (Figure 5A). The upregulation effect of CD5L treatment on IL-6 and IL-8 was rescued by CD36 knockdown (Figure 5B,C). In addition, CD36 knockdown also reversed the promoted effect of CD5L treatment on RASFs migration and invasion (Figure 5D).

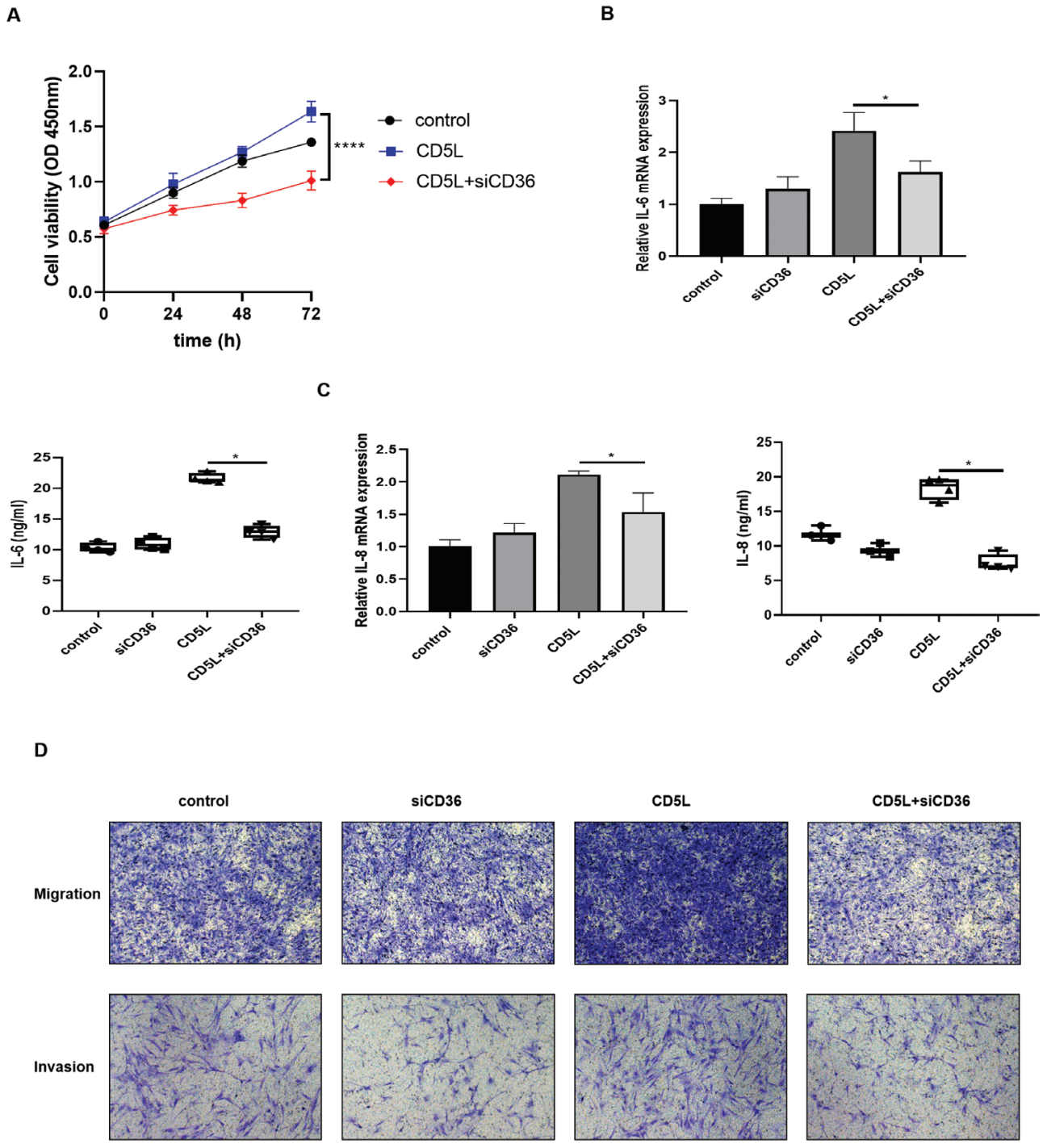

Figure 5. CD36 knockdown significantly rescued the upregulation effect of CD5L on RASFs activity. (A) Cell proliferation was evaluated by CCK-8 assays. (B, C) The expression of inflammatory cytokines (IL-6 and IL-8) at the transcription and protein levels in RASFs. (D) Cell migration and invation were evaluated by Transwell assays. Scale bar = 100 μm

CD5L Induces IL-6 and IL-8 Levels by Potentiating PI3K/Akt Pathway

Since the activation of PI3K/Akt pathway is closely associated with RA synovial inflammation, we evaluated the effect of CD5L treatment on PI3K/Akt pathway activation in RASFs. As shown in Figure 6A, there were no significant difference of total PI3K p85α and Akt levels between control and CD5L treated RASFs. However, the levels of phosphorylated PI3K p85α (p-PI3Kp85α, Y607) and Akt (p-Akt, T308) were significantly upregulated in RASFs treated with CD5L. And moreover, CD5L i.p. promoted the levels of p-PI3Kp85α and p-Akt in the synovial tissue of CIA rats (Figure 6B). To confirm whether CD5L regulated the inflammation of RA through PI3K/Akt signaling, rescue experiment was performed by using LY294002, which is an PI3K/Akt signaling inhibitor. LY294002 treatment significantly inhibited the expression of IL-6 and IL-8 at both mRNA and protein levels in CD5L-treated RASFs (Figure 6C,D).

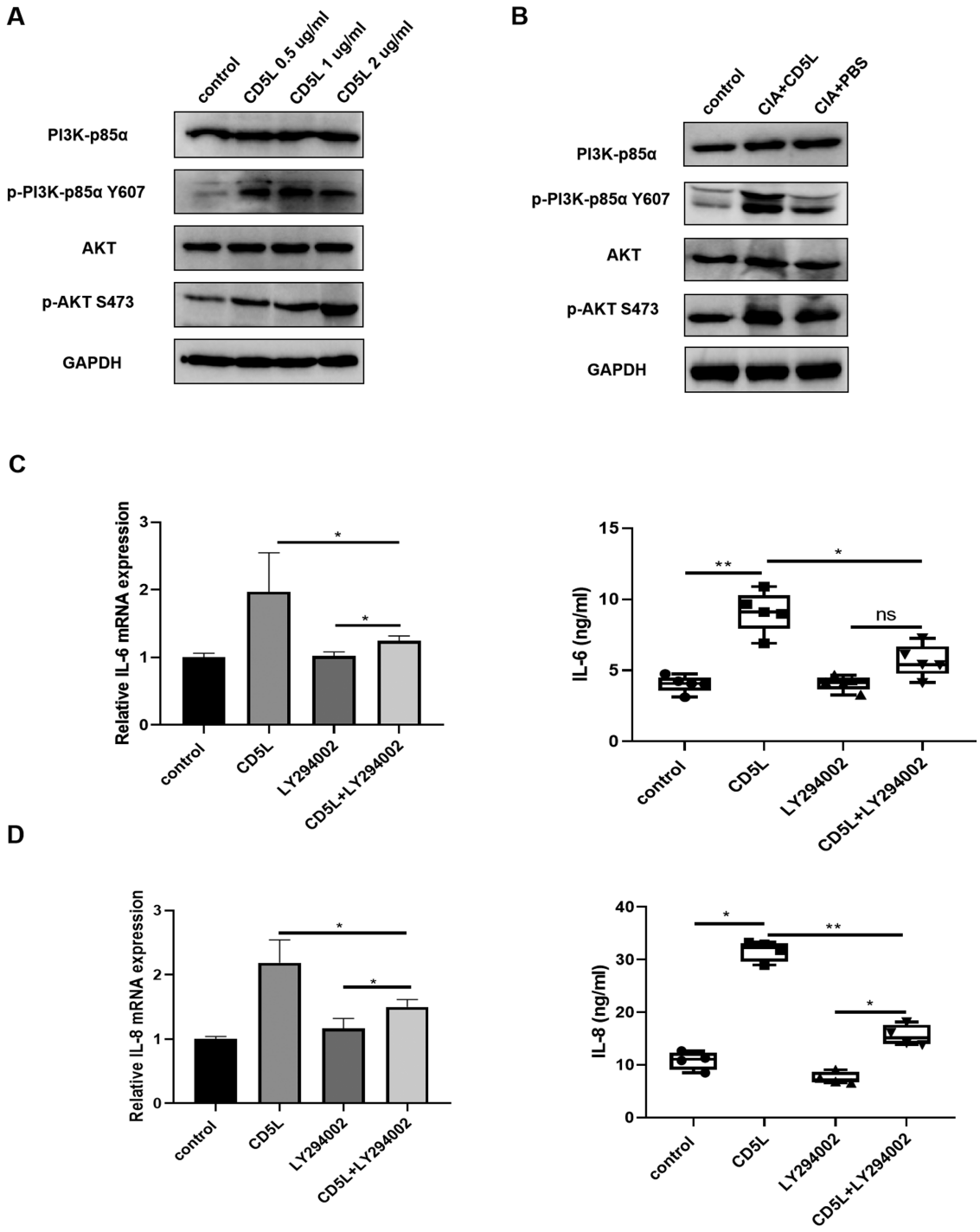

Figure 6. CD5L induces IL-6 and IL-8 expression by regulating PI3K/Akt Signaling Pathway. (A, B) Total protein and phosphorylation levels of PI3K/Akt signaling molecules in RASFs and rat synovial tissues. (C, D) The mRNA and protein expression of IL-6 and IL-8 in RASFs after treatment with culture media, CD5L and LY294002 for 48 h, was determined using qRT-PCR and ELISA

Discussion

Our previous study have found that CD5L protein levels were significant higher in serum and synovial fluid from RA patients than that in OA and healthy subjects, and were positively correlated with RA disease activity, which indicated that CD5L might be a biomarker for diagnosis and monitoring of RA [15]. However, in addition to being a concomitant biomarker, weather the increased CD5L levels contributed to RA progression remained unclear.

In this study, firstly, we showed that CD5L mRNA expression levels were increased in PBMC and synovial tissue from RA patients, which could explain the cause of higher CD5L levels in serum and synovial fluid from RA patients. More importantly, we demonstrated that CD5L promoted disease progression of CIA rats, a commonly used rat model of RA. Compared to control rats, CD5L i.p. groups developed more severe arthritis, including greater clinical score, lower bone density, increased synovial inflammation, more serious

cartilage erosions and cartilage damage. Moreover, CD5L i.p. in CIA rats significantly triggered the production of IL-6 and IL-8 levels in the joint synovial tissue, which might be involved in the increased immune inflammation. Moreover, by using anti-CD5L antibody, we found that CD5L blockage alleviated disease of CIA rats and decreased the levels of proinflammatory cytokines. Notably, CD5L could not induce RA in rats, which suggested that CD5L were not involved in RA occurrence, but were involved in disease progression. These data demonstrated that increasing CD5L levels contributed to RA disease progression and CD5L could be an therapeutic target for RA.

Synovial fibroblasts (SFs) are the main stromal cells responsible for synovial hyperplasia and bone destruction in joints [20, 21]. RASFs tended to exhibit excessive proliferation and secrete more inflammatory cytokines. The increased SFs, both in quantity and activity, were characteristics of RA patients and associated with the RA progression. Our data showed that CD5L was highly expressed in the SFs of RA patients. There were multiple effects of CD5L on cell's behaviors and activity. Aran et al. reported that CD5L could promote liver cancer cell proliferation and antiapoptotic responses in hepatocellular carcinoma [22]. Bamodu et al. highlighted that CD5L knockout significantly attenuated the migration and invasive capability of PC3 and DU145 cells [23]. Thus, we investigated the effects of CD5L on RASFs biological behaviors. The results showed that CD5L protein treatment could significantly promote proliferation, migration, and invasion of RASFs. As is well known, inflammatory cytokines, such as IL-1β, IL-6, IL-8, IL-10 and TNF-α, are upregulated during the pathogenesis of RA. Several studies have revealed that CD5L could regulate the inflammatory response by inducing the production of cytokines and participates in disease pathology [24–26]. Accordingly, our data supported that the CD5L could induce the increased production of the pro-inflammatory factors IL-6 and IL-8 in RASFs, but had no effect on TNF-α expression. Notably, target knockdown of CD36, which is the receptor of CD5L, significantly rescued the effect of CD5L treatment on RASFs. These data demonstrated that CD5L/CD36 signaling pathway could promote RA disease progression via enhancing the number and activity of RASFs.

As we know, PI3K/Akt signaling have believed to involved in the regulation of SFs and the development of RA [27, 28]. Recently, Song et al. found that bone morphogenetic protein 9 (BMP9) regulates the proliferation, migration, and invasion of RASFs by activating PI3K/Akt signaling, and inhibits the expression of inflammatory genes Cyclin D1, MMP-2, and MMP-9 [29]. Aihaiti et al. demonstrated Peroxiredoxin-4 (PRDX4) may regulate the tumor cell-like biological characteristics of RASFs and matrix metalloproteinases (MMPs) expression through PI3K/Akt pathway [30]. Moreover, Yang et al. confirmed that IL-34 can activate PI3K/Akt signaling pathway, promote the massive release of IL-8 and TNF-α, induce the abnormal proliferation of SFs, and aggravate the progression of RA [31]. However, the correlation between CD5L and PI3K/AKT remains poorly understood. Our results revealed that the levels of p-PI3K p85α (Y607) and p-Akt (T308) were upregulated in CD5L treated RASFs. Moreover, the promoted effect of CD5L on IL-6 and IL-8 expression in RASFs was significantly reversed by PI3K/Akt signaling inhibitor.

In conclusion, we demonstrated that CD5L expression was increased in RA patients. The enhanced CD5L could promote RA disease progress, via promoting the proliferation, migration, and inflammatory cytokines expression of RASFs. These findings revealed that CD5L possibly representing a potential therapeutic target for RA.

Supplementary data

Supplementary data is available at *Clinical and Experimental Immunology* online.

Ethical approval

The studies involving human participants were approved by the Ethics Committee of Tangdu Hospital, Fourth Military Medical University. The animal study was approved by the Air Force Medical University's Institutional Animal Care and Use Committee. Ethical approval details can be found in the article.

Funding

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Conflict of Interest

The author reports no conflicts of interest in this work.

Data availability

The original contributions presented in the study are included in the article/ Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

X-nW and Z-wG designed the this work; X-nW, Z-wG and JZ performed the cell experiments and analyzed data; X-nW, LY and CL performed the animal experiments; X-nW and Z-wG wrote the original draft preparation; H-zZ and KD supervised the this work. All authors contributed to the article and approved the submitted version.

Permission to reproduce

Not applicable.

Clinical trial registration

Not applicable.

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