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Abatacept alleviates rheumatoid arthritis development by inhibiting migration of fibroblast-like synoviocytes via MAPK pathway

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Abstract. – OBJECTIVE: To investigate whether Abatacept could regulate the occurrence and progression of rheumatoid arthritis (RA) by mediating cell migration of fibroblast-like synoviocytes (FLS) via mitogen-activated protein kinase (MAPK) pathway.

PATIENTS AND METHODS: Levels of MMP1, MMP3 and MMP13 in RA-FLS treated with Abatacept or MAPK pathway inhibitor were detected by quantitative Real-time-polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. The regulatory effect of Abatacept on MAPK pathway was detected by Western blot. Transwell assay was performed to access the role of Abatacept in regulating cell migration of RA-FLS.

RESULTS: Abatacept treatment remarkably downregulated levels of MMP1, MMP3 and MMP13 in FLS, which were confirmed by qRT-PCR and ELISA. Migratory ability of FLS was inhibited by Abatacept treatment. Western blot results suggested that Abatacept treatment downregulated MAPK pathway-related genes in FLS. The effects of Abatacept on MMPs expressions and cell migration were partially reversed by SB203580 treatment, the MAPK pathway inhibitor.

CONCLUSIONS: Abatacept inhibits FLS migration and MMPs expressions via inhibiting MAPK pathway, thereby inhibiting RA development.

Key Words:
Abatacept, Rheumatoid arthritis, Fibroblast-like synoviocytes, MAPK pathway.

Introduction

Rheumatoid arthritis (RA) is a common arthritis disease with unclear etiology. RA mainly involves the proximal small joints of the extremities. Synovitis is the basic pathological manifestation of RA. The global incidence of RA is about 1%, which is about 0.8% in adult RA patients. Women are more frequently affected than men[1]. Compared with other chronic diseases, the early diagnosis rate of RA is lower and its disability rate is higher. Advanced RA patients may lose the basic labor capacity or even the self-care ability if they could not be treated in time. As a result, RA has become one of the major disabling diseases. It is reported that the life expectancy of RA patients is shortened by 3-18 years in comparison with healthy controls[2]. Due to its high disability rate, the social and economic burdens of RA on affected patients and their families are well concerned. The basic pathological lesions in RA-affected joints are hyperplasia of the synovial lining layer and abundant infiltration of inflammatory cells. It is currently believed that the proliferation of synovial membranes is mainly caused by the abnormal proliferation of fibroblast-like synoviocytes (FLS). Researches[3,4] have shown that the activated RA-FLS exerts crucial role in the occurrence and progression of RA. Multiple inflammatory factors in joints, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), can stimulate the proliferation of synovial cells and induce the secretion of cytokines, chemokines, adhesion molecules and metalloproteinases (MMPs)[5]. Therefore, RA-FLS is the main effector cells leading to synovial inflammatory response and destruction of joint bone and cartilage in RA patients. Abatacept is the first synthetic co-stimulatory pathway blocker consisting of the extra-

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cellular domain of CTLA4 and the human IgG1 Fc segment. It blocks the activation of T cells by inhibiting the binding of co-stimulatory molecule CD28 and CD80/CD86[6]. Abatacept is approved for treating moderate to severe RA that are unresponsive to tumor necrosis factor antagonists or SAARD (slow-acting antirheumatic drug). Kremer et al[7] have shown that one-year treatment of Abatacept remarkably improves joint function and structure in RA patients. In addition, Abatacept treatment exerts great effect on RA patients invalid to MTX treatment. Combination of Abatacept and MTX further improves the therapeutic efficacy on RA patients[8]. Clinical trials found that low-dose Abatacept treatment could not achieve a satisfactory outcome in active RA patients. It is not recommended to administrate with Abatacept combined with TNF-α due to the remarkable infection risk and poor therapeutic effect[9,10]. Abatacept exerts a safer efficacy than that of Infliximab in RA patients. The efficacy of Abatacept is gradually emerging in Phase II and Phase III clinical trials. It can be used not only for monotherapy but also for combination therapy after failure of MTX treatment[11]. However, in addition to inhibiting T cell activation, whether Abatacept could affect the function of RA-FLS needs to be further explored.

Patients and Methods

Sample Collection

Synovial tissues were harvested from RA patients undergoing joint replacement. Patients with cardiovascular diseases, malignancies, liver diseases, liver and renal dysfunction within 3 months, hypertension, diabetes, other acute and chronic inflammation, hepatitis C, tuberculosis, HIV infection, other primary diseases or autoimmune diseases were excluded. All RA patients were in accordance to the standard of ACR/EULAR published in 2010[12]. Among the included RA patients, there were 2 males and 7 females with the age of 42-64 years (mean age of 54.3±10.5 years). Patients signed informed consent and this study was approved by the Yantaishan Hospital Ethics Committee.

Cell Culture

The sterile synovial tissue was transferred to a 100-mm cell culture dish and adipose tissue on the surface of the synovial tissue was peeled off. After that phosphate-buffered saline (PBS) wash twice, tissues were cut into small pieces of about 1 mm^3. The small pieces were transferred in the new culture bottle with an interval of 1 cm. The culture bottle was inverted in the incubator. 4 hours later, 5 mL of DMEM (Dulbecco's Modified Eagle's Medium) containing 15% FBS (fetal bovine serum), 100 U/mL penicillin and 100 μg/mL streptomycin (HyClone, South Logan, UT, USA) was added. Culture medium was replaced every other day. Cell passage was performed until the 90% of cell confluence.

RNA Extraction And Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol kit (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA, which was then reversely transcribed into complementary Deoxyribose Nucleic Acid (cDNA). After the cDNA was amplified, qRT-PCR was performed to detect the expressions of related genes. Primers used in this study were as follows: MMP1, F: AAAATTACACGCCAGATTTGCC, R: GGTGTGACATTACTCCAGAGTTG; MMP3, F: CTCTGGAGTAATGTCACACCTCT, R: TGTTGGTCCACCTTTCATCTTC; MMP13, F: ACTGAGAGGCTCCGAGAAATG, R: GAACCCCGCATCTTGGCTT.

ELISA (Enzyme-Linked Immuno Sorbent Assay)

Cells were seeded in the 96-well plates at a density of 5×10^4 per well. Serum-free medium was replaced when the cell confluence was up to 80-90%. After specific treatment, the supernatant of each group was collected for detecting levels of MMP1, MMP3 and MMP13 using ELISA kits (R&D Systems, Minneapolis, MN, USA). The absorbance value was recorded at the wavelength of 492 nm with a microplate reader.

Transwell Assay

Cells were centrifuged and resuspended in serum-free DMEM at a density of 5.0×10^5/mL. Transwell chambers pre-coated with Matrigel were placed in 24-well plates. 200 μL of cell suspension and 900 μL of medium containing 20% FBS were added in the upper and lower chamber, respectively. After cell culture for 48 h, cells were fixed with 4% paraformaldehyde for 15 min and stained with crystal violet for 15 min. Inner cells were carefully cleaned. Penetrating cells were captured in 5 randomly selected fields of each sample.

Western Blot

Cells were lysed for protein extraction. The concentration of each protein sample was determined by a BCA (bicinchoninic acid) kit (Abcam, Cambridge, MA, USA). Protein sample was separated by gel electrophoresis and transferred to PVDF (polyvinylidene difluoride) membranes (Roche, Basel, Switzerland). After incubation with primary and secondary antibody (Cell Signaling Technology, Danvers, MA, USA), immunoreactive bands were exposed by enhanced chemiluminescence method.

Statistical Analysis

We used Statistical Product and Service Solutions (SPSS) 18.0 software (IBM, Armonk, NY, USA) for statistical analysis. The quantitative data were represented as mean ± standard deviation ($\bar{x} \pm s$). The *t*-test was used for comparing differences between the two groups. $p < 0.05$ was considered statistically significant.

Results

Abatacept Inhibited MMPs Expressions in FLS

MMPs play an important role in cell migration. In the present study, we detected expressions of MMPs in FLS after Abatacept treatment. QRT-PCR data showed that mRNA levels of MMP1, MMP3, and MMP13 in FLS were remarkably decreased after Abatacept treatment, which achieved the lowest levels at 48 h (Figure 1A). ELISA results obtained the similar results in detecting MMPs levels in the supernatant of culture medium (Figure 1B). Transwell assay showed that Abatacept treatment significantly inhibited cell migration of FLS (Figure 1C).

Abatacept Inhibited Mitogen-Activated Protein Kinase (MAPK) Pathway

Abnormal activation of MAPK pathway is a typical feature of chronic synovitis in RA.

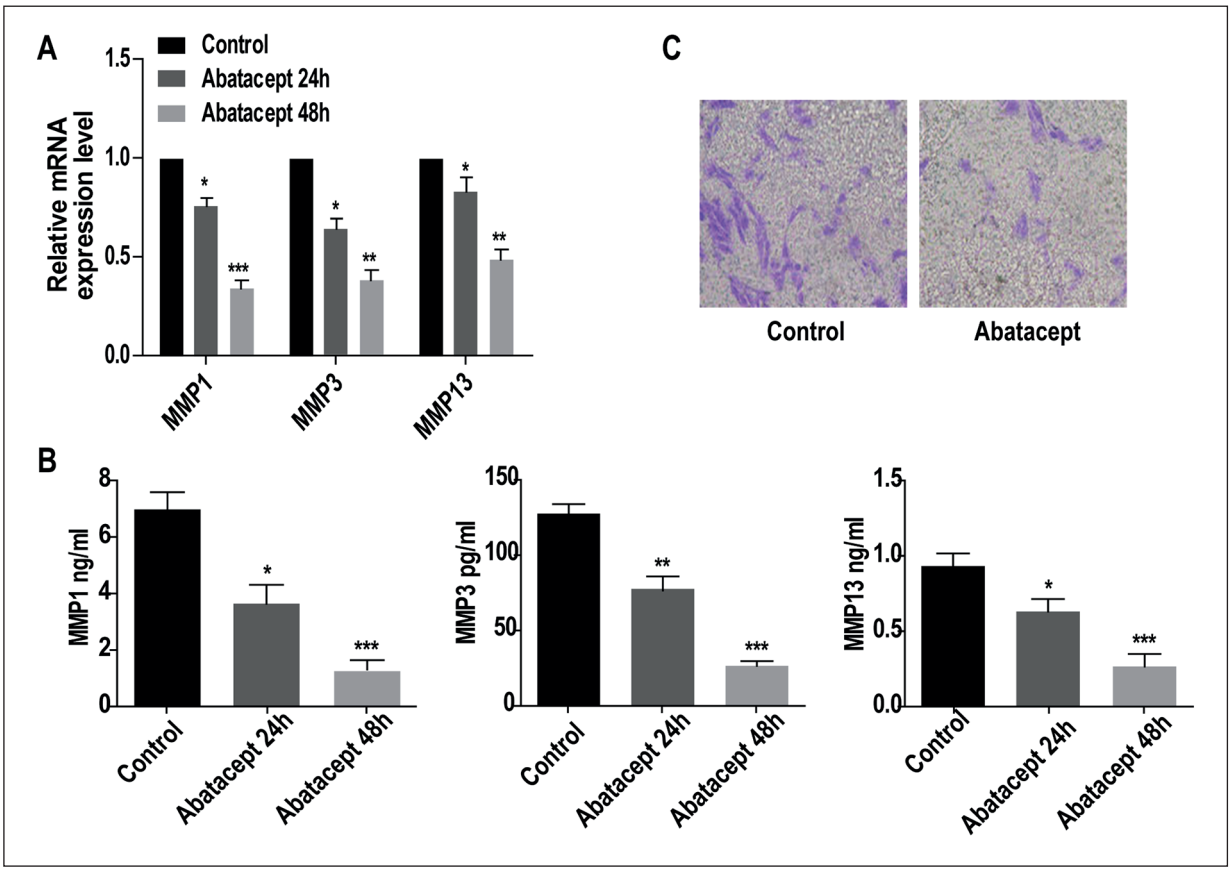

Figure 1. Abatacept inhibited MMPs expressions in FLS. ***A,*** QRT-PCR data showed that mRNA levels of MMP1, MMP3, and MMP13 were remarkably decreased after Abatacept treatment, which achieved the lowest levels at 48 h. ***B,*** ELISA results showed levels of MMP1, MMP3, and MMP13 in the supernatant of culture medium were decreased after Abatacept treatment. ***C,*** Transwell assay showed that Abatacept treatment significantly inhibited cell migration of FLS.

We hypothesized whether Abatacept could activate MAPK pathway. Western blot results showed that the phosphorylation levels of p-p38, p-Erk1/2, and p-p65 were remarkably decreased after Abatacept treatment (Figure 2). These results indicated that Abatacept may inhibit cell migration of FLS by inhibiting MAPK pathway.

Treatment of MAPK Pathway Inhibitor Upregulated MMPs Expressions

To investigate the role of MAPK pathway in the regulation of MMPs expressions, FLS were treated with the MAPK pathway inhibitor, SB203580. The mRNA levels of MMP1, MMP3, and MMP13 were remarkably increased after SB203580 treatment (Figure 3A). ELISA data obtained the similar results (Figure 3B).

Treatment of MAPK Pathway Inhibitor Reversed Abatacept-Induced MMPs Downregulation

We speculated whether SB203580 could reverse the inhibitory effect of Abatacept on cell migration. We found that the downregulated MMP1, MMP3 and MMP13 in FLS treated with Abatacept were reversed by SB203580 treatment (Figure 4A). ELISA results also found levels of MMP1, MMP3 and MMP13 in the supernatant of culture medium were reversed by SB203580 treatment (Figure 4B).

Discussion

Rheumatoid arthritis (RA) is a diffuse connective tissue disease. Due to the impaired joint structure and function caused by synovial inflammation, RA severely restricts daily life of affected patients[13,14]. Previous investigations have suggested that Abatacept can alleviate the symptoms of RA. This study aims to further investigate the molecular mechanism of Abatacept in treatment of RA. Mitogen-activated protein kinase (MAPKs) pathway responds to external stress and its abnormal activation is typical in RA-induced chronic synovitis[15,16]. The MAPKs family is a highly conserved threonine/tyrosine protein kinase composed of three distinct kinase families, including JNK, p38 and ERK[17,18]. The abnormally proliferating FLS in RA secrete a large number of inflammatory cytokines and MMPs, further leading to hypertrophy, hyperplasia and inflammatory cell infiltration. RA-FLS subsequently promotes the decomposition of the basement membrane matrix by proteases, especially MMPs, which is beneficial to endothelial cell survival and angiogenesis[19]. Studies have shown that the ERK pathway regulates the production of TNF-α and IL-1 through stimulating T cell proliferation. Cytokines are further activated, leading to accumulation and activation of monocytes and macrophages. As a result, multiple inflammatory factors are released in synovial tissues, such as TNF-α, IL-1 and IL-6, which in turn promoting hyperproliferation and abnormal apoptosis of RA-FLS. Pillinger et al[20] have found that inhibition of ERK pathway remarkably reduces RA-FLS proliferation via suppressing IL-1β and TNF-α production. Tagoe et al[21] have shown that MMP-1 production is associated with TNF-α secretion via regulating annexin-1 in FLS. ERK, JNK and NF-κB pathways are involved in annexin-1-induced MMP-1 secretion. Abnormal activation of MAPK pathway in RA-FLS leads to inflammatory reaction of synovial cells, hyperproliferation of synovial cells and MMPs upregulation. Series pathological changes eventually lead to long-term chronic synovitis, thereafter influencing RA development. In this work, we explored the effect of Abatacept on migration of FLS. Our results suggested that Abatacept treatment remarkably downregulated expressions of MMP1, MMP3 and MMP13, thereby inhibiting cell migration. Western blot results found that Abatacept treatment in RA-FLS inhibited expressions of relative genes in MAPK pathway. Rescue experiments further demonstrated that Abatacept treatment alleviated RA development through inhibiting cell migration via MAPK pathway.

Conclusions

We found that Abatacept inhibits migration of FLS and expressions of MMPs via inhibiting MAPK pathway, thereby alleviating RA development.

Conflict of Interest

The Authors declare that they have no conflict of interests.

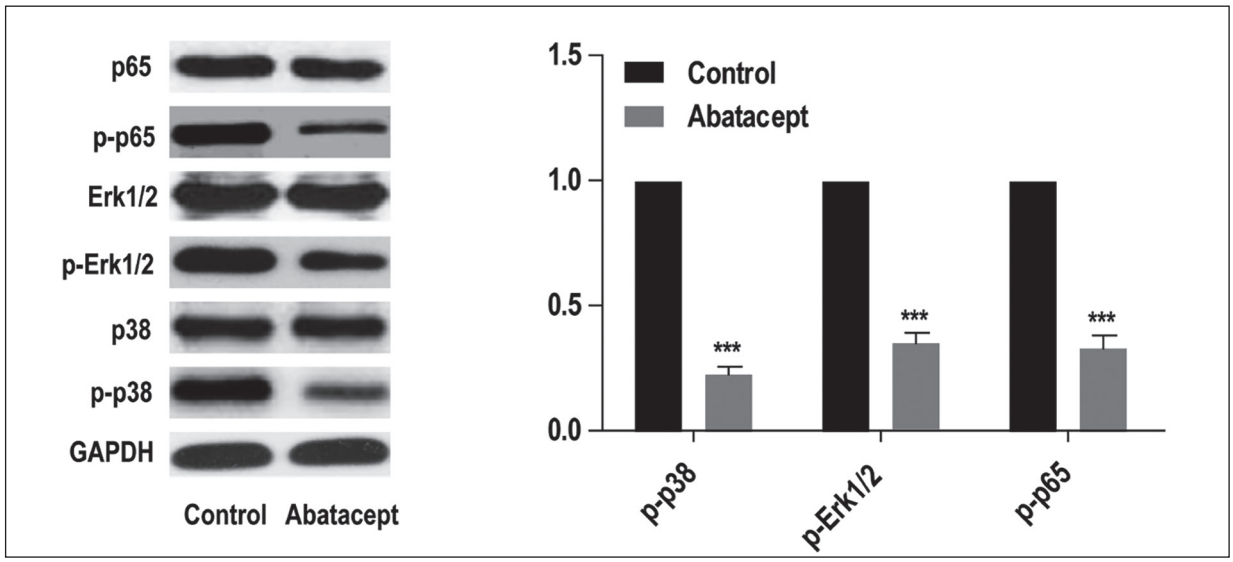

Figure 2. Abatacept inhibited MAPK pathway. Western blot results showed that the phosphorylation levels of p-p38, p-Erk1/2, and p-p65 were remarkably decreased after Abatacept treatment.

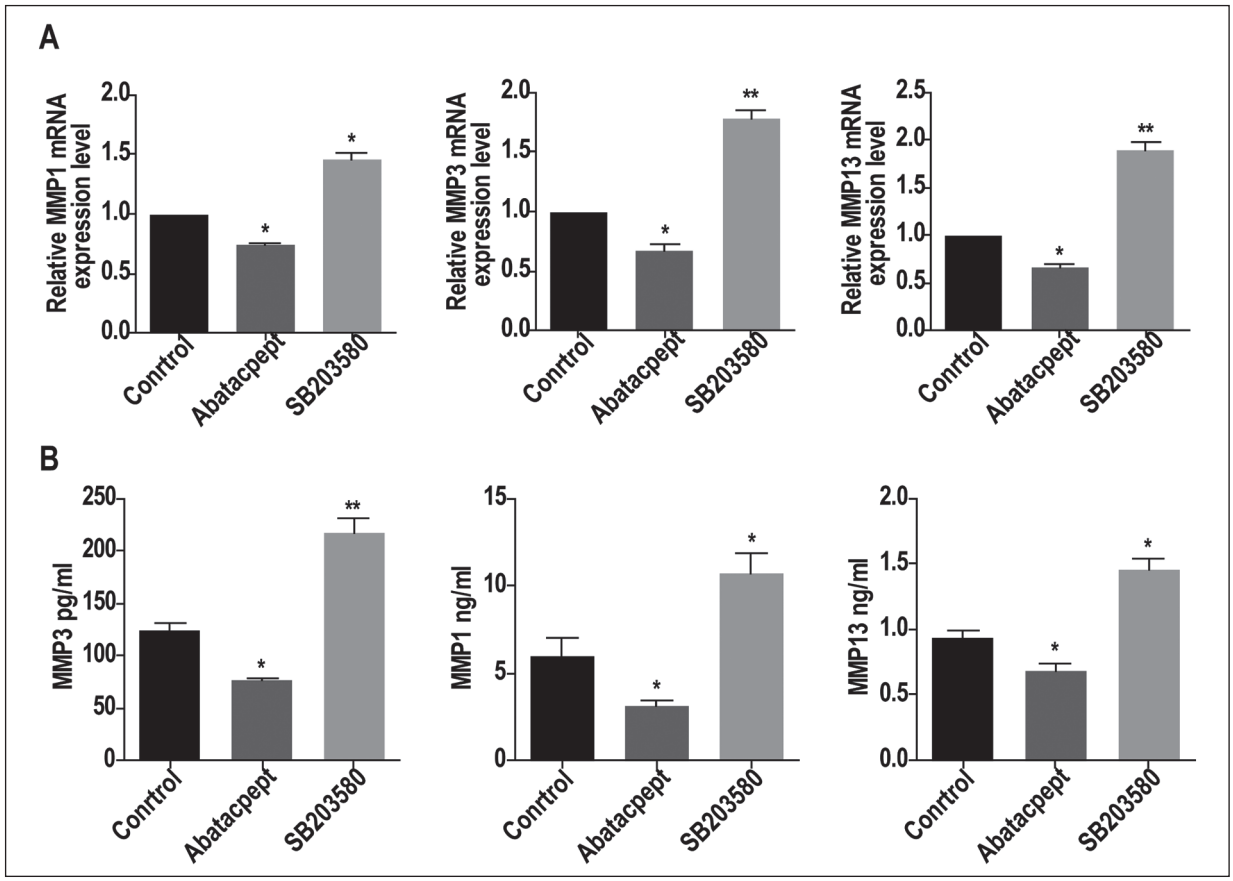

Figure 3. Treatment of MAPK pathway inhibitor upregulated MMPs expressions. ***A,*** The mRNA levels of MMP1, MMP3, and MMP13 were remarkably increased after SB203580 treatment. ***B,*** ELISA data showed that levels of MMP1, MMP3, and MMP13 in the supernatant of culture medium were remarkably increased after SB203580 treatment.

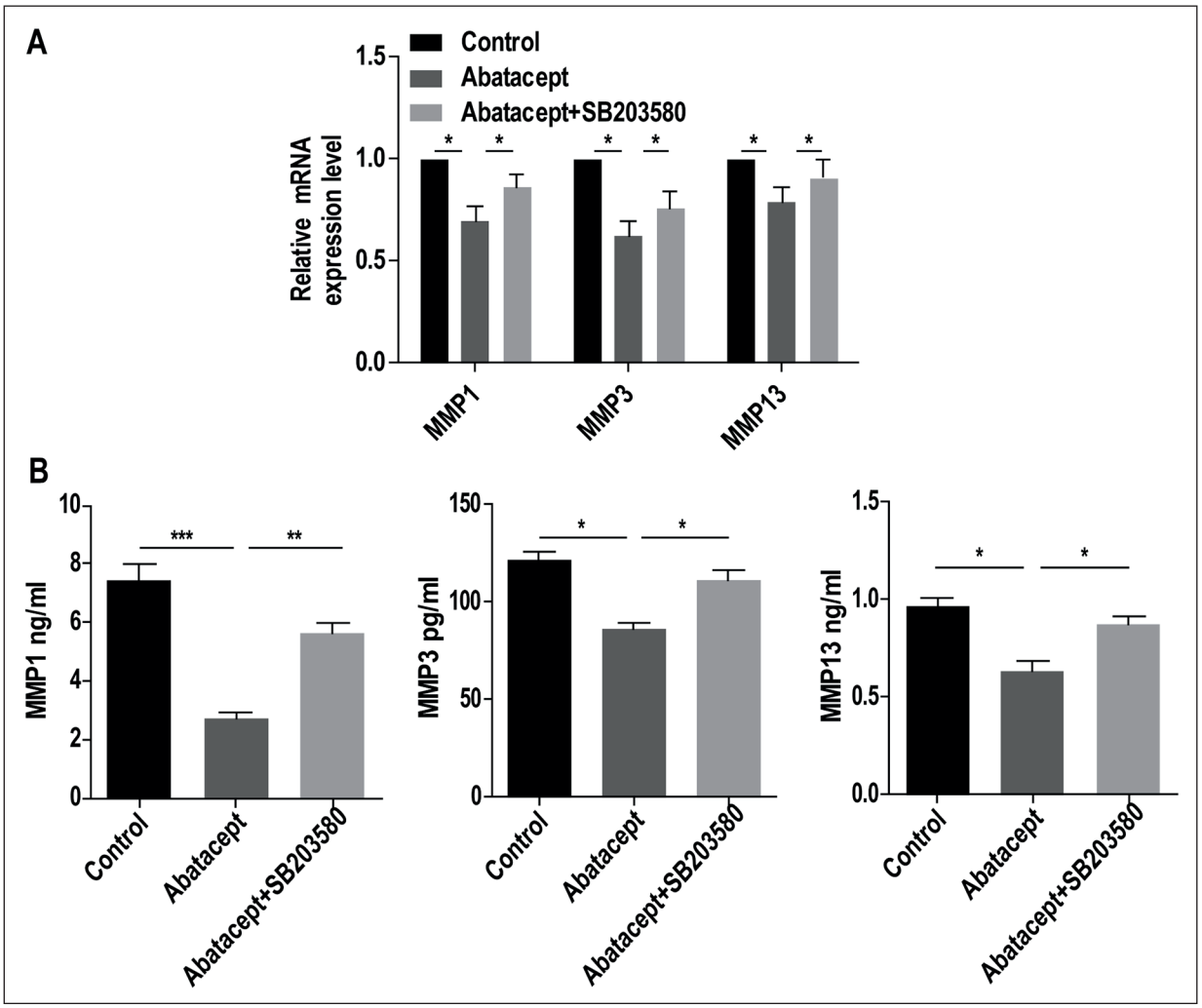

Figure 4. Treatment of MAPK pathway inhibitor reversed Abatacept-induced MMPs downregulation. ***A,*** Downregulated MMP1, MMP3 and MMP13 in FLS treated with Abatacept were reversed by SB203580 treatment. ***B,*** ELISA results found levels of MMP1, MMP3 and MMP13 in the supernatant of culture medium were reversed by SB203580 treatment.

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