



Transforming growth factor β 1 promotes fibroblast-like synoviocytes migration and invasion via TGF- β 1/Smad signaling in rheumatoid arthritis

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Abstract

Migration and invasion are important characteristics of rheumatoid arthritis fibroblast-like synoviocytes (RA-FLSs), which are involved in joint damage and contribute to rheumatoid arthritis (RA) pathology. However, the underlying mechanisms remain unclear. Because epithelial–mesenchymal transition (EMT) is a key mechanism related to migration and invasion in cancer cells, we investigated the relationship between EMT and RA-FLSs and explored whether the transforming growth factor β 1 (TGF- β 1)/Smad signaling pathway is involved. In vivo, fibroblast-like synoviocytes (FLSs) were isolated from the synovium of RA or osteoarthritis (OA) patients and cultured for 4–8 passages. EMT markers were detected by immunofluorescence and Western blotting. RA-FLSs were treated with TGF- β 1 or Smad2/3 small interfering RNA (siRNA), EMT markers were detected, and migration and invasion were assessed by Transwell assays. EMT markers could be detected in FLSs; when compared with osteoarthritis fibroblast-like synoviocytes (OA-FLSs), E-cadherin and vimentin decreased, while N-cadherin and α -smooth muscle actin (α -SMA) increased in RA-FLSs. Furthermore, TGF- β 1 enhanced migration and invasion by inducing EMT via activating Smad2/3 in RA-FLSs. Phosphorylation of Smad2/3 was accompanied by degradation of Smad3. Silencing Smad2/3 blocked EMT and inhibited the migration and invasion induced by TGF- β 1. Matrix metalloproteinase 9 (MMP9) and vimentin were not affected when cells were treated with TGF- β 1 or Smad2/3 siRNA. The TGF- β 1/Smad signaling pathway is involved in EMT and contributes to migration and invasion in RA-FLSs. Interestingly, vimentin decreased in RA-FLSs, but there is no correlation between vimentin and TGF- β 1/Smad signaling pathway. Thus, further research on vimentin should be conducted.

Keywords Rheumatoid arthritis · Epithelial–mesenchymal transition · Transforming growth factor β 1 · Migration · Invasion

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Introduction

Rheumatoid arthritis (RA) is a complex autoimmune disease. It affects mainly the joints and is characterized by synovial hyperplasia and inflammatory cell infiltration [1]. In fact, it should be considered a syndrome that includes extra-articular manifestations, such as rheumatoid nodules, pulmonary involvement or vasculitis, and systemic comorbidities [2]. Its clinical features include chronic pain and joint destruction that usually progresses from distal to more proximal joints [3]. According to the etiology of RA, the annual incidence has been reported to be 0.5% to 1%; women (2:1 to 3:1) are more likely to be affected than men [4], and genetic admixture, environmental factors, age, gender, and smoking may contribute [5]. Treatment strategies involve traditional disease-modifying anti-rheumatic drugs and novel biologic

agents. Although clinical symptoms can be improved if patients are treated early and appropriately, this disease carries a substantial burden for both the individual and society [6]. To date, considerable research has been conducted, but the exact pathogenesis remains unclear. Neumann E reported joint resident rheumatoid arthritis fibroblast-like synoviocytes (RA-FLSs) as key players in the pathogenesis of RA. RA-FLSs have similar biological properties as tumor cells. For example, they undergo tumor-like proliferation and produce matrix metalloproteinases that could in turn promote their migration and invasion, finally leading to progressive articular cartilage and bone degradation [7, 8]. RA-FLSs can also spread by migrating through the bloodstream and invade distant exposed cartilage matrix [9].

Epithelial–mesenchymal transition (EMT) is a phenomenon present in both physiological and pathological processes. It plays an important role in organ formation, tissue differentiation, and repair. Additionally, EMT contributes to organ fibrosis and promotes carcinoma progression [10]. Many studies have demonstrated that transforming growth factor β 1 (TGF- β 1) could induce EMT. This process induces cell proliferation and extracellular matrix deposition, increases the expression of α -smooth muscle actin (α -SMA), fibronectin, and collagen type I (collagen I) in vitro, and leads to organ fibrosis [11]. In addition, some research also demonstrated that TGF- β 1 could upregulate vimentin and fibronectin and downregulate E-cadherin, contributing to migration and invasion capabilities [12–14]. Some studies have demonstrated that the expression of TGF- β 1 was high in the fibroblast-like synoviocytes (FLSs) and synovial fluids from RA patients [15–17]. All of these findings indicate that RA-FLSs may undergo a EMT process induced by TGF- β 1. However, the role and molecular mechanisms of EMT in RA remain unidentified.

In this study, we demonstrated RA-FLSs undergo EMT by detecting the expression of the epithelial marker E-cadherin and the mesenchymal markers N-cadherin, vimentin, and α -SMA. We found that TGF- β 1 exerts its role in EMT by activating RA-FLSs. Finally, we investigated the involvement of the TGF- β /Smad signaling pathway in the induction EMT phenotype transformation with Smad2/3 interference.

Materials and methods

Reagents

Human TGF- β 1 (8915LC), polyclonal rabbit anti-Smad2/3 (8685), polyclonal rabbit anti-phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4), and polyclonal rabbit anti-Snail (3879) were purchased from Cell Signaling Technology (USA). Monoclonal mouse anti-N-cadherin (sc-59987), anti-p53/Smad2/3 small interfering RNA (siRNA)

(sc-37238), and control siRNA (sc-36869) were purchased from Santa Cruz (USA). Polyclonal rabbit anti-E-cadherin (20874-1-AP), polyclonal rabbit anti-vimentin (10366-1-AP), polyclonal rabbit anti- α -SMA (55135-1-AP), and monoclonal mouse anti-GAPDH (60004-1-Ig) were obtained from Proteintech (Wuhan, China).

FLSs culture

Osteoarthritis (OA) is a degenerative disease. Compared with RA, the clinical symptoms and pathological manifestations of OA are milder. OA has been frequently used as control to study the pathobiology of RA. Fibroblast-like synoviocytes were obtained from the synovium of RA or OA patients undergoing knee replacement surgery. The present study was approved by the Ethics Committee of Southern Medical University (Guangzhou, China, NFEC-20120201). Written informed consent was obtained from all patients. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.) and antibiotics (100 mg/ml streptomycin and 100 U/ml penicillin) in a humidified incubator at 37 °C with 5% CO₂. The cells used for experiments were from passages 3–8. RA-FLSs were identified by immunofluorescence (phenotype: >98% vimentin, data not shown). RA-FLSs were cultured in zero serum conditions for 12 h, and then treated with TGF- β 1 in 1% serum conditions.

Immunofluorescence

RA-FLSs were seeded in confocal dishes and incubated for 24 h. Then, the culture medium was discarded, and the cells were washed three times with phosphate buffer (PBS). The cells were fixed with 4% paraformaldehyde for 20 min and permeabilized in 0.2% Triton X-100/PBS for 5 min at room temperature. After washing with PBS three times, the cells were blocked with 10% normal goat serum (Bioss) for 1 h at room temperature and incubated with specific primary antibodies at 4 °C overnight. The primary antibodies included anti-E-cadherin (1:100), anti-N-cadherin (1:100), anti- α -SMA (1:100), and anti-vimentin (1:100). Then, the cells were washed with PBS three times and incubated with goat anti-rabbit IgG/Alexa Fluor 488 antibody (1:200) (Bioss, bs-0295G-AF488), rabbit anti-mouse Kappa light chain/Alexa Fluor 488 antibody (1:200) (Bioss, bs-0330R-AF488), or goat anti-rabbit IgG/Alexa Fluor 647 antibody (1:200) (Bioss, bs-0295G-AF647) for 1 h at room temperature. Finally, fluoroshield with DAPI (Abcam, ab104139) was used to distinguish the cellular nuclei. Immunofluorescence was viewed with a laser scanning confocal microscope.

For phosphorylated-Smad2/3 detections, cells were incubated with TGF- β 1 for 48 h in DMEM with 1% FBS. Triton X-100/PBS (0.5%) was used to permeabilize the cells for 20 min at room temperature. Then, the cells were blocked and incubated with phospho-Smad2/3 (1:200). The rest of the procedure was performed as described above.

Small interfering RNA transfection

To knock down Smad2/3, RA-FLSs were cultured in six-well plates (1.0×10^5 per well). Transfection with siRNA was performed using LipofectAMINE 3000 (Invitrogen) according to the manufacturer's instructions. The cells were transfected with control siRNA or Smad2/3 siRNA (Santa Cruz Biotechnology) at a final concentration of 30 nM for 48 h.

Western blot analysis

Cells were lysed with a Minute™ total protein extraction kit (SD-001/SN-002, Invent Biotechnologies). The cells were washed with PBS and then lysed in SD-001 lysis buffer containing protease inhibitor cocktail and phosphatase inhibitors (Sigma-Aldrich) on ice. The extracts were centrifuged at 15,000 rpm for 1 min, and the protein concentrations were quantified using a BCA Protein Assay Kit (Cwbio, Beijing, China). Equal amounts (25 μ g) of protein were separated by 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat dry milk in Tris-buffered Saline-Tween (TBST) at room temperature for 1 h and incubated overnight at 4 °C with the primary antibodies. The membranes were then washed three times with TBST and then incubated with the secondary antibody. GAPDH was used as an internal control. Immunoreactive bands were detected with the chemiluminescent substrate, and the intensities of the bands were quantified by Quantity One Software (Bio-Rad).

Transwell migration and invasion assays

First RA-FLSs were treated with TGF- β 1 or Smad2/3 siRNA for 48 h. For the migration assays, 2×10^4 cells in 200 μ l of 1% FBS-containing medium were placed in the top chamber of the Transwell plate (pore size, 8 μ m; BD Bioscience). For the invasion assays, 3×10^4 cells in 200 μ l of 1% FBS-containing medium were placed in the top chamber coated with Matrigel (BD Bioscience) following the manufacturer's protocol. The lower chamber was filled with 900 μ l of 10% FBS-containing medium. After incubation at 37 °C for 24 h, the cells on the upper surface were wiped away with a cotton swab, while the cells invading the bottom of the membranes were fixed in a methanol solution for 20 min and stained with a 0.1% crystal violet solution for 20 min and counted

under a microscope. Five random fields were counted. The experiments were repeated three times.

Statistical analysis

All the results are expressed as the mean \pm standard deviation (SD). The results from different groups were analyzed by one-way analysis of variance (ANOVA) with Fisher's probable least-squares difference test or Student's *t* test. $p < 0.05$ was considered statistically significant.

Results

Comparison of EMT markers in RA-FLSs and OA synovial fibroblasts (OA-FLSs)

To ensure that RA-FLSs can express epithelial–mesenchymal transition markers, we cultured cells until passage 4 and then measured EMT markers (including N-cadherin, E-cadherin, vimentin, and α -SMA) by immunofluorescence. As shown in Fig. 1a, N-cadherin and E-cadherin are expressed in mainly the cell membrane, while vimentin and α -SMA are expressed in mainly the cytoplasm. To confirm that RA-FLSs had undergone EMT, we isolated FLSs from the synovium of RA and OA patients. Then, we lysed the cells, measured the total cellular protein, and finally analyzed the EMT markers by Western blotting. The results are shown in Fig. 1b. Compared with those in OA patients, N-cadherin ($p < 0.01$) and α -SMA ($p < 0.01$) protein levels were increased, and E-cadherin ($p < 0.01$) and vimentin ($p < 0.01$) protein levels were decreased in RA patients. As for vimentin, the results of immunofluorescence were consistent with western blot (Fig. 1c).

TGF- β 1 induces EMT in RA-FLSs

TGF- β 1 is a known factor that promotes EMT in cancer cells, and when EMT occurs, the migration and invasion capabilities of cancer cells are enhanced. The previous results indicate that RA and OA-FLSs could express EMT markers and likely RA-FLSs undergo EMT. To investigate whether TGF- β 1 could induce EMT in RA-FLSs, we treated RA-FLSs with TGF- β 1, and the results are shown in Fig. 2. First, RA-FLSs were treated with a control or different concentrations of TGF- β 1 (0.1, 1, 10 ng/ml) for 48 h. Figure 2b indicates that compared with the PBS control, 1 ng/ml ($p < 0.01$) and 10 ng/ml ($p < 0.01$) TGF- β 1 significantly increased N-cadherin, and 10 ng/ml ($p < 0.01$) TGF- β 1 increased α -SMA and matrix metalloproteinase 2 (MMP2). However, 1 ng/ml ($p < 0.05$) and 10 ng/ml ($p < 0.01$) TGF- β 1 decreased E-cadherin. Second, RA-FLSs were treated with 10 ng/ml TGF- β 1 for 12 h, 24 h, and 48 h. Figure 2c

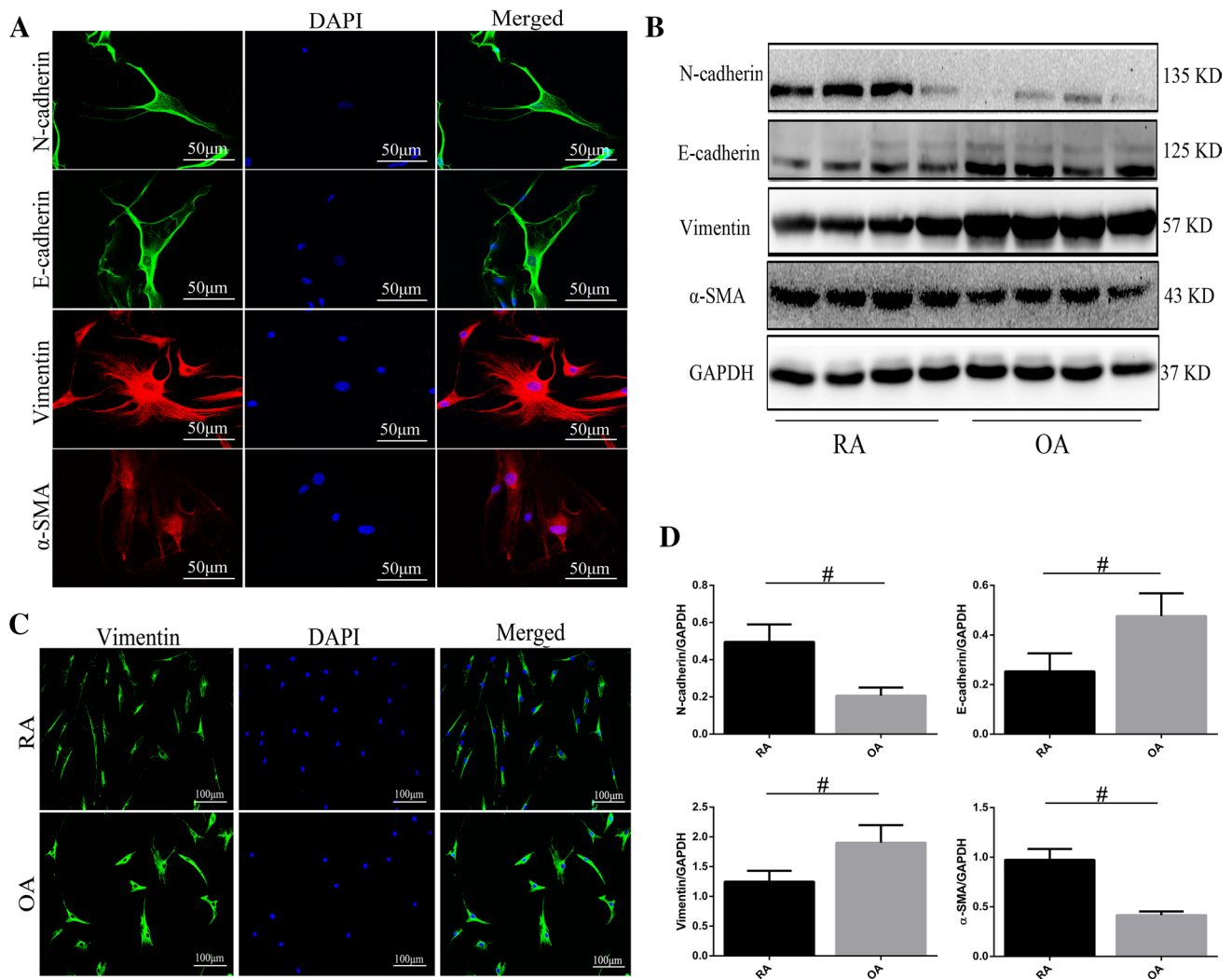


Fig. 1 Expression analysis of EMT markers in FLs. **a** Fluorescence micrographs with staining for N-cadherin (green), E-cadherin (green), vimentin (ren), α -SMA (red), and nuclei (blue) in RA-FLSs at $\times 400$. **b** Western blot analyses for N-cadherin, E-cadherin, vimen-

tin, and α -SMA expression in RA-FLSs and OA-FLSs. **c** Fluorescence micrographs with staining for vimentin in RA-FLSs and OA-FLSs at $\times 200$. **d** Densitometric analysis of the Western blot results ($n = 4$) * $p < 0.05$, # $p < 0.01$. (Color figure online)

indicates that compared with the PBS control, TGF- β 1 increased N-cadherin ($p < 0.01$) and MMP2 ($p < 0.01$) when the cells were treated for 48 h and increased α -SMA ($p < 0.01$) when the cells were treated for 24 h and 48 h. At the same time, E-cadherin ($p < 0.01$) was decreased when the cells were treated for 48 h. However, the present study also showed that TGF- β 1 had no effect on the expression of vimentin ($p > 0.05$) and matrix metalloproteinase 9 (MMP9) ($p > 0.05$) in RA-FLSs.

Snail is involved in TGF- β 1-induced EMT in a Smad2/3-dependent manner

Smad2/3 and Snail are important factors that regulate EMT through TGF- β . To confirm that TGF- β 1 can activate

Smad2/3 and regulate Snail in RA-FLSs, we treated cells with 10 ng/ml TGF- β 1 for 48 h and then detected Phospho-Smad2/3 and Snail by Western blot and immunofluorescence assays. As shown in Fig. 3, compared with the PBS control, TGF- β 1 upregulated the expression of phospho-Smad2/3 ($p < 0.01$) and downregulated the expression of Smad2/3 ($p < 0.05$), thus promoting phospho-Smad2/3 entry into the nucleus. Ultimately, the expression of Snail ($p < 0.05$) was increased. These results demonstrated that TGF- β 1 could induce RA-FLSs to undergo EMT by activating Smad2/3 and regulating Snail.

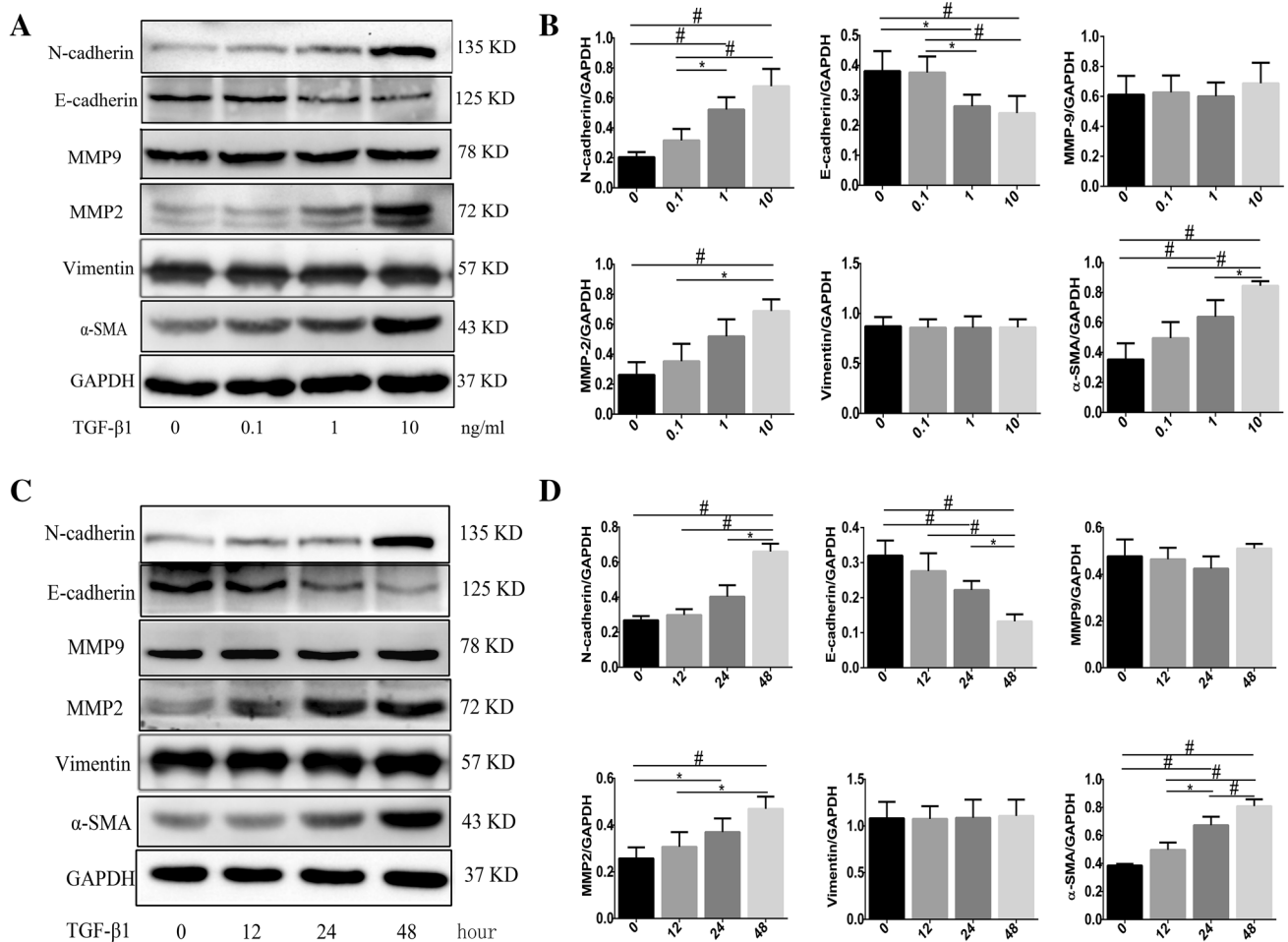


Fig. 2 TGF- β 1 induces EMT in RA-FLSs. **a** Western blot analyses for N-cadherin, E-cadherin, vimentin, α -SMA, MMP9, and MMP2 expression after RA-FLSs were treated with a vehicle control or different concentrations of TGF- β 1 (0.1, 1, or 10 ng/ml) for 48 h. **c** Western blot analyses for N-cadherin, E-cadherin, vimentin, α -SMA,

MMP9, and MMP2 expression after RA-FLSs were treated with a vehicle control or TGF- β 1 (10 ng/ml) for different times (12, 24 or 48 h). **b, d** Densitometric analysis of the Western blot results ($n=4$). * $p < 0.05$, # $p < 0.01$

Smad2/3 knockdown affected EMT by regulating the expression of EMT markers

Previous results indicate that Smad2/3 is a key factor in EMT and activating Smad2/3 could induce EMT in RA-FLSs. Therefore, we investigated whether RA-FLSs would undergo mesenchymal–epithelial transition (MET) when treated with Smad2/3 siRNA. As shown in Fig. 4a, Smad2/3 protein expression levels were significantly decreased after Smad2/3 siRNA transfection for 48 h. As a result, E-cadherin protein expression was increased ($p < 0.05$), and N-cadherin ($p < 0.05$) and α -SMA ($p < 0.05$) protein expression was decreased (Fig. 4d). However, there were no changes in the protein expression levels of MMP2 ($p > 0.05$), MMP9 ($p > 0.05$), or vimentin ($p > 0.05$).

Smad2/3 knockdown inhibited the migration and invasion of RA-FLSs by blocking TGF- β 1-induced EMT

The previous results demonstrated that the pathological changes in RA-FLSs were related to EMT. TGF- β 1 could induce RA-FLSs to undergo EMT by activating Smad2/3. Smad2/3 knockdown reversed the expression of EMT markers, including N-cadherin, E-cadherin, and α -SMA. As migration and invasion are important characteristics of RA-FLSs, it is necessary to investigate whether the characteristics will be affected when Smad2/3 are knocked down. Cells were treated with Smad2/3 siRNA or control siRNA for 48 h and then treated with TGF- β 1 (10 ng/ml) for another 48 h. EMT markers were detected by Western blotting, and the migration and invasion capabilities were detected by

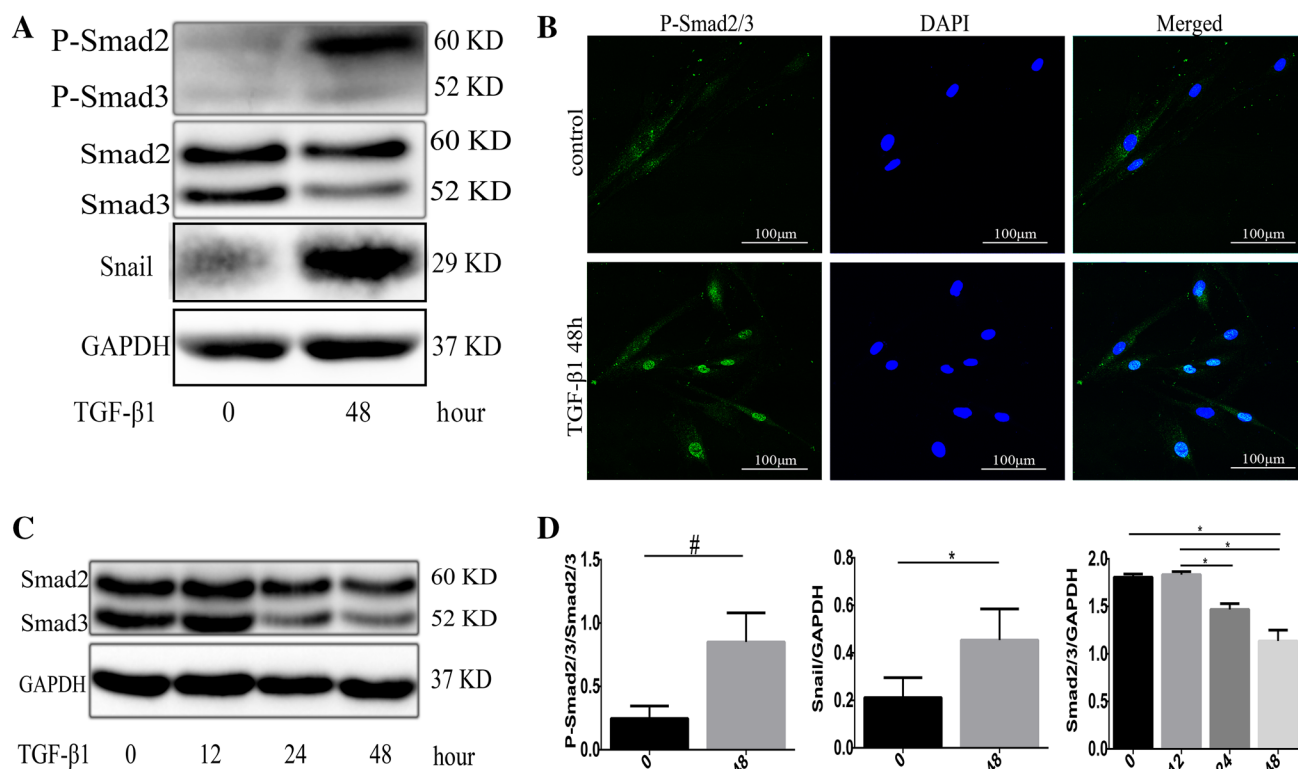


Fig. 3 TGF- β 1 increases Snail levels via activating Smad2/3 in RA-FLSs. **a** Western blot analyses for Smad2/3, P-Smad2/3, and Snail after RA-FLSs were treated with TGF- β 1 (10 ng/ml) for 48 h. **b** Fluorescence micrographs with staining for P-Smad2/3 (green) and nuclei

(blue) after RA-FLSs were treated with TGF- β 1 (10 ng/ml) for 48 h. **c** Western blot analyses for Smad2/3 after RA-FLSs were treated with TGF- β 1 (10 ng/ml) for 12, 24, 48 h. **d** Densitometric analysis of the Western blot results ($n=4$). * $p < 0.05$, # $p < 0.01$. (Color figure online)

Transwell assays. As shown in Fig. 5a, compared with the TGF- β 1 control, Smad2/3 knockdown significantly blocked the TGF- β 1-induced expression of N-cadherin ($p < 0.05$), E-cadherin ($p < 0.05$), MMP2 ($p < 0.05$), and α -SMA ($p < 0.05$). However, the protein expression levels of MMP9 ($p > 0.05$) and vimentin ($p > 0.05$) remained unchanged. At the same time, as shown in Fig. 5c, TGF β 1-induced migration and invasion in RA-FLSs were also inhibited upon Smad2/3 knockdown. The Transwell assay results were consistent with the Western blot analysis results.

Discussion

It has been shown that the migration and invasion of RA-FLSs are related to EMT. For example, hypoxia could induce EMT, resulting in the migration and invasion of RA-FLSs [18]. In this study, we demonstrated that TGF- β 1 was involved in the EMT of RA-FLSs by activating Smad2/3, which results in their migration and invasion.

It is necessary to confirm the relationship between RA and EMT, as there are differing opinions on the issue. Some research showed that classical E-cadherin expression was scant [19], but E-cadherin was found in healthy and arthritic

synovial tissue [20]. E-cadherin is an important component of extracellular connections. As a key protein in EMT, its decreased expression is accompanied by an increase in N-cadherin, and it facilitates cell migration and invasion [21]. In this study, we provided evidence that E-cadherin was present in RA and OA-FLSs. As shown in Western blots, the expression level of E-cadherin was decreased, while that of N-cadherin was increased in RA-FLSs when compared with those of OA-FLSs. Regarding α -SMA, the tumor microenvironment is rich in carcinoma-associated fibroblasts, which are characterized by the expression of α -SMA and contribute to tumor progression [22]. α -SMA-positive FLSs reportedly play a key role in the pathogenesis of RA [23, 24]. According to immunofluorescence and Western blotting assays, α -SMA could be detected in both RA and OA-FLSs. In addition, α -SMA was increased in RA-FLSs. These results indicate that RA-FLSs can express EMT markers and that phenotypic cell characteristics are related to EMT.

TGF- β 1 is a multifunctional cytokine whose effects are different, even opposite, depending on the cell type and the conditions. For example, TGF- β 1 can inhibit cell proliferation but also promote cell growth, enhance stem cell pluripotency but also differentiation, and regulate muscle genes in myoblasts and neural genes in neuroblasts [25, 26]. TGF- β 1

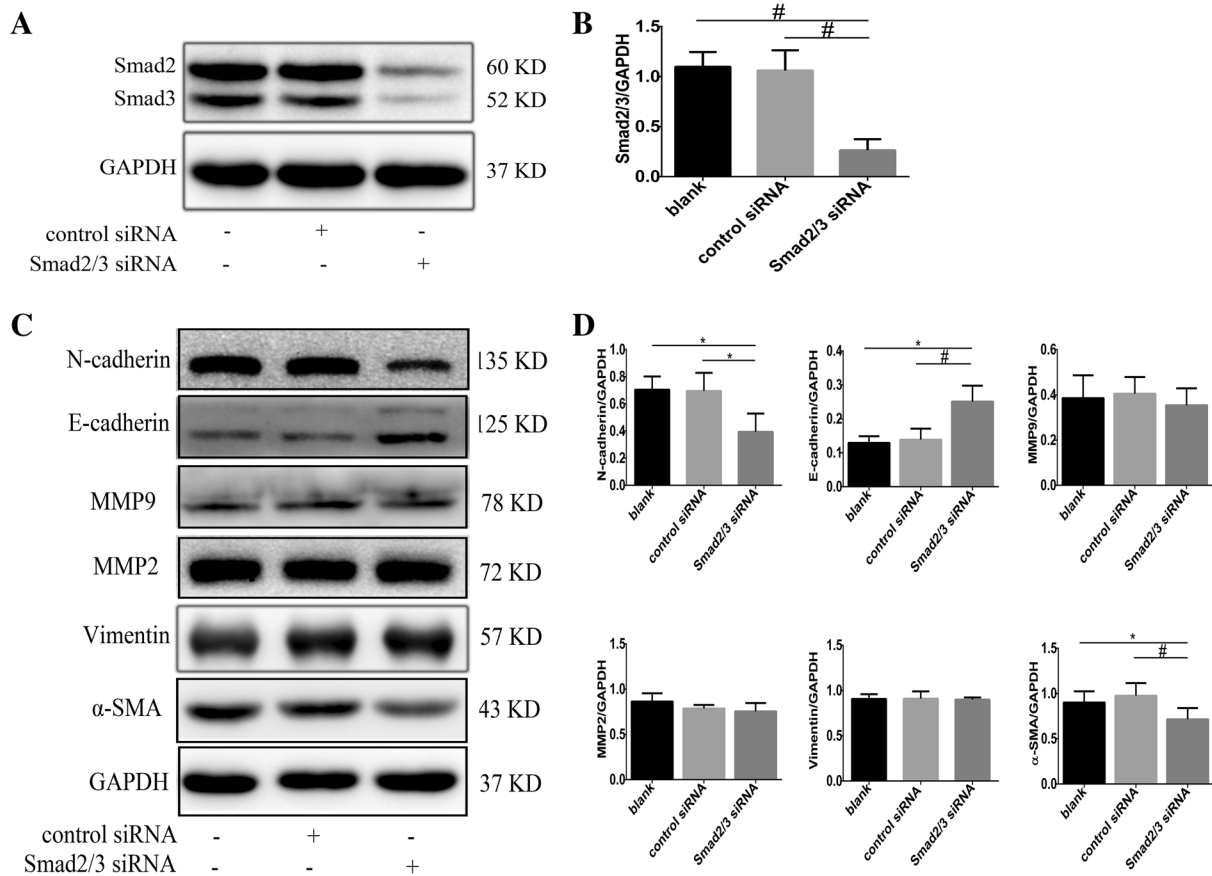


Fig. 4 Knockdown of Smad2/3 reversed EMT in RA-FLSs RA-FLSs were pretreated with scrambled siRNA (30 nM) or Smad2/3 siRNA (30 nM) for 48 h. **a** Western blot analyses for Smad2/3 and Snail. **b** Densitometric analysis of the Western blot results ($n=4$). **c** Western

blot analyses for N-cadherin, E-cadherin, vimentin, α -SMA, MMP9, and MMP2 expression. **d** Densitometric analysis of the Western blot results ($n=4$). * $p < 0.05$, # $p < 0.01$

is secreted by many types of cells, including FLSs. Some studies demonstrated that TGF- β 1 expression was upregulated in the synovial fluid and fibroblasts of arthritis patients [15–17]. However, it is not clear whether TGF- β 1 has an effect on RA-FLSs or contributes to the progression of RA. In this study, we evaluated the effect of TGF- β 1 on RA-FLSs by treating cells for various times and with different concentrations. The results demonstrated that N-cadherin, MMP2, and α -SMA expression levels were increased, E-cadherin expression levels were decreased, and cell migration and invasion capacity were enhanced when cells were treated with 10 ng/ml TGF- β 1 for 48 h. These findings confirmed that TGF- β 1 had an effect on RA-FLSs, and might lead to joint damage by inducing EMT.

TGF- β 1 exerts biological functions by activating downstream effectors via either a Smad-dependent pathway or a Smad-independent (TAK1-p38MAPK) signaling axis [26]. Increased P-Smad-2/3 levels with a nuclear distribution were observed in blood vessels, synovial lining cells, and mononuclear cell infiltrates of RA and OA synovial

tissues compared to those in healthy controls [27]. The present study confirmed that Smad2/3 could be phosphorylated and enter the nucleus when cells were treated with TGF- β 1. Smad2/3 knockdown led to mesenchymal-epithelial transition (MET). Furthermore, TGF- β 1-induced EMT was blocked, and cell migration and invasion were inhibited after Smad2/3 was silenced. All these results showed that TGF- β 1 enhanced migration and invasion and contributed to RA pathology by inducing EMT via activating Smad2/3 in RA-FLSs. In addition, we observed that Smad2/3, especially smad3 decreased when RA-FLSs were treated with TGF- β 1. Smad ubiquitination regulatory factor 2 (SMURF2), one of the HECT (homologous with E6-APC terminus) family of E3 ubiquitin ligases, regulates ubiquitination-mediated Smad3 degradation [28]. Meanwhile, SMURF2 could be induced by TGF- β 1 [29, 30]. So, we speculated that TGF- β 1 might contributed to the expression of SMURF2, leading to ubiquitination-mediated Smad3 degradation RA-FLSs.

MMP9 and vimentin were not affected in RA-FLSs treated with TGF- β 1 or Smad2/3 siRNA. MMP2 and

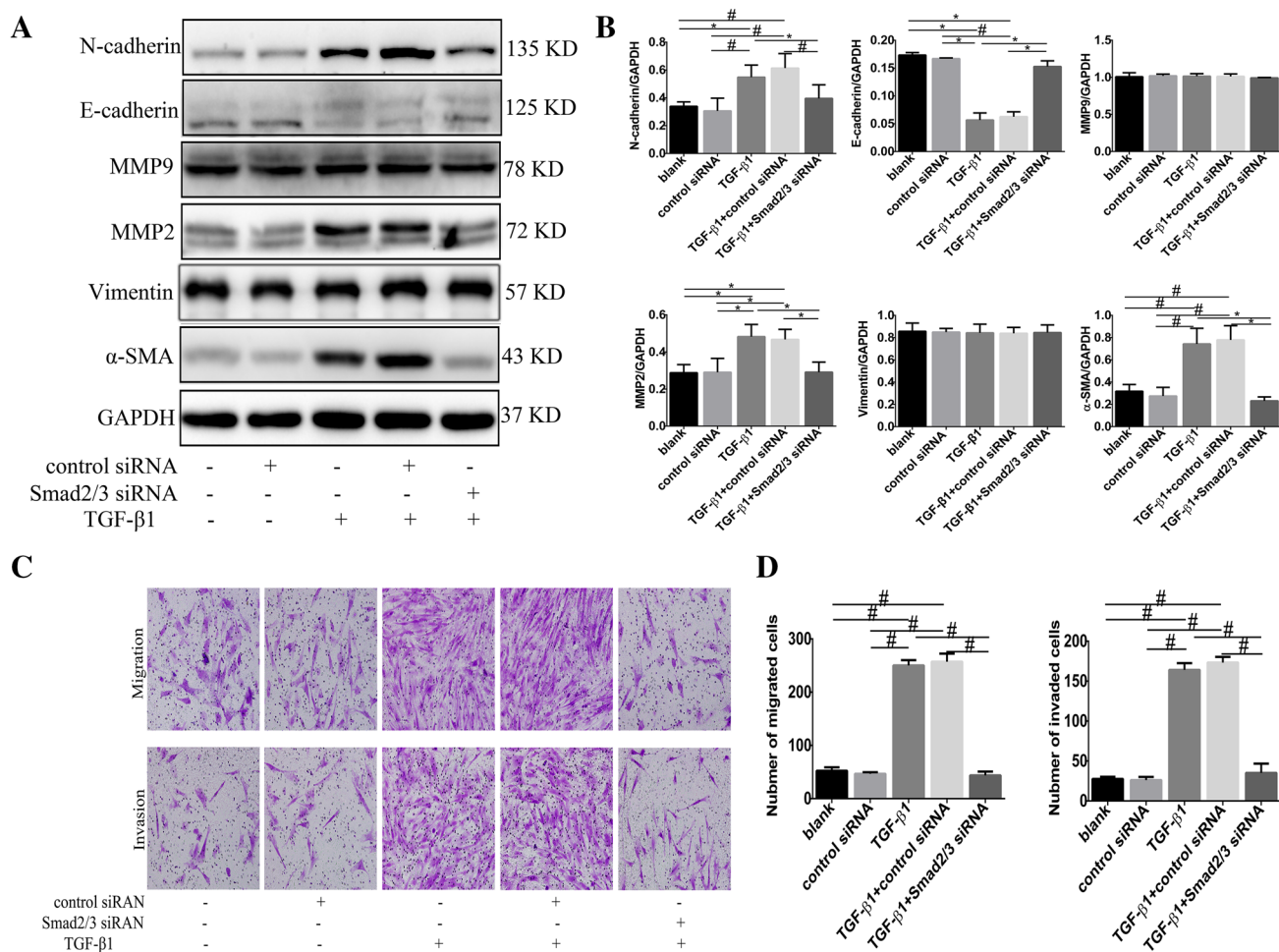


Fig. 5 Knockdown of Smad2/3 blocked TGF-β1-induced EMT and inhibited migration and invasion in RA-FLSs. RA-FLSs were pre-treated with scrambled siRNA (30 nM) or Smad2/3 siRNA (30 nM) for 48 h and then treated with TGF-β1 (10 ng/ml) for 48 h. **a** Western blot analyses for N-cadherin, E-cadherin, vimentin, α-SMA, MMP9, and MMP2 expression. **b** Densitometric analysis of the Western blot

results ($n=4$). **c** Transwell migration and Matrigel invasion assays for the migration and invasion abilities of RA-FLSs after treatment. The migrated and invaded cells were photographed ($\times 200$ magnification). **d** The migrated and invaded cells were counted in five random fields for each treatment ($n=3$). $*p < 0.05$, $\#p < 0.01$

MMP9 are both type IV collagenases, which are involved in the invasion and metastasis of various tumor cells. Previous studies showed that they could be detected in synovial tissue and cells [31], and the present study confirmed these findings. Although both MMP2 and MMP9 can be induced by TGF-β1 in tumor cells [32–34], we provided evidence that MMP2, but not MMP9, is involved in TGF-β1/Smad signaling in RA-FLSs. Vimentin is also a marker of EMT and associated with epithelial and non-epithelial tumor spread, tumor cell migration, and angiogenesis [35]. Vimentin contributes to cell migration by regulating focal adhesion-associated proteins [36]. The loss of vimentin alters signaling through focal adhesion and mechanotransduction, resulting in reduced cancer cell adhesion and migration [37]. Therefore, we predicted that

the expression of vimentin would increase when RA-FLSs undergo EMT. In fact, our study showed that the expression level of vimentin decreased in RA-FLSs. This result is consistent with those of Kim [38]. Previous observations also demonstrated that the induction of cellular vimentin synthesis in MPC-11 mouse myeloma cells was correlated with cell progression inhibition at the G1 phase of the cell cycle [24]. In addition, vimentin inhibited proliferation, while citrullinated vimentin significantly stimulated proliferation capacity in RA-FLSs [39]. As FLs proliferation is an important characteristic of RA [40], present study demonstrated that decrease in vimentin might contribute to proliferation and provide new insight into the pathology of RA.

Conclusion

The present study demonstrated that the RA-FLS phenotype was related to EMT. TGF- β 1 could induce EMT via activating Smad2/3 and contributing to migration and invasion in RA-FLSs. Silencing Smad2/3 blocked TGF- β 1-induced EMT and inhibited cell migration and invasion. Vimentin and MMP9, special EMT markers in RA-FLSs, were not regulated by TGF- β 1 and need further investigation.

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Compliance with ethical standards

Conflict of interest The authors have declared that no conflicts of interest exist.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Southern Medical University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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