

Fibrosis factor CTGF facilitates VCAM-1-dependent monocyte adhesion to osteoarthritis synovial fibroblasts via the FAK and JNK pathways

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Abstract. Osteoarthritis (OA) is a long-term, degenerative joint disease that presents significant clinical challenges and imposes considerable financial burdens. Fibrosis is closely intertwined with the pathogenesis of various degenerative diseases, including OA. Using data from the GDS5401 dataset, the present study determined that expression levels of the fibrosis factor connective tissue growth factor (CTGF) were significantly higher in OA patients than in normal individuals. The present study also identified CTGF elevated expression levels in both OA patients compared with healthy controls and in rats with anterior cruciate ligament transection-induced OA versus controls. Stimulating OA synovial fibroblasts (OASFs) with CTGF was shown to promote vascular cell adhesion molecule-1 (VCAM-1) production, thereby facilitating monocyte adhesion to OASFs. Analysis of a large dataset revealed that monocytes are the only mononuclear cells with significantly elevated levels in OA patients. It also appeared

that CTGF-induced VCAM-1 production and monocyte adhesion were mediated via the focal adhesion kinase and JNK pathways. These findings suggest that CTGF contributes to OA progression by enhancing monocyte adhesion to the synovial membrane.

Introduction

Osteoarthritis (OA) is a long-term, degenerative joint disease that presents significant clinical challenges and imposes considerable financial burdens. Currently, >300 million individuals worldwide suffer from OA and by 2030, it is expected to become the leading cause of disability globally (1-3).

The lack of a clear pathophysiology has hindered efforts to develop effective treatments. OA was once thought to affect only cartilage; however, it is now characterized as a low-grade inflammatory disease affecting the entire joint. Long-term OA can induce changes in the meniscus, subchondral bone remodeling, osteophyte synthesis, and progressive cartilage destruction (3-5). These mechanical processes should be considered alongside the inflammation, such as synovitis, which plays a major role in the pathogenesis of the disease (2,6).

The complex system referred to as the arthritic microenvironment comprises a number of cell types, including fibroblasts and inflammatory cells, such as dendritic cells, macrophages, and monocytes (7). Among these, monocytes and macrophages make up most of the cells observed in the arthritic milieu (8). Cytokines attract specific subsets of these cells, which influence the course of OA. Note that the migration of monocytes and macrophages into the joint and their subsequent attachment to the synovial membrane are important steps in the

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development and progression of OA (9). Vascular cell adhesion molecule-1 (VCAM-1) is one of several molecules that control the adhesion of monocytes/macrophages to the synovium (10). Researchers have determined that the synovial tissue of OA patients synthesizes more VCAM-1 than does normal synovial tissue (11). Thus, one potential strategy to reducing OA-related inflammation is to moderate the production of VCAM-1 in the synovium (7,12).

Fibrosis plays a crucial role in the etiology of several degenerative illnesses, including OA; however, the complex underlying processes have yet to be elucidated. Fibrotic processes can be triggered or exacerbated by inflammatory responses and chronic inflammation is frequently associated with the persistent deposition of extracellular matrix (ECM) and the ensuing tissue scarring (13). The interaction between fibrotic tissue and inflammatory reactions is a reciprocal process, often resulting in a vicious cycle that accelerates OA progression (14). Connective tissue growth factor (CTGF), also known as CCN2, is a key factor in the pathogenic processes driving inflammation and fibrotic tissue formation in OA (15,16). CTGF expression is higher in the cartilage from OA patients than in normal cartilage and expression levels are associated with disease severity (17).

It has been established that CTGF promotes osteoclastogenesis and enhances the production of proinflammatory cytokines in OASFs (18-20); however, the effect of monocyte adherence to the synovium during OA remains poorly understood. The current study revealed that CTGF and monocyte marker expression levels were higher in OA patients compared with normal individuals. It was also determined that CTGF promoted VCAM-1 expression in OASFs and facilitated the adhesion of monocytes to synovial fibroblasts via the focal adhesion kinase (FAK) and JNK signaling cascades. These findings suggested a plausible mechanism to explain the association between CTGF and OA, while offering a novel therapeutic target for OA.

Materials and methods

Materials. CTGF recombinant protein was obtained from PeproTech, Inc. Antibodies against phosphorylated (p-)FAK (cat. no. 3283S) and p-JNK (cat. no. 9251S) were acquired from Cell Signaling Technology, Inc. Antibodies against VCAM-1 (cat. no. GTX110684), FAK (cat. no. GTX50489), JNK (cat. no. GTX52360) and β -actin (cat. no. GTX109639) were acquired from GeneTex International Corporation. Specific inhibitors of FAK (cat. no. 324878) were obtained from Calbiochem (Merck KGaA). Specific inhibitors of JNK; SP600125 (cat. no. BML-E1305-0010) was obtained from Enzo Life Sciences, Inc. Supplements for cell culture were purchased from Invitrogen (Thermo Fisher Scientific, Inc.). Small interfering (si)RNAs targeting FAK and JNK were purchased from GE Healthcare Dharmacon, Inc. The source of all other reagents was MilliporeSigma.

Cell cultures. OA synovial tissue specimens were obtained from patients with OA undergoing knee replacement surgery (n=5) and patients undergoing arthroscopy after trauma or joint derangement, who served as normal controls (n=5), at the Department of Orthopedic Surgery, China Medical University

Hospital, Taichung, Taiwan. The specimens were collected between January 2021 and January 2022. The ages of OA patients ranged from 65 to 82 years. The male-to-female ratio was 2:3. Prior to participation, all patients provided written informed consent, with approval from the Institutional Review Board (IRB) of China Medical University Hospital (approval no. CMUH109-REC2-181). OASFs were digested in Collagenase Type II and then cultured in accordance with the methods in a previous study (21). Cells from the human monocyte cell line THP-1 from the American Type Culture Collection were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS, penicillin, and streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) in a humidified environment with 5% CO₂ at 37°C.

Examination of monocyte adhesion to OASFs. Monocytes were stimulated with BCECF-AM (Invitrogen, Thermo Fisher Scientific, Inc.) (10 μ M) at 37°C for 1 h. OASFs were exposed to CTGF for 24 h, and then exposed to the monocytes for 1 h. Nonadherent monocytes were rinsed off and adherent monocytes were quantified using a fluorescence microscope.

Bioinformatic analysis. Synovial tissue samples from healthy donors and OA patients were examined in terms of CTGF expression levels and fibrosis-related variables using the GDS5401 dataset from the Global Data Services database (ncbi.nlm.nih.gov/sites/GDSbrowser?acc=GDS5401).

Anterior cruciate ligament transection (ACLT)-induced OA model. Male Sprague Dawley (SD) rats (eight-weeks-old; weighing 300-350 g) were purchased from the National Laboratory Animal facility (Taipei, Taiwan). The animals were housed in the animal care facility of China Medical University (Taichung, Taiwan) and were housed in a specific pathogen-free environment with controlled temperature (22-24°C) and humidity (45-55%). Rats had ad libitum access to sterilized rodent chow and autoclaved water throughout the experiment, and they were maintained on a 12/12-h light/dark cycle. A total of 12 Rats were used in the experiment.

Rats in the experiment group (n=6) underwent anterior cruciate ligament (ACL) transection during arthrotomy in accordance with the methods outlined in previous studies (22,23). In brief, the left knee was prepared using a surgically sterile technique. The ACL fibers were transected with a scalpel, and the entire medial meniscus was excised through a medial parapatellar mini-arthrotomy. The joint surface was irrigated with sterile saline solution, and both the capsule and skin were sutured following ACL transection and medial meniscectomy. Ampicillin (50 mg/kg body weight) was administered for five days postoperatively. Rats in the control group (Control; n=6) did not have their ACLs severed during arthrotomy. All animal experiments were conducted in strict accordance with a protocol approved by the Institutional Animal Care and Use Committee of China Medical University (approval no. CMUIACUC-2021-050-2).

Immunohistochemical (IHC) staining. Slices were obtained from serial sections of human synovial tissue. After fixing the synovial tissue in 1% formaldehyde at 37°C overnight, the formalin-fixed, paraffin-embedded tissues were sectioned at a

thickness of 4 μm and baked in an oven at 65°C for >2 h. The paraffin sections were deparaffinized using xylene and washed with gradient alcohol and then distilled water. The sections were placed in a citrate-based solution, heated in a microwave oven, and cooled to room temperature for antigen retrieval. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide (37°C, 10 min). Then, bovine serum albumin (BSA; Thermo Fisher Scientific, Inc.) was used to block and prevent nonspecific staining (37°C, 10 min). All sections were stained with anti-CTGF (cat. nos. MAB660, R&D, MN, USA) or anti-CD11b (cat. nos. MAB1699, R&D, MN, USA) antibodies (1:100) and incubated overnight at 4°C. The staining results were measured as previously described (19). After incubation with the primary antibodies, peroxidase-labeled second antibodies (1:100) (cat. nos. RE7280-K, Leica Biosystems, Newcastle-upon-Tyne, UK) were applied at room temperature for 60 min to visualize antigens. The slices were rinsed in PBS, stained with 3,3'-diaminobenzidine; DAB (liquid DAB + substrate, Leica Biosystems), and counterstained with hematoxylin (37°C, 3 min) followed drying, and mounting. The staining procedures were carried out using the Leica Novolink Polymer Detection system (Leica Biosystems Inc.). The tissue sections were finally observed under an optical photographic light microscopy (magnifications, x10 and x20) and the final staining scores were calculated by summing the staining intensity and the percentage of positive cells (24-26).

Reverse transcription-quantitative (RT-q) PCR. 3×10^5 OASF cells were seeded in 6-well dishes, and the RNA was extracted using a TRIzol kit (Invitrogen, Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. The quality and quantity of the RNA were assessed using a Nanovue Spectrophotometer (GE Healthcare) based on A260 values. cDNA synthesis was performed using an M-MLV RT kit (Invitrogen; Thermo Fisher Scientific, Inc.) with 1 μg of total RNA in accordance with the manufacturer's recommendations. RT-qPCR was performed using the KAPA SYBR® FAST qPCR Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) (23,24,27). RT-qPCR assays were carried out in triplicate using a StepOnePlus sequence detection system. The cycling conditions were as follows: Initial 10-min polymerase activation at 95°C followed by 40 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C for 60 sec. The expression levels of VCAM-1 and ICAM-1, relative to GAPDH, were determined using the ΔCq comparative methods (18). The primer sequences used were as follows: VCAM-1 forward primer (TTCCAGGGACTTCCTGTC TG) and reverse primer (TCCGTCTCATTGACTTGACG); intercellular adhesion molecule-1 (ICAM-1) forward primer (ATGCCAGACATCTGTGTC) and reverse primer (GGG GTCTCTATGCCCAACAA); GAPDH forward primer (ACC ACAGTCCATGCCATCAC) and reverse primer (TCCACC ACCCTGTTGCTGTA).

Western blot analysis. OASFs were lysed in RIPA lysis buffer (cat. no. P0013; Beyotime Institute of Biotechnology), and the BCA Protein Assay Kit (Thermo Fisher Scientific Inc., IL, USA) was used to quantify the protein in the extracts. SDS-PAGE on 10% gels was used to resolve the extracted proteins (30 μg), followed by transfer to PVDF membranes in

accordance with the procedures outlined in our earlier publications (28-31). Briefly, the membranes were blocked for 1 h in PBST containing 4% non-fat milk at room temperature and incubated overnight at 4°C with primary antibodies targeting p-FAK (cat. no. 3283S; 1:1,000; Cell Signaling Technology, Inc.), p-JNK (cat. no. 9251S; 1:1,000; Cell Signaling Technology, Inc.), VCAM-1 (cat. no. GTX110684; 1:1,000; GeneTex International Corporation.), FAK (cat. no. GTX50489; 1:1,000; GeneTex International Corporation.), JNK (cat. no. GTX52360; 1:1,000; GeneTex International Corporation.), and FAK (cat. no. 324878; 1:1,000; Calbiochem, Merck KGaA). After washing using a PBST washing buffer, the blots were incubated for 60 min at 37°C with horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit IgG, cat. no. sc-2357; 1:1,000; goat anti-mouse IgG, cat. no. sc-516102; 1:1,000; Santa Cruz Biotechnology, Inc.). β -actin (cat. no. GTX109639; 1:5,000; GeneTex International Corporation.) were used as internal references for total protein. Immunoreactive bands were detected using an enhanced chemiluminescence reagent (ECL) from Merck Millipore. The blot membranes were visualized using a Fujifilm LAS-3000 imaging system (FUJIFILM Wako Pure Chemical Corporation). After standardization to β -actin, ImageJ v1.48 software (National Institutes of Health) was used to measure the optical density of the blot.

ELISA. The levels of CTGF in the serum samples of all subjects were determined using commercial ELISA kits (Human CTGF/CCN2 DuoSet ELISA kit, DY9190, R&D, MN, USA) according to the manufacturer's instructions. The plates were read at 450 nm, and the absorbance values were measured using a Multiskan GO microplate reader (Thermo Fisher Scientific, Inc.). Calculations were performed based on the standard curve to determine sample concentrations.

Statistical analysis. Quantified data were analyzed using GraphPad Prism 8.0 software (Dotmatics). All values are presented as the mean $\pm$ SD. Statistical significance between experiment groups was evaluated using unpaired Student's t-test. Comparisons involving more than two groups were conducted using one-way analysis of variance followed by Bonferroni's post hoc test. Spearman's rank correlation was used to assess IHC staining scores in human synovial tissue. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

CTGF and monocyte expression levels are higher in tissue from OA patients compared with normal tissue. Fibrosis has been shown to play a key role in the pathogenesis of degenerative disorders, such as OA (14). GDS5401 dataset was used to explore the expression of fibrosis factors during OA development. Among the various fibrosis factors, CTGF expression was higher among OA patients than among normal individuals; however, no difference was observed between the two groups in terms of type I collagen, C/EBP homologous protein, glucose-regulated protein 78, Inositol-requiring enzyme 1 (IRE1), Eukaryotic Initiation Factor 2 and Activating transcription factor 4 (ATF4) expression (Figs. 1A and B and S1). IHC analysis of synovial tissues

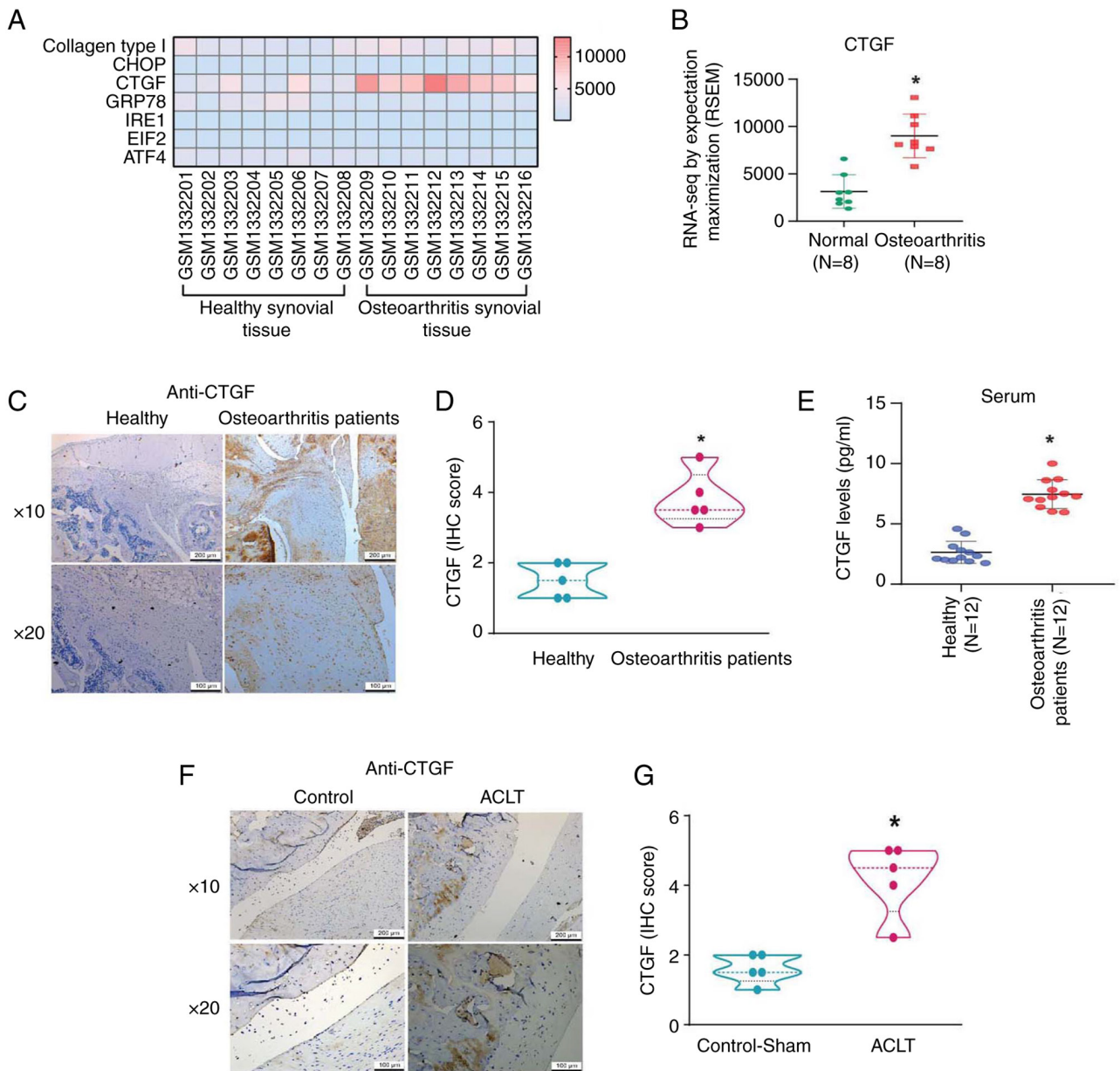


Figure 1. High CTGF levels in OA patients. (A) Heatmap showing differentially expressed fibrosis-related genes from the GDS5401 dataset. (B) CTGF gene expression levels in normal (n=8) vs. OA patients (n=8) in the GDS dataset. (C and D) IHC staining (n=5) of synovial tissue and (E) ELISA assay (n=12) results for CTGF in serum from OA patients vs. healthy controls. (F and G) IHC staining results for CTGF in ACLT-induced OA rats (n=5) vs. controls (n=5). *P<0.05 compared with the normal controls. CTGF, connective tissue growth factor; OA, osteoarthritis; IHC, immunohistochemical; ACLT, anterior cruciate ligament transection.

and ELISA of serum from clinical samples confirmed that CTGF expression levels were higher in OA patients than in normal controls (Fig. 1C-E). Moreover, CTGF expression levels were higher in rats with ACLT-induced OA compared with the controls (Fig. 1F and G). Mononuclear cell migration and adhesion through the synovial membrane is a crucial step in regulating the progression of OA (32). Analysis of mononuclear cell expression in the GDS5401 dataset revealed that the expression of monocyte marker CD11b was significantly higher in OA patients than in normal controls (Fig. 2A and B). Elevated CD11b expression levels were also detected in OA samples from clinical patients and the ACLT model (Fig. 2C and D). When individual IHC staining scores were analyzed by GraphPad Prism 8.0 software, significant

positive correlations were found between CTGF and CD11b IHC staining scores from human synovial tissue (Fig. 2E).

CTGF promotes VCAM-1 synthesis and monocyte adhesion via the FAK and JNK pathways. ICAM-1 and VCAM-1 are known to control monocyte adhesion (7). Stimulating OASFs with CTGF was shown to promote the mRNA expression of VCAM-1, but not ICAM-1 (Fig. 3A). Western blot analysis revealed that the synthesis of VCAM-1 protein was upregulated after treatment with CTGF (Fig. 3B). Stimulating OASFs with CTGF also enhanced monocyte adhesion in a concentration-dependent manner (Fig. 3C and D).

Monocyte adhesion has been linked to FAK activation (33). In the current study, treating OASFs with an FAK inhibitor

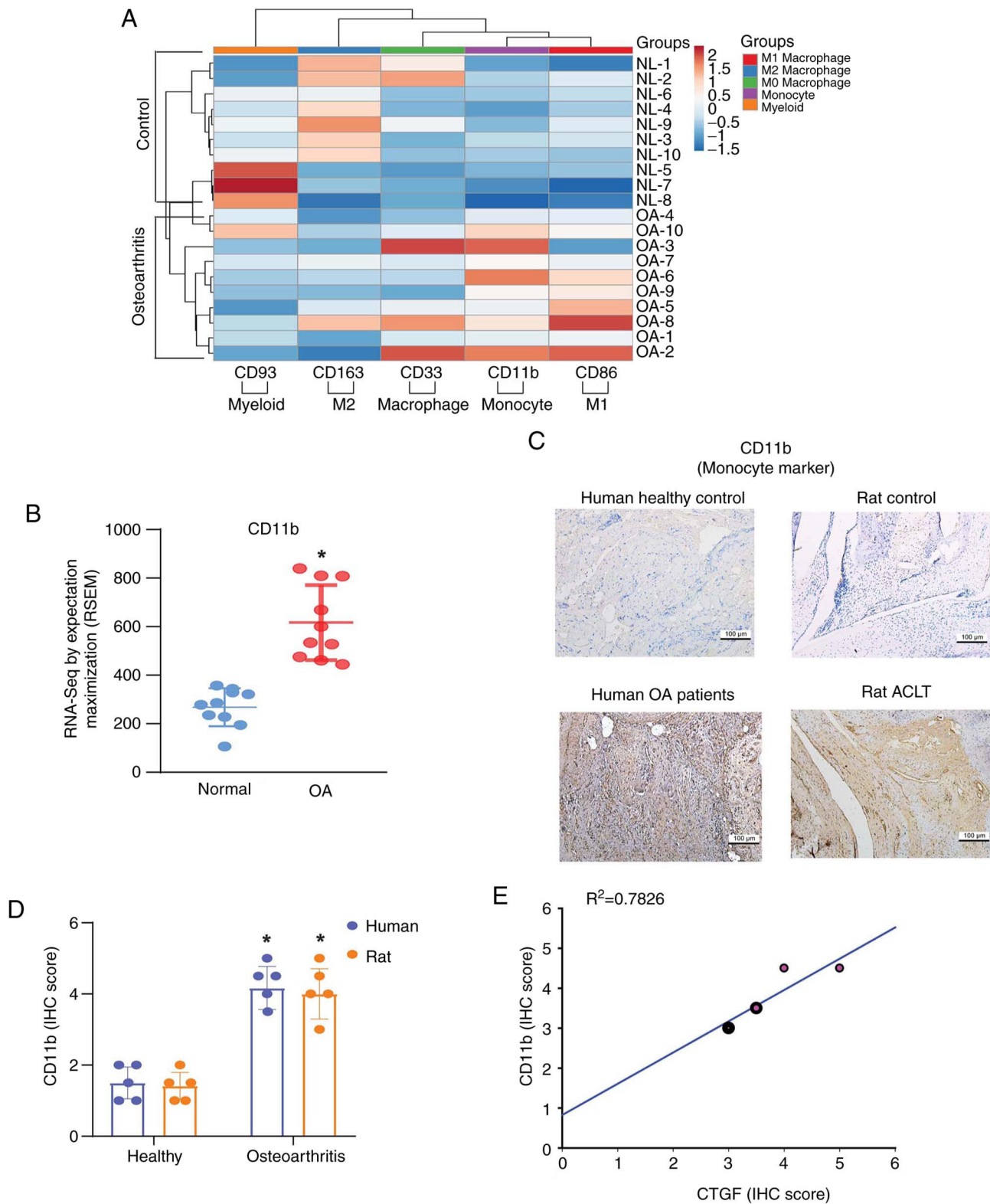


Figure 2. High CD11b monocyte marker levels in OA patients. (A) Heatmap showing differentially expressed mononuclear cell genes from the GDS5401 dataset. (B) CD11b gene levels in OA patients vs. normal individuals in the GDS dataset; (C and D) IHC staining results for CD11b in OA patients (n=5) vs. healthy controls (n=5) and ACLT-induced OA rats (n=5) vs. controls (n=5). Magnification 20x; scale bar, 100 μ m. (E) Spearman's rank correlation coefficient testing identified significant, positive correlations between CTGF and CD11b IHC staining scores in synovial tissue retrieved from human synovial tissue. * $P<0.05$ vs. healthy controls. OA, osteoarthritis; IHC, immunohistochemical; ACLT, anterior cruciate ligament transection; CTGF, connective tissue growth factor.

antagonized CTGF-promoted VCAM-1 mRNA expression and protein synthesis (Fig. 4A and B). The FAK inhibitor also inhibited CTGF-enhanced monocyte adhesion to OASFs

(Fig. 4C). Stimulating OASFs with CTGF was shown to enhance FAK phosphorylation in a time-dependent manner (Fig. 4D).

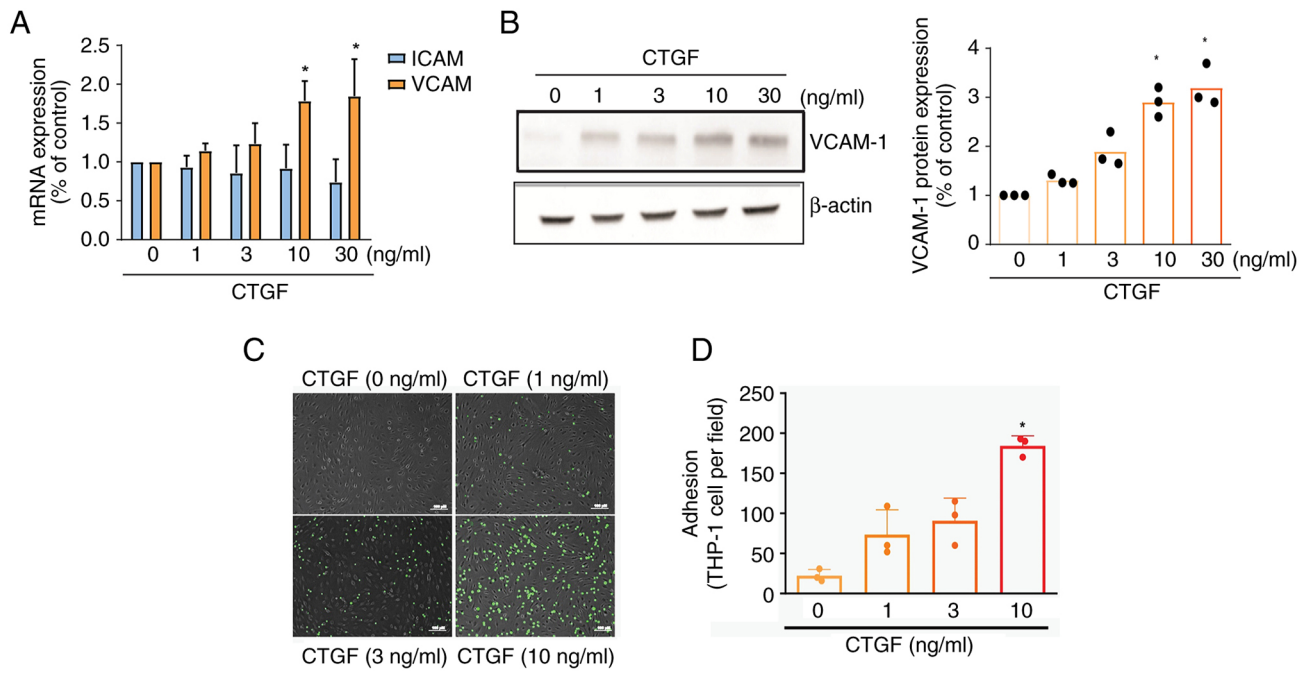


Figure 3. CTGF promotes VCAM-1 production and monocyte adhesion. (A) quantitative PCR results (n=5) showing ICAM-1 and VCAM-1 mRNA expression levels in OASFs after 24-h treatment with CTGF at various concentrations (1-30 ng/ml); (B) Western blot analysis (n=3) showing VCAM-1 expression levels in OASFs after 24-h treatment with CTGF at various concentrations (1-30 ng/ml); The densitometry analysis of (B) was quantified (right panel). (C) Fluorescence microscope images (n=3) of THP-1 cells adhered to OASFs following incubation with CTGF for 24 h and (D) analysis. Magnification 20x; Scale bar, 100 μ m. *P<0.05 vs. control group. CTGF, connective tissue growth factor; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; OASFs, osteoarthritis synovial fibroblasts.

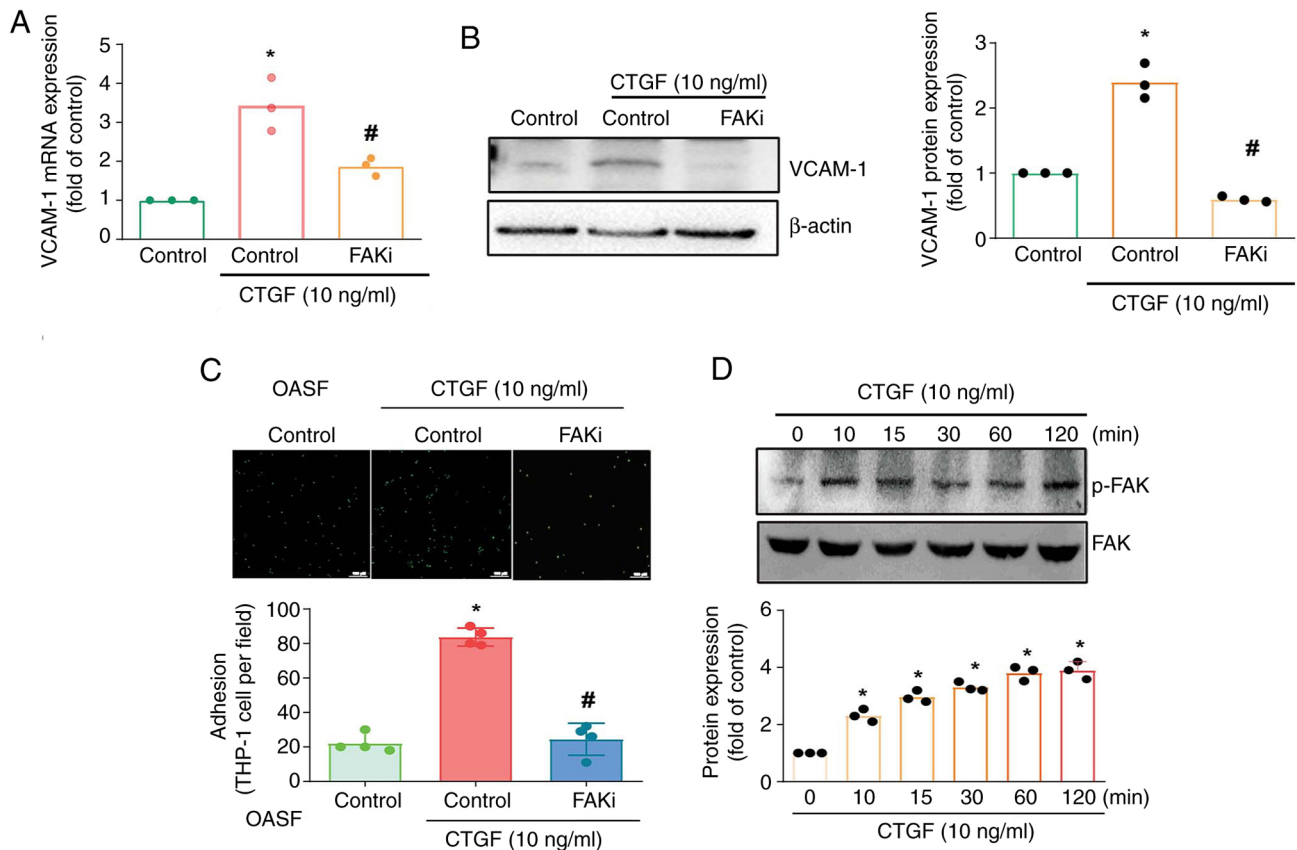


Figure 4. FAK activation is involved in CTGF-induced VCAM-1 synthesis and monocyte adhesion. (A and B) VCAM-1 expression (n=3) and (C) THP-1 adhesion (n=4) in OASFs incubated with a FAK inhibitor (10 μ M) and then stimulated with CTGF for 24 h. The densitometry analysis of (B) was quantified (n=3; right panels). (D) Western blot analysis showing FAK phosphorylation in OASFs treated with CTGF. The densitometry analysis of (D) was quantified (n=3; lower panels). *P<0.05 vs. control group. #P<0.05 vs. CTGF-treated group. FAK, focal adhesion kinase; CTGF, connective tissue growth factor; VCAM-1, vascular cell adhesion molecule-1; p-, phosphorylated.

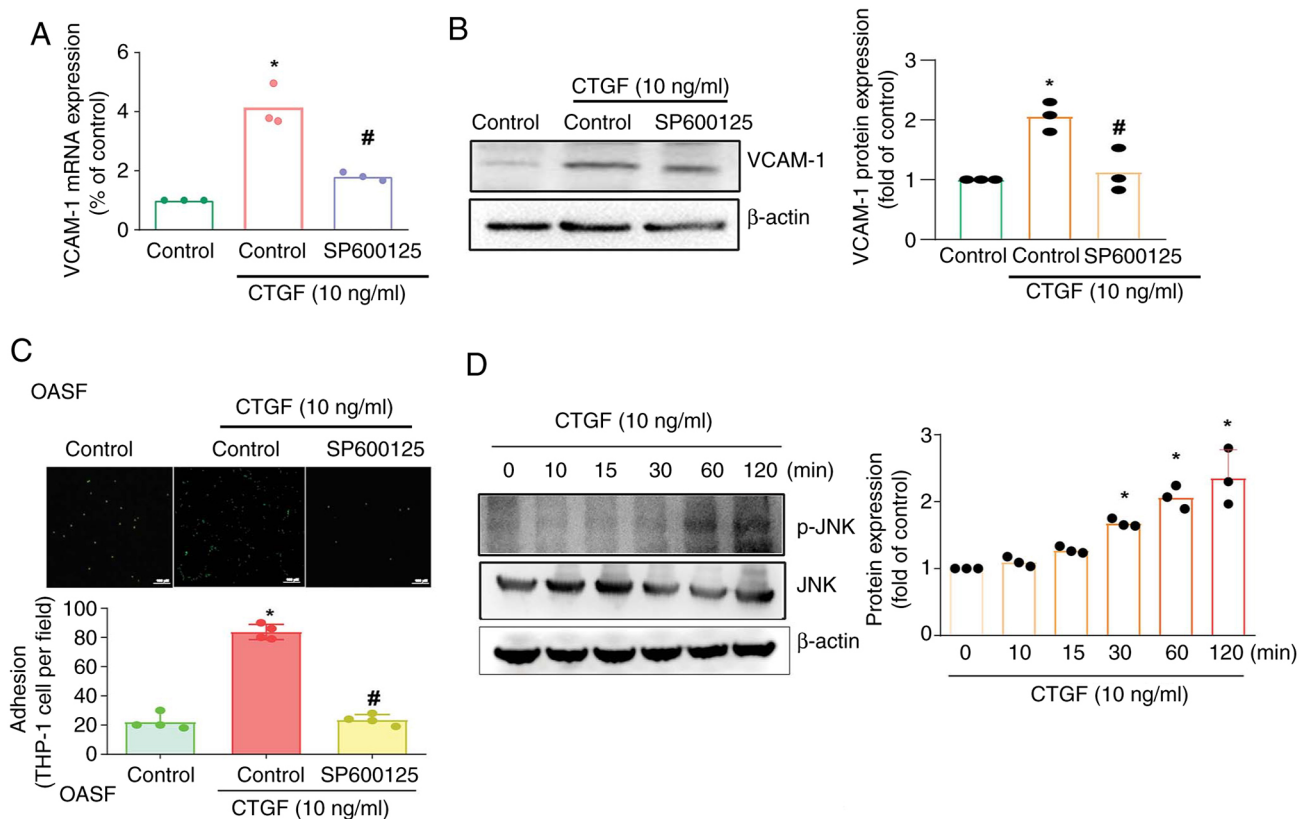


Figure 5. CTGF enhances VCAM-1 synthesis and monocyte adhesion via the JNK pathway. (A and B) VCAM-1 expression (n=3) and (C) THP-1 adhesion (n=4) in OASFs incubated with SP600125 (10 μ M) and then stimulated with CTGF for 24 h; The densitometry analysis of (B) was quantified (n=3; right panels). Magnification 20x; scale bar, 100 μ m. (D) Western blot analysis showing JNK phosphorylation in OASFs treated with CTGF. The densitometry analysis of (D) was quantified (n=3; right panels). *P<0.05 vs. control group. #P<0.05 vs. CTGF-treated group. CTGF, connective tissue growth factor; VCAM-1, vascular cell adhesion molecule-1; OASFs, osteoarthritis synovial fibroblasts; p-, phosphorylated.

JNK signaling has been shown to regulate monocyte migration and adhesion in the development of OA (11). In the present study, the JNK inhibitor SP600125 antagonized CTGF-induced VCAM-1 synthesis and monocyte adhesion (Fig. 5A-C). Treating OASFs with CTGF facilitated JNK phosphorylation (Fig. 5D). These findings indicated that the FAK and JNK pathways mediate CTGF-induced, VCAM-1-regulated monocyte adhesion.

Discussion

Numerous factors have been implicated in the development of OA, including weight-bearing activities, joint damage and advanced age. The pathological characteristics of OA include bone resorption and remodeling, cartilage degradation, and inflammation of the synovium. These pathogenic characteristics affect the progression of OA through similar molecular regulatory mechanisms (34,35). Inflamed synovial cells generate large quantities of pro-inflammatory compounds (36), which induce the secretion of inflammatory mediators by monocytes, macrophages, chondrocytes, and osteoblasts, resulting in the joint pain, swelling, and redness typical of OA (37,38). The present study determined that the fibrosis factor CTGF participated in OA progression by promoting monocyte adhesion to the synovial membrane. This is a potentially valuable insight into the connection between fibrosis and OA.

Fibrosis is usually a result of incomplete healing of damaged tissue. In cases of normal healing, ECM organization and baseline levels are nearly restored; however, in cases of fibrosis, ECM production is excessive and uncontrolled (39). Fibrotic lesions and the excessive deposition of resilient fibers can cause an imbalance in the structure and function of the tissue (40). Fibrosis factor CTGF significantly affects arthritic disease by increasing inflammatory processes (18). Its elevated levels in OA cartilage correlates with disease severity (41). CTGF promotes cartilage degradation, stimulates proinflammatory cytokine IL-6 synthesis (42), enhances monocyte migration through MCP-1 expression (20) and influences angiogenesis by modulating VEGF production in osteoarthritic synovial fibroblasts (18). Analysis of the online GDS5401 dataset revealed that CTGF was the only fibrosis factor that was higher in OA patients than in normal controls. These findings are supported by clinical samples and the ACLT-mimic OA model, indicating that CTGF regulates the progression of OA.

In cases of OA, chemokines secreted by active synovial fibroblasts, such as CCL2, draw inflammatory monocytes from the bloodstream into the synovium, where a number develop into inflammatory macrophages. These macrophages produce inflammatory factors that further stimulate fibroblasts, creating a sustained feedback loop of synovial inflammation. In this loop, activated synovial cells release inflammatory factors that activate macrophages, driving them to transform

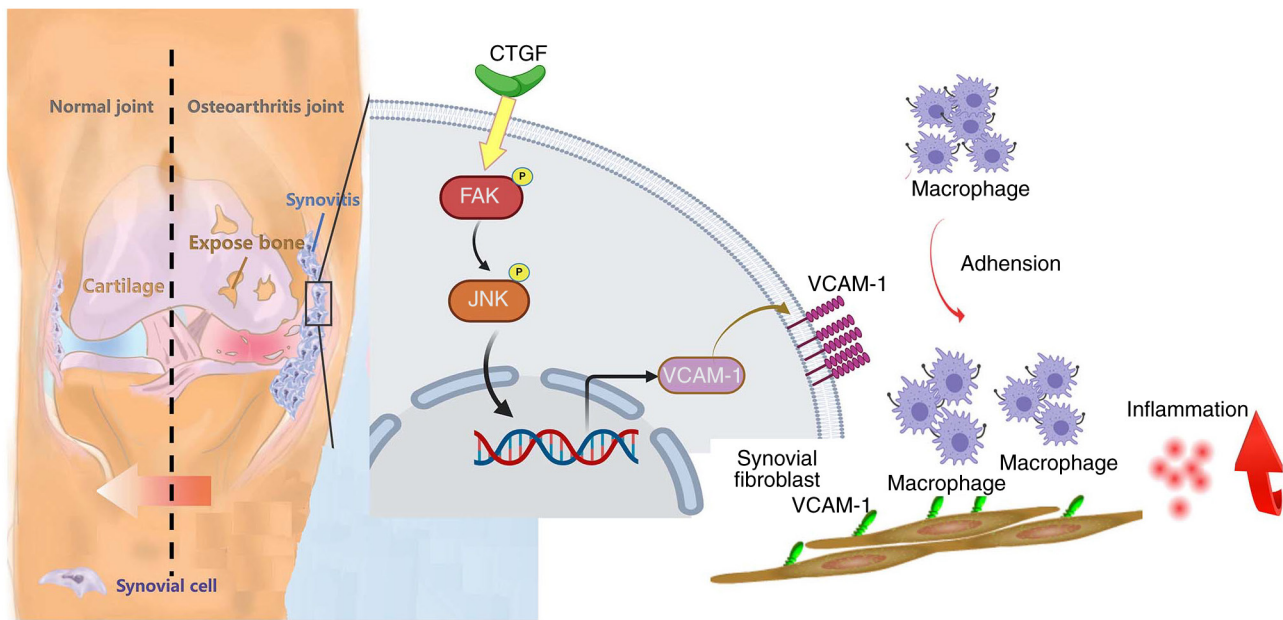


Figure 6. Illustration depicting the effects of CTGF on monocyte adhesion during OA progression. CTGF-induced VCAM-1 production in human OASFs promotes monocyte adhesion via the FAK and JNK pathways.

into the pro-inflammatory M1 macrophage state (43,44). Analysis of the GDS5401 dataset suggests that monocytes play a more significant role than other mononuclear cells, such as myeloid cells, macrophages, M1 macrophages and M2 macrophages, based on elevated expression levels in OA patients. IHC staining of specimens from clinical patients and an ACLT-mimic OA rat model revealed similar results. It was also determined that CTGF facilitates monocyte adhesion to OASFs by promoting VCAM-1 production.

The FAK and JNK signaling pathways are known to control various cellular functions, such as bone formation and metastasis (45). The present study demonstrated that a FAK inhibitor can suppresses CTGF-induced VCAM-1 synthesis in OASFs as well as monocyte adhesion (46). Treating OASFs with CTGF facilitated the phosphorylation of FAK. Similar effects were observed with the JNK inhibitor SP600125, which moderated CTGF-enhanced VCAM-1 synthesis and monocyte adhesion. These results provided evidence that the FAK/JNK pathway contributes to CTGF-mediated VCAM-1 production in OASFs and monocyte adhesion.

In summary, the present study demonstrated that CTGF enhances VCAM-1 synthesis in human OASFs, thereby promoting monocyte adhesion via the FAK and JNK pathways (Fig. 6). Taken together, these findings suggest that CTGF is a promising novel target for the treatment of OA.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SCL, YYL, WCC and CHTa conceived the research project. SCL, YYL, WCC, YYW, YLH and CHTs provided reagents/materials and conducted data analysis. SCL and CHTa wrote the manuscript and confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures were conducted in strict adherence to the principles of the Declaration of Helsinki and received approval from the Institutional Review Board of China Medical University Hospital (approval no. CMUH109-REC2-181). All patients provided written informed consent prior to participation in the study. All animal experiments were conducted in strict accordance with a protocol approved by the Institutional Animal Care and Use Committee of China Medical University (approval no. CMUIACUC-2021-050-2).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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