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RESEARCH

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Baricitinib improves pulmonary fibrosis in mice with rheumatoid arthritis-associated interstitial lung disease by inhibiting the Jak2/Stat3 signaling pathway

Hongli Liu[1†], Yan Yang[1†], Jie Zhang[2*†] and Xuelin Li[2†]

Abstract

Objective The study explored improvements in pulmonary inflammation and fibrosis in a bovine type II collagen-induced rheumatoid arthritis-associated interstitial lung disease mouse model after treatment with baricitinib and the possible mechanism of action.

Methods A rheumatoid arthritis-associated interstitial lung disease mouse model was established, siRNA Jak2 and lentiviral vectors were transfected with human embryonic lung fibroblast cells. And the levels of relevant proteins in mouse lung tissue and human embryonic lung fibroblasts were detected by Western blotting.

Results The levels of JAK2, p-JAK2, p-STAT3, p-SMAD3, SMA, TGFβR2, FN and COL4 were increased in the lung tissues of model mice ($P < 0.5$) and decreased after baricitinib intervention ($P < 0.05$). The expression levels of p-STAT3, p-SMAD3, SMA, TGFβR2, FN and COL4 were reduced after siRNA downregulation of the JAK2 gene ($P < 0.01$) and increased after lentiviral overexpression of the JAK2 gene ($P < 0.01$).

Conclusion Baricitinib alleviated fibrosis in the lung tissue of rheumatoid arthritis-associated interstitial lung disease mice, and the mechanism of action may involve the downregulation of Smad3 expression via inhibition of the Jak2/Stat3 signaling pathway, with consequent inhibition of the profibrotic effect of transforming growth factor-β1.

Keywords Rheumatoid arthritis, Interstitial lung disease, Baricitinib, Jak2/Stat3

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that affects 1% of the general population. RA attacks the joints, causing progressive erosive destruction of cartilage and bone. The most common extra-articular manifestation is pulmonary involvement, which can affect up to 60% of patients with RA during the disease course [1–3]. Interstitial lung disease (ILD) is the most common manifestation of lung involvement in RA, and it is also an important cause of excess mortality among RA patient [4, 5]. The risk of death among patients with Rheumatoid arthritis-associated interstitial lung disease (RA-ILD) is 3 times higher than that among RA patients without ILD, and the median survival after diagnosis of RA-ILD is approximately 2.6–3.5 years [6]. However, the treatment for RA-ILD is controversial, and there is a lack of randomized clinical trials and standardized treatment

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guidelines [7, 8]. RA-ILD presents with pulmonary fibrosis and inflammation. The most common pathological type is interstitial pneumonia, which manifests as obvious dense fibrosis and pulmonary structural remodeling [9, 10].

The Jak/Stat signaling pathway plays an important role in the pathogenesis of ILD and can be activated via interactions with profibrotic/proinflammatory cytokines, such as IL-6, IL-11 and IL-13 [11, 12]. Similarly, various growth factors overexpressed in ILD, such as platelet-derived growth factor, transforming growth factor-β1 (TGFβ1), and fibroblast growth factor, can also activate Jak/Stat through canonical or noncanonical pathways, indicating that Jak/Stat plays an important role in ILD. Beyer [13] et al. proposed that inhibiting Jak2 can effectively inhibit the development of fibrosis and that Jak2 inhibitors may provide new options for the treatment of fibrotic diseases. Baricitinib is an oral inhibitor of Jak1/2 approved by the FDA for RA patients [14]. It can block the intracellular signaling pathways of inflammatory factors in the host defense mechanism, but there is less research on RA-ILD [15]. The aims of this study were to determine whether baricitinib improves the extent of pulmonary fibrosis and to evaluate the possible mechanism of action in a bovine type II collagen-induced RA-ILD mouse model.

Materials and methods

Animals and cells

Specific-pathogen free -grade male DBA/1 mice (30 mice, 6–8 weeks old, body weight 24 ± 2 g) were purchased from Changzhou Cavens Laboratory Animal Co., Ltd. (Quality Certificate No. 202243188, Laboratory Animal License No. SCXK (Su) 2021–0013) and were adaptively fed for 1 week. Human embryonic lung fibroblasts (HLFs,#CL-0106) were kindly provided by Procell Life Science & Technology Co., Ltd.

Drugs and reagents

Bovine type II collagen was purchased from Chondrex (#20,022). Freund's complete adjuvant (#F5881) and Freund's incomplete adjuvant (#F5506) were purchased from Sigma. Baricitinib (LY3009104) was purchased from Adooq Bioscience. The following antibodies were used: mouse antibodies: β-actin (HuaBio,#EM21002); TGF-βR2 (Proteintech,#66,636–1-Ig); rabbit antibodies were purchased from affinity: SMA(#14,395–1-AP),Jak2, collagen 4 (Col4, #AF0510),fibronectin(FN,#AF5335),p-Jak2(#AF3002),Stat3(#10,253–2-AP), p-Stat3(#AF3293), Smad3(#AF6362), and p-Smad3(#AF8315); goat anti-mouse IgG antibody (EarthOx, #E030110-01) and goat anti-rabbit IgG antibody (EarthOx, #E030120-01); and transforming growth factor-β1 (Peprotech). si-Jak2 (#stB0004834B, refseq GCCGAGTTGTTACTATCCA) was purchased from Ribo. A Jak2 overexpression lentiviral vector was purchased from Obio Technology (H27516,pcSLenti-EF1-EGFP-P2A-Puro-CMV-JAK2-3Xflag-WPRE). Upstream and downstream primers were purchased from Sangon Biotech (Table 1 for the primer sequences). Reverse transcription kits were purchased from Takara(#RR036A). Wound healing plug-ins were purchased from Ibidi(#80,209).

Main equipment

The following instruments were used: carbon dioxide incubator (ESCO Singapore CCL-0508–8-IVF), microplate reader (Shenzhen Huakerui HR801), real-time fluorescence quantitative PCR instrument (Bioer LineGene 9600Plus), chemiluminescence imaging system (Clinx, Shanghai, Chemi Scope e6000EXP), high-speed refrigerated centrifuge (Zonkia, Anhui, HC-3016R), nanospectrophotometer (Kairo, Beijing, K5500PLUS), and integrated ultrasonic cell disruptor (Xiao Mei Chao Sheng, Kunshan).

Experimental groups

Cell experiments

HLFs at passages 3–5 were obtained and divided into the following groups for the cell scratch experiment: (1) control group, normal HLFs; (2) model group, HLFs + TGFβ1 (10 ng/ml, best concentration determined in previous experiment); and (3) intervention group, HLFs + TGFβ1 (10 ng/ml) + baricitinib (2000 nmol/L). Photographs were taken after 48 h of culture.

For downregulation of the Jak2 gene, HLFs were divided into the following groups and transfected with Jak2 siRNA: (1) the control group, in which HLFs were cultured normally; (2) the siNC group, in which HLFs were transfected with the siRNA non-specific control (siNC) and treated with TGFβ1 (10 ng/ml); and (3) the si-Jak2 group, in which HLFs were transfected with siRNA and treated with TGFβ1 (10 ng/ml).

Table 1 List of primers

Primers	Forward (5′ to 3′)	Reverse (5′ to 3′)
Gapdh	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA
JAK1	CTCTCTGTCACAACCTCTTCGC	TTGGTAAAGTAGAACCTC
JAK2	TGGAATGGCCTGCCTACAATG	ATGCG
JAK3	CCATCACGTTAACTTTGCCA	TGGCTCTAATCTGCTTAGAAT
SMA	CCCAGACATCAGGGAGTA	GGCGGAGGATATAGGTGCCTG
Col1	ATGG	TCTATCGGATACTTCAGCGTCA
Col4	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
FN	ATGCCCTTTCTCTTCTGCAA	GAAGGAATAGCCGATCCACA
	AAGACCATACCTGCCGAATG	GAACATGACCGATTTGGACC

For overexpression of the Jak2 gene, HLFs were divided into the following groups and transfected with lentiviral vectors: (1) the empty virus group, in which HLFs were transfected with empty virus (multiplicity of infection (MOI) = 80) and treated with TGFβ1 (10 ng/ml) and (2) the lentiviral vector group, in which HLFs were transfected with Jak2-expressing lentiviral vector (MOI = 100) and treated with TGFβ1 (10 ng/ml).

Animal experiments

Nine mice in the control group were received normal saline by gavage. There were 21 mice in the model group. These mice received injections of 0.1 ml Freund's adjuvant complete (FAC) emulsified bovine collagen type II (bCII) in the base of the tail on day 0 and were boosted with 0.1 ml Freund's incomplete adjuvant (FIA) emulsified bCII on day 21. The severity of arthritis was assessed three days after the second immunization using a scoring system ranging from 0 to 4 points [16], with a maximum total score of 4 points per mouse, and the assessment was performed once every 3 days. The mice in the model group were equally divided into two groups with similar arthritis scores, one of which served as the intervention group; the mice in this group received baricitinib suspended in 0.5% methylcellulose (3 mg/kg 0.1 ml per mouse) by gavage. Mice in the control group and the model group received 0.1 ml of normal saline every other day by gavage. One mouse was killed to confirm successful modeling.

Histological examination

Lung tissue was fixed in 4% paraformaldehyde for 24 h, dehydrated with gradient alcohol, embedded in paraffin, and sliced into 4-μm sections; the sections were subjected to conventional H&E staining, and alveolar and inflammatory cell infiltration were observed under a light microscope. Lung tissue inflammation was scored using the Szapiel score [17]. Masson staining was used to observe alveolar morphology and collagen deposition under a microscope. Pulmonary fibrosis was scored using the Modified scale [18].

Immunohistofluorescence staining

Lung tissue sections were obtained and blocked with serum, incubated with anti-p-Jak2 antibody (1:50) at 4 °C overnight in the dark, and incubated with fluorescent secondary antibody (1:100, FITC, labeled, green) at 37 °C for 1 h in a humidified box. DAPI was added dropwise, and the sections were incubated in the dark for 5 min. The specimens were stained and mounted with anti-fluorescence quenching reagent. Images were obtained under a fluorescence microscope.

Real-time quantitative PCR (RT–qPCR)

RNA extraction RNA was extracted from lung tissue following the kit instructions, air dried for 5–10 min, dissolved in 20 μl of enzyme-free water, and stored in a − 80 ℃ freezer for future use.

Reverse transcription of RNA to cDNA RT reaction solution was prepared following the reverse transcription kit instructions. The total volume of the reaction system was 10 μl, and the reaction conditions were 37 °C for 15 min, 85 °C for 5 s, and 4 °C for 59 min. RT−qPCR:The total volume of the PCR system was 20 μl: upstream and downstream primers, 0.4 μl each; cDNA, 2 μl; enzyme-free water, 8.2 μl; and SYBR Green, 10 μl. A dissolution curve was created, and the data were analyzed using the 2-ΔΔCt method.

Western blotting analysis

Total protein was extracted from adherent cell monolayers and lung tissue. The protein concentration was determined using the BCA method. After equivalent amounts of protein were denatured, the protein was electrophoresed and then transferred to a PVDF membrane. The PVDF membrane was soaked in blocking solution and placed on a shaking table at room temperature for 30 min. The membrane was incubated with primary antibodies (β-actin, TGF-βR2, SMA, Jak2, p-Jak2, Col4, FN, Stat3, p-Stat3, Smad3, p-Smad3) overnight at 4 °C, after which excess primary antibodies were washed off. Then, the membrane was incubated with goat anti-rabbit secondary antibody or goat anti-mouse secondary antibody at 37 °C for 1 h on a shaker. The secondary antibodies were washed off, and the protein bands were visualized. The gray values of the protein bands were analyzed using ImageJ. β-Actin was used as an internal reference to calculate the relative protein expression levels of SMA, TGF-βR2, Jak2, p-Jak2, Col4, FN, Stat3, p-Stat3, Smad3, and p-Smad3.

Statistical analysis

GraphPad Prism 9.0 software was used for the data analysis and to create graphs. The data are expressed as the mean ± standard error. One-way analysis of variance (one-way ANOVA) was used to compare multiple groups, and the t test was used for pairwise comparisons. $P < 0.05$ was regarded as a statistically significant difference.

Results

Body weight change and arthritis score for mice in each group

The body weight of the mice in the control group increased with time, and the body weight of the mice in the model and intervention groups gradually decreased from day 30, with a significant difference between the model group and the control group; from day 54, compared with the model group, the intervention group showed slower weight loss, but the difference was not significant (Fig. 1a).

There was no redness or swelling in the joints and toes of the mice in the control group, and the score was 0 points. In the model and intervention groups, from the 24th day of modeling, the joint scores showed upward trends with time and increased significantly after day 36 of modeling. Baricitinib was administered on day 34. After approximately 1 week, the joint scores began to gradually decrease, but some joints were still red and swollen before sacrifice (Fig. 1b). The toes of the mice in the model group were red, swollen and stiff, and the toe symptoms of the mice in the intervention group subsided (Fig. 1c).

Baricitinib improves the degree of pulmonary fibrosis in RA-ILD mice

The lung tissue of mice in each group was collected after 56 days of modeling. H&E and Masson staining revealed that a large number of inflammatory cells infiltrated the alveoli and alveolar septa of the mice in the model group; additionally, the alveolar walls and alveolar septa were thickened, with increased collagen deposition around the blood vessels and alveolar walls. The abovementioned

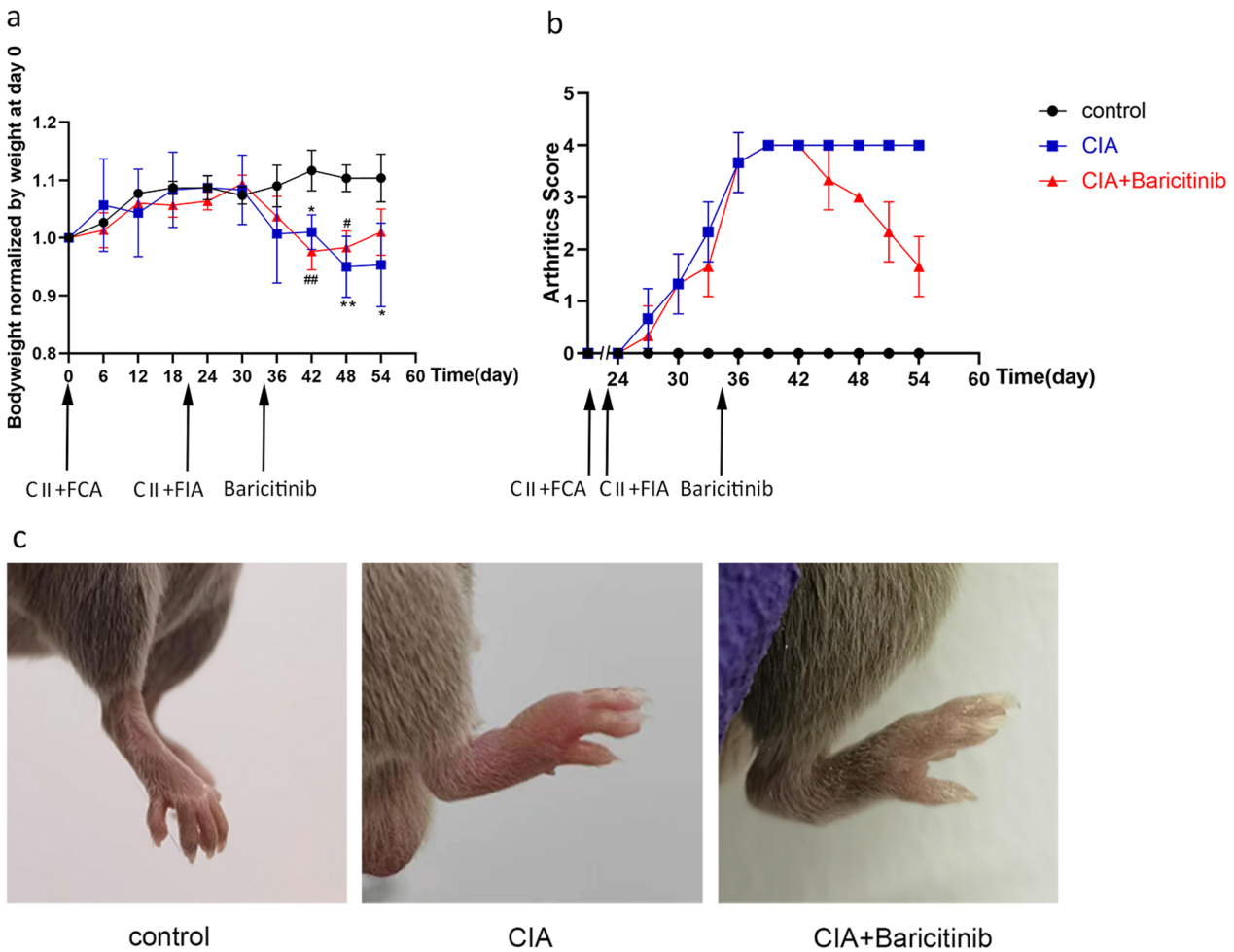

Fig. 1 Changes in the general condition of the mice. Control: control group; CIA: model group; CIA + baricitinib: intervention group. **a** Changes in body weight of mice in each group: *control group vs. model group, # control group vs. intervention group; *$P < 0.05$, **$P < 0.01$, #$P < 0.05$, ##$P < 0.01$. **b** Changes in joint scores of the mice in each group. **c** Mouse joint pathology in each group: Representative images of the paws of mice in the control, model and intervention groups. The paws of mice in the model group were obviously red, swollen and stiff

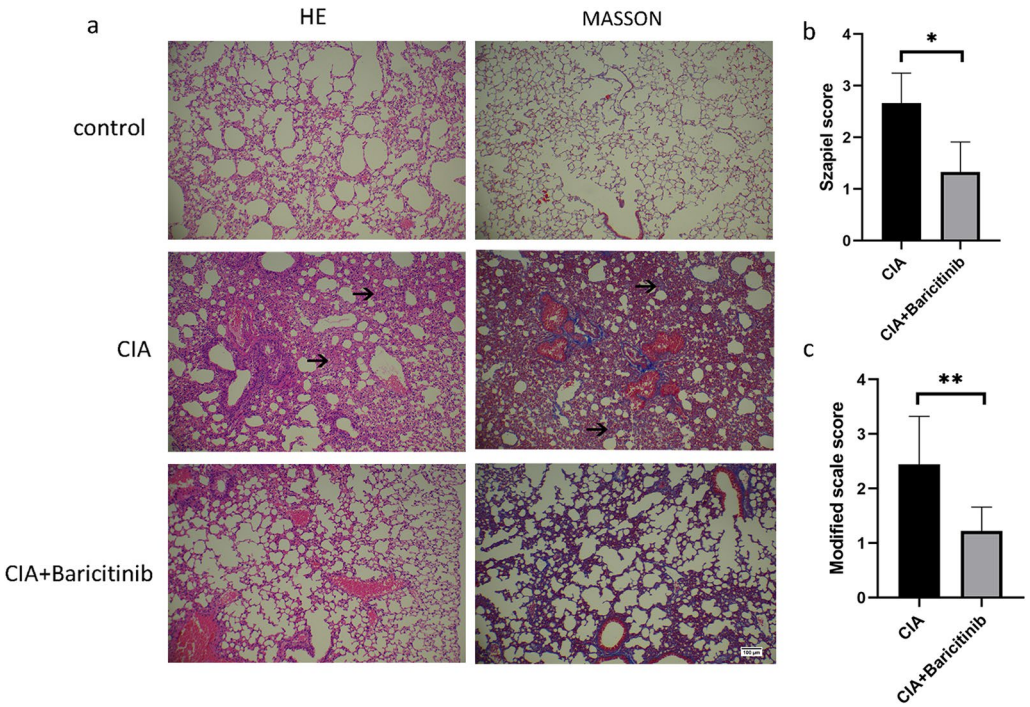

Fig. 2 Baricitinib affects lung tissue changes in mice. H&E staining and Masson staining of mouse lung tissue: **a** HE staining model group, ▼inflammatory cell infiltration in alveolae and the alveolar septum, septal thickening; Masson staining model group, ▼increased vascular and alveolar wall collagen deposition. **b** Mouse lung tissue inflammation scores (n = 3), $^{*}P < 0.05$. **c** Mouse lung fibrosis scores (n = 3), $^{**}P < 0.01$

manifestations were significantly reduced in the intervention group (Fig. 2). To determine the inhibitory effect of baricitinib on Jak2 in the lung tissue of the model mice, we assessed the levels and localization of p-Jak2 via immunofluorescence. The levels of p-Jak2 increased in the lung tissue of mice in the model group, and the protein was localized mainly to the cytoplasm. After baricitinib intervention, the fluorescence signal for p-Jak2 significantly decreased (Fig. 3).

mRNA levels and changes in related genes in the lung tissue of mice in the model group

The expression of Jak1/2 mRNA showed an increasing trend with the prolongation of the modeling time, peaking in the 3rd week and being higher than that the control group until the 8th week. SMA mRNA peaked in the first week and was still lower than that in the control group until the 8th week. FN/Col4 mRNA increased in the first week, decreased in the 3rd week and was lower than that in the control group in the 8th week. The Jak3/Col1 mRNA level of the model group was lower than that of the control group before the 8th week (Fig. 4). This indicated that Jak1/2 mRNA was highly expressed during the entire RA-ILD modeling process.

Changes in the protein expression levels of Jak2, p-Jak2, Stat3, p-Stat3, Smad3, p-Smad3 and ECM in the lung tissue of mice in the intervention group

Compared with those in the control group, the protein levels of Jak2, p-Jak2, p-Stat3, p-Smad3, SMA, TGFβR2 and FN in the lung tissue of the mice in the modeling group were significantly higher. After treatment with baricitinib, the expression levels of the above proteins significantly decreased (Fig. 5a, b).

After siRNA- and lentivirus-transfection of HLFs, the protein expression of Stat3, p-Stat3, Smad3, p-Smad3 and ECM was affected by downregulating and upregulating Jak2 gene expression

After downregulating the Jak2 gene using siRNA, the protein levels of p-Stat3 and p-Smad3 in HLFs decreased, and the corresponding ECM levels decreased. Lentiviral vector transfection upregulated the expression of Jak2, resulting in significantly increased levels of p-Stat3 and p-Smad3 and ECM production (Fig. 6a, b). It is speculated that the inhibition of Jak2 expression by baricitinib may affect the expression of p-Smad3 through p-Stat3 and reduce ECM production.

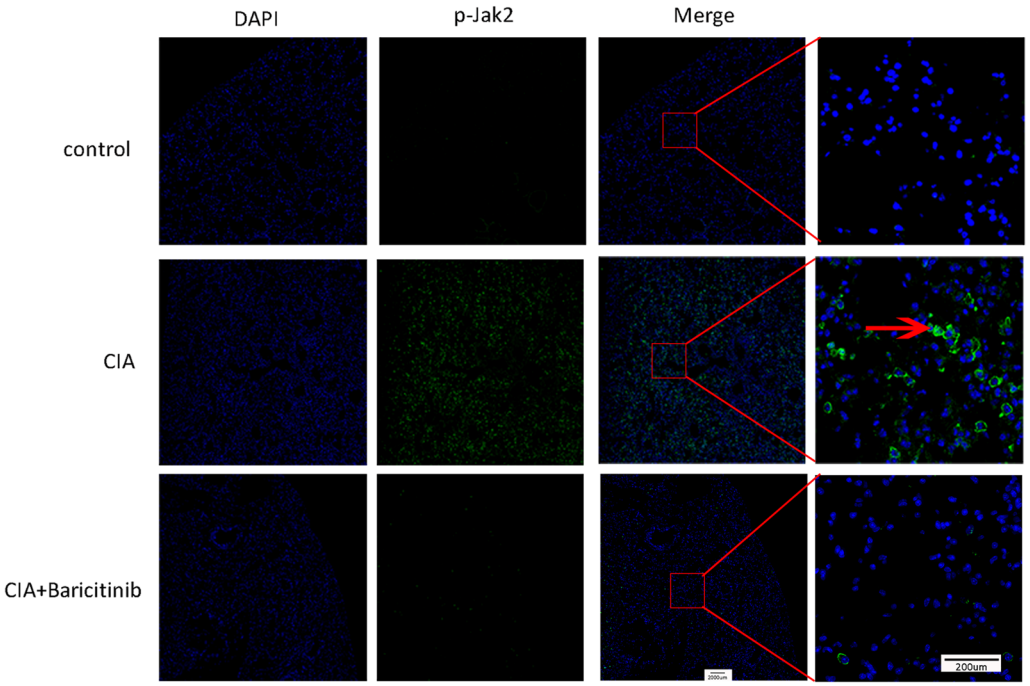

Fig. 3 Increased expression of p-jak2 in mouse lung tissue. Immunofluorescence of p-Jak2 in mouse lung tissue: p-Jak2 (green) immunofluorescence staining of lung tissue from each group of mice (nucleus (blue)). p-Jak2 levels were almost undetectable in the lung tissue of the control group; ▼ the cytoplasmic expression of p-Jak2 was increased in the model group, and the expression of p-Jak2 was decreased in the intervention group

The effect of baricitinib on the migration of HLFs (TGFβ1 10 ng/ml)

After 24 h and 48 h of coculture, compared with that in the control group, the migration ability of HLFs in the model group was significantly enhanced, and baricitinib significantly inhibited the migration of these cells (Fig. 7).

Discussion

RA-ILD is the main reason for the increased mortality from RA. In clinical practice, most RA patients are diagnosed after obvious manifestations in the lungs and significantly impaired lung function. Therefore, early identification and delay of disease progression are very important. Wang [19] et al. performed immunohistochemistry and RNA sequencing on lung tissue from RA-ILD patients and found that the Jak/Stat signaling pathway was involved in the pathogenesis of RA-ILD, but the small sample size and lack of RA-ILD animal models limited further mechanistic studies. Among the commonly used RA-ILD models, the collagen-induced model combines two disease states, arthritis and ILD, and closely resembles human RA-ILD, making it suitable for studying the mechanism of early disease development [20, 21]. In this study, in pathological sections of lung tissue from 56-day-old model mice, there was a large amount of inflammatory cell infiltration in the alveoli, alveolar septa, and surrounding blood vessels and increased collagen deposition around the bronchi and blood vessels. Additionally, the significant increase in the mRNA level of ECM was confirmed by RT–qPCR. The abovementioned inflammatory cells and collagen deposition were significantly reduced after baricitinib treatment. These results confirmed that the collagen-induced mouse model can be used to study the pathogenesis of RA-ILD. Interestingly, we also found that at the late stage of modeling, the gene expression of ECM in the model group was lower than the protein expression level. It may be that the transcription-to-translation process is more complex, and the amount of gene expression is not necessarily positively correlated with the amount of protein expression. In addition, fibrosis is characterized by long-term deposition of proteins in the tissues, whereas gene changes in the tissues occur earlier and more rapidly, which ultimately leads to inconsistent gene and protein expression levels in the tissues.

RA-ILD includes alveolar inflammation, epithelial to mesenchymal transition (EMT), fibroblast to myofibroblast transition (FMT), which pathologically manifests as interstitial pneumonia [9]. A variety of inflammatory factors and cytokines can bind to Jak2 through various pathways and phosphorylate the protein; the phosphorylation site can also be a docking site for signal transducers

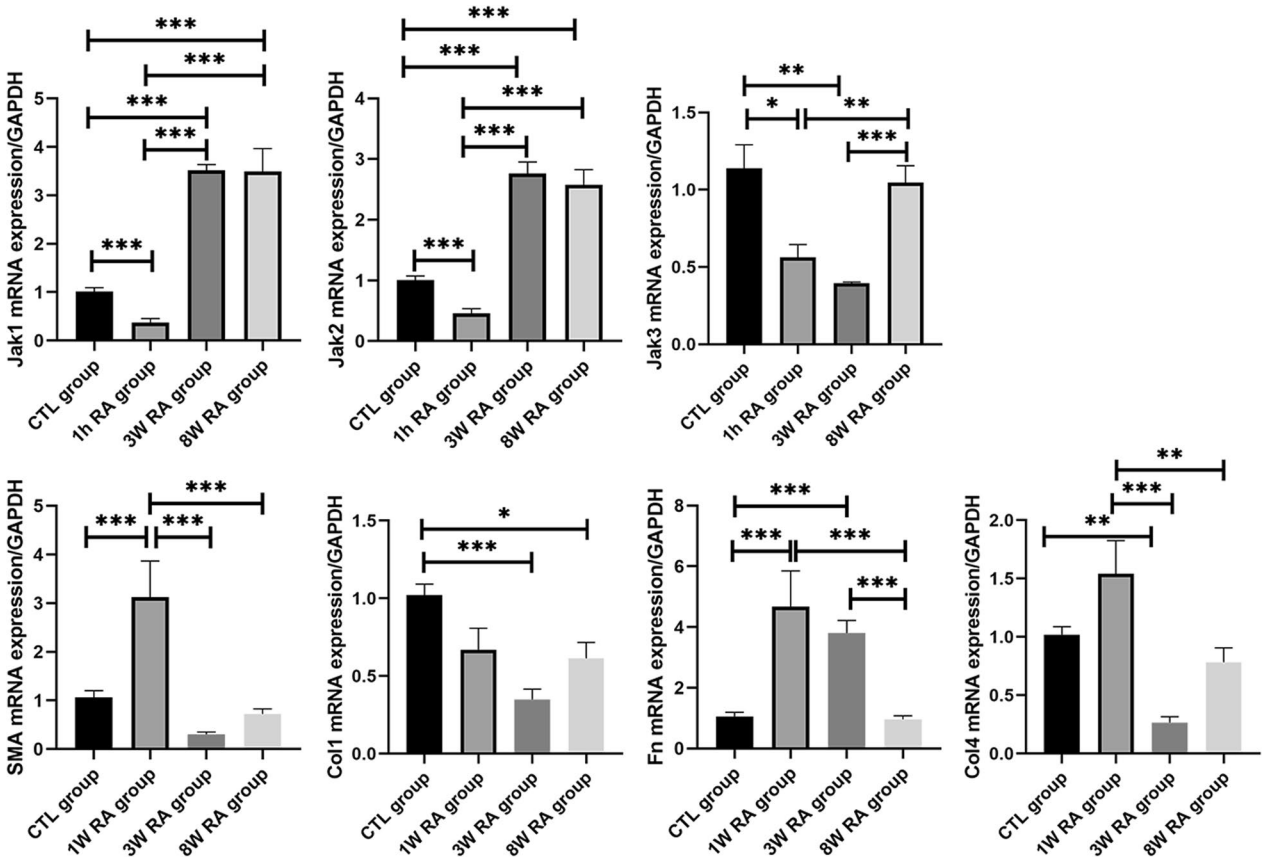

Fig. 4 Changes in RNA expression levels in the lung tissue of the model group. RT–qPCR of mouse lung tissue at different times: Control group and model group (RA) at different times (1 h, 1 w, 3 w, 8 w); gene expression of Jak1-3 and collagen markers SMA, Col1, Col4, and FN was detected by RT–qPCR, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

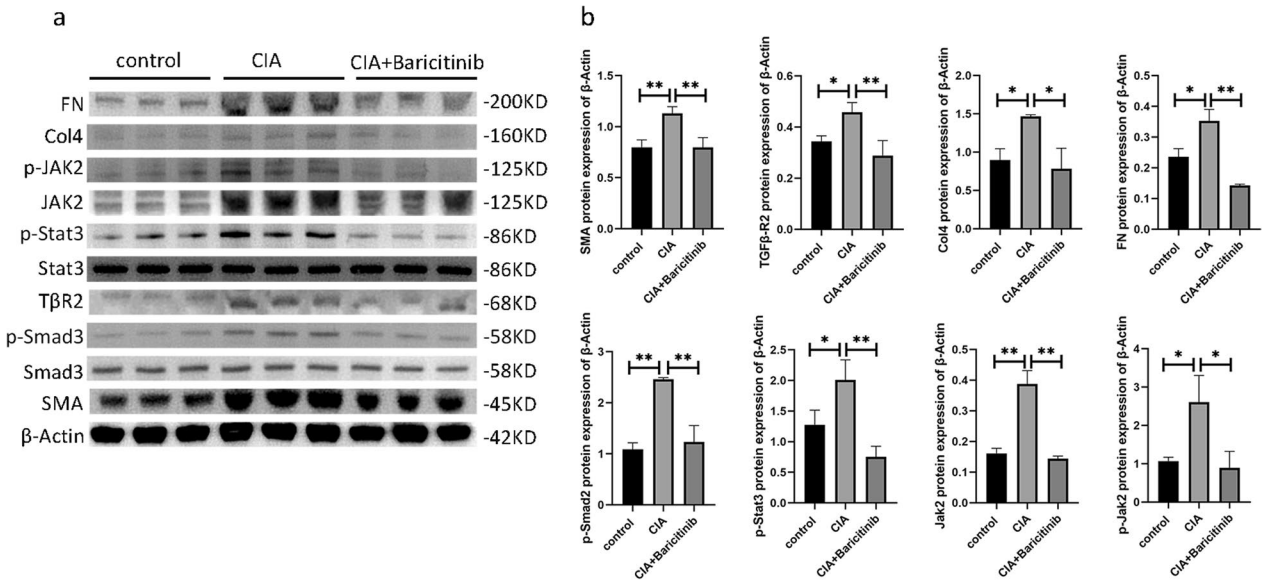

Fig. 5 Changes in protein levels in mouse lung tissue. **a** Protein expression in mouse lung tissue: CIA, model group; CIA + baricitinib, intervention group. WB (**b**) and semi-quantification of the result (**c**) measurement of SMA, Jak2, p-Jak2, Stat3, p-Stat3, Smad3, p-Smad3 and ECM protein levels in the lung tissue of the mice in each group, n = 3 per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.01$

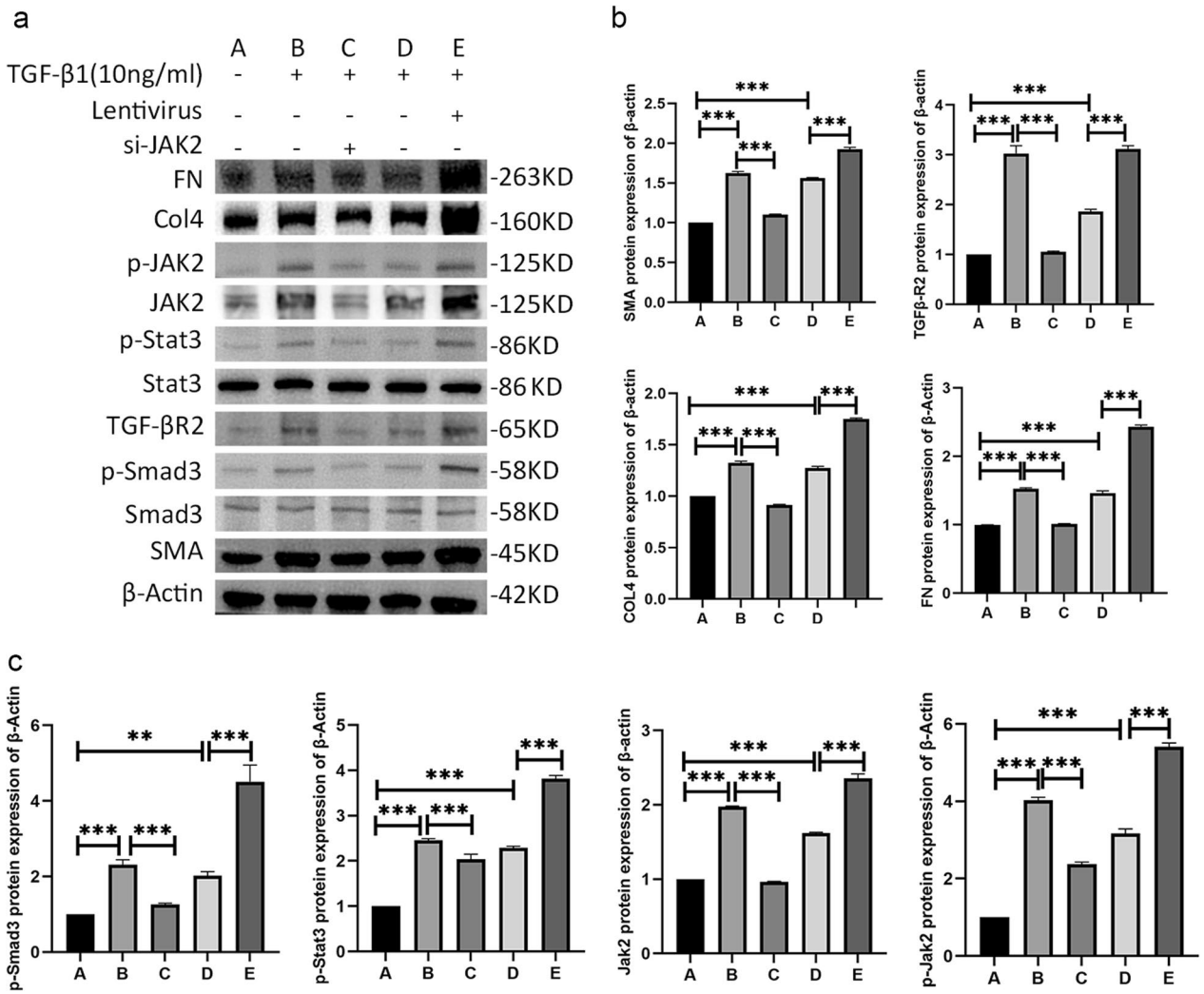

Fig. 6 Effects of siRNA and lentivirus on protein levels in HLFs.c/d. Effects of siRNA and lentivirus on protein levels in HLFs: **a**. Control group **b**. si-NC group **c**. si-Jak2 group D. Empty virus group E. Lentiviral vector group. WB(a) and semi-quantification of the result (b) measurement of the protein levels of SMA, Jak2, p-Jak2, Stat3, p-Stat3, Smad3, p-Smad3 and ECM in HLFs, one sample was measured three times by three experimenters in a single-blind method for the intensity of the bands. *$P < 0.05$, **$P < 0.01$, ***$P < 0.01$

and activator of transcription (Stat) proteins, which bind to Jak2 through their SH2 structure domain, followed by Stat dimerization and translocation into the nucleus to regulate gene expression [13, 22, 23]. Jak2 interacts with and phosphorylates Stat3, and p-Stat3 participates in TGFβ1-induced epithelial cell repair and myofibroblast transformation by affecting p-Smad3, ultimately leading to fibrosis [11, 22]. Our research group found that the expression of p-Jak2 increased in the lung tissue of RA-ILD and IPF patients [19]. Similarly, the expression of p-Jak2 in the lung tissue of the mice in the model group in this study also increased. It is speculated that Jak2 is involved in the occurrence of RA-ILD.

Previous studies have found that Jak2/Stat3 is involved in the occurrence of ILD [24]. Jak2/Stat3 activation is involved in FMT, EMT, senescence, autophagy, apoptosis and proliferation [12, 25]. FMT is characterized by the overexpression of SMA and FN [26]. Therefore, targeted inhibition of the Jak2/Stat3 signaling pathway can be used as a new strategy to treat pulmonary fibrosis. We found that the protein expression levels of Jak2, p-Jak2, p-Stat3, SMA and ECM were increased in the lung tissue of model mice by WB and that the levels of these proteins decreased after baricitinib treatment. It is speculated that the Jak2/Stat3 signaling pathway may also be involved in the occurrence of RA-ILD. In cell experiments, treatment of HLFs with baricitinib significantly weakened

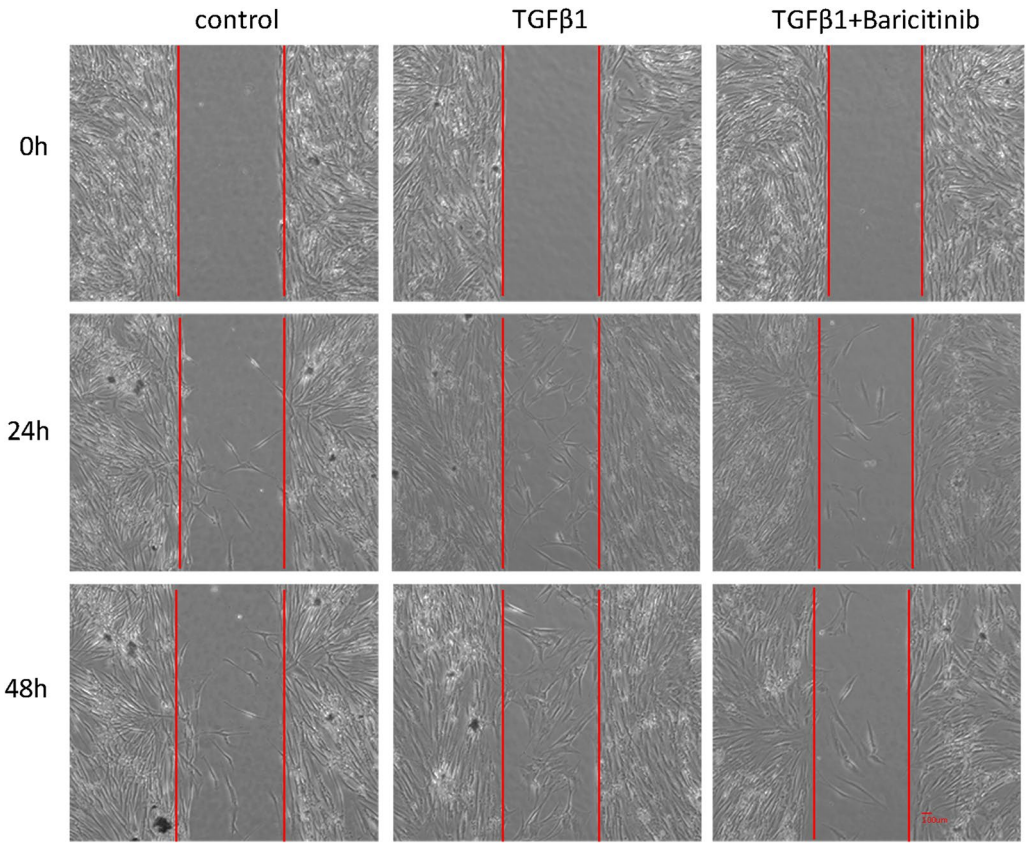

Fig. 7 HLF scratch assay. control, control group; TGFβ1, model group; TGFβ1 + baricitinib, intervention group. HLFs cocultured with TGFβ1 (10 ng/ml) and baricitinib (2000 nmol/ml) were subjected to a wound healing assay. Wound closure was recorded at 0, 12, and 24 h after injury

their migration ability and slowed cell proliferation. It is suggested that baricitinib can alleviate the degree of pulmonary fibrosis in RA-ILD mice; however, the specific mechanism is still unclear.

Some studies have found that Jak2 can interfere with the TGFβ1 signaling pathway through a "crosstalk" mechanism, thereby affecting excessive epithelial cell repair as well as FMT [22]. TGFβ1 expression leads to ECM deposition and fibrosis by activating its downstream Smad signaling pathway [27]. Smad2 and Smad3 are two major downstream regulators of TGFβ1-mediated tissue fibrosis [28]. Ren [29] and Sun [30] found that by inhibiting the Jak2/Stat3 signaling pathway, TGFβ/Smad3 are indirectly affected, thus reducing renal inflammation and fibrosis. In a mechanistic study of myocardial fibrosis, Eid [31] also found decreased TGF-β1 and Smad3 levels after applying a Jak2 inhibitor, confirming that TGF-β1/Smad3-induced myocardial fibrosis in rats can be alleviated by altering the Jak2/Stat3 pathway. Zhang [32] et al. found that myocardial remodeling caused by the TGFβ1/Smad2&3 signaling pathway was similarly ameliorated by inhibition of Jak2/Stat3. In the inflammatory microenvironment, IL-6 regulates downstream Jak2/Stat3, synergistically activating TGF-β/Smad3 and leading to peritoneal fibrosis [33]. Yu [34] found that nanoparticles affected TGF-β/Smad3 via the Jak2/Stat3 pathway in vivo, inducing lung inflammation and collagen deposition. Shi [35] also showed that there are two important pathways, Jak/Stat and TGF-β/Smad3, in a mouse model of pulmonary fibrosis and that they influence each other. The intervention of baricitinib as a Jak1/2 inhibitor in RA-ILD mice resulted in a significant reduction in p-Jak2 fluorescence and a decrease in p-Jak2, p-Stat3 and p-Smad3 protein expression in mouse lung tissue. This finding is consistent with previously reported results. Gu [36] showed that after treating mouse lung fibroblasts with baricitinib, p-Stat3 and p-Smad3 expression decreased. Therefore, we speculate that in the treatment of RA-ILD with baricitinib, Jak2 inhibitors not only affect the Jak2/Stat3 signaling pathway but may also affect TGFβ1/Smad3. For further verification, we silenced the Jak2 gene and overexpressed the Jak2 gene in TGFβ1-induced HLFs. The results showed that p-Stat3 and p-Smad3 protein expression was inhibited after downregulating Jak2 gene expression and that lentivirus transfection upregulated Jak2 gene expression

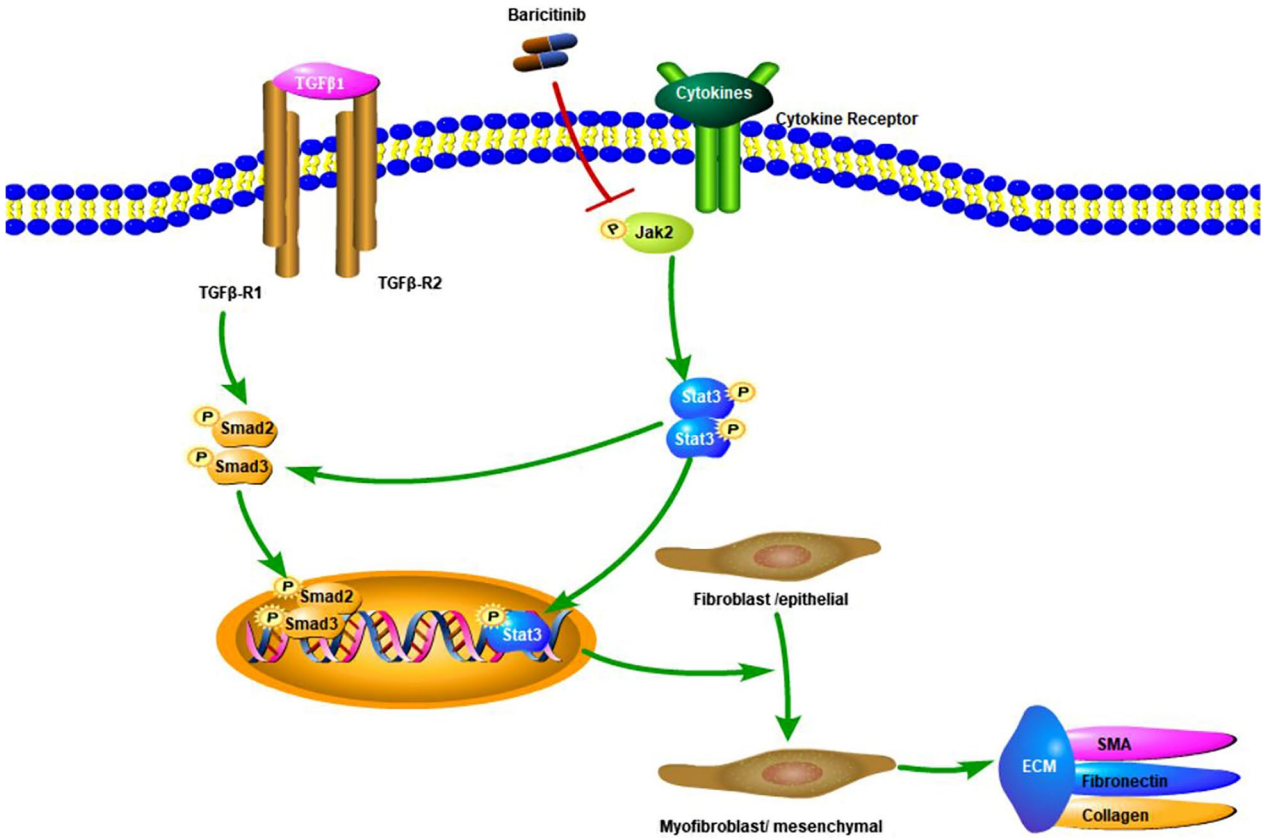

Fig. 8 Mechanism of action of baricitinib in the Jak2/Stat3 signaling pathway Baricitinib inhibits the Jak2/Stat3 pathway and interferes with p-Smad3 via p-Stat3, ultimately attenuating ECM deposition. One pathway is that TGFβR1 affects downstream Smad3/pSMAD3. The other pathway is that cytokine stimulation of downstream Jak2/p-Jak2 affects Stat3/p-Stat3. Baricitinib affects p-Smad3 via p-Jak2/p-Stat3. Inhibition of p-Smad3 and p-Stat3 promotes the transformation of fibroblasts to myofibroblasts, thereby further reducing the deposition of FN, SMA and ECM. Inhibition of p-Smad3 and p-Stat3 promotes the transformation of fibroblasts to myofibroblasts, which further attenuates the deposition of FN, SMA, and ECM, and ultimately reduces pulmonary fibrosis

and subsequently p-Stat3 and p-Smad3 levels. Again, after Jak2 inhibitors affect the Jak2/Stat3 signaling pathway, they may also interfere with TGFβ1/Smad3 through a "cross-talk" mechanism, thereby reducing the deposition of collagen in the lungs.

This study has some limitations. First, regarding RA-ILD animal model used in this experiment, the modeling and treatment observation time was up to 8 weeks, and the treatment of rheumatoid interstitial lung disease in the clinic is a long-term process; thus, future studies are required to assess the sustained efficacy of baricitinib in improving the lung fibrosis in the RA-ILD animal model. Second, pulmonary fibrosis affects lung function, which is also often used as an indicator to assess drug efficacy, a part of the study that we neglected. In addition, baricitinib is a clear JAK1/2 inhibitor, upadacitinib has been approved as a selective JAK1 inhibitor for the treatment of rheumatoid joints, and the JAK1/STAT3 pathway also plays an important role in the proliferation of tumor cells. The mechanism underlying the role of the JAK1 pathway in RA-ILD was not studied here, and we only detected the JAK1 gene changes in the CIA model. Thus, the specific pathway in pulmonary fibrosis needs to be explored in future studies.

Conclusion

In summary, baricitinib can improve the degree of fibrosis in the lung tissue of RA-ILD mice. The possible mechanism involves not only inhibition of the Jak2/Stat3 signaling pathway but also interference with Smad3 expression through Stat3, thereby affecting the profibrotic function of TGFβ1 (Fig. 8).

Abbreviations

RA	Rheumatoid arthritis
ILD	Interstitial lung disease
RA-ILD	Rheumatoid arthritis-associated interstitial lung disease
TGFβ1	Transforming growth factor-β1
HLFs	Human embryonic lung fibroblasts
Col4	Collagen 4
FN	Fibronectin
MOI	Multiplicity of infection
bCII	Bovine collagen type II
RT–qPCR	Real-time quantitative PCR
EMT	Epithelial to mesenchymal transition
FMT	Fibroblast to myofibroblast transition
siNC	SiRNA non-specific control
FAC	Freund's adjuvant complete
FIA	Freund's incomplete adjuvant
ECM	Extracellular matrix

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Author contributions
HL: Conception, Methodology, Validation, Writing Original draft. YY: Conceptualization, Methodology, Investigation. JZ: Writing-Review and Editing, Supervision, Project administration, Resources, Funding acquisition. XL: Writing-Review and Editing, Supervision.

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Declarations

Ethics approval and consent to participate
All animal care procedures and treatments complied with the ARRIVE guidelines. All animal procedures were conducted in accordance with the guidelines of Institutional Animal Care. Ethics approval was obtained from the Greentech Committee on the Management and Use of Laboratory Animals (IAC-B2019011-P-01). Substantial efforts were made to minimize the suffering of animals.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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CORRECTION

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Correction to: Baricitinib improves pulmonary fibrosis in mice with rheumatoid arthritis-associated interstitial lung disease by inhibiting the Jak2/Stat3 signaling pathway

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In the original version of this article [1], the affiliations were published incorrectly.

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The affiliations have been updated above and the original article has been corrected.

The publisher would like to apologize for any inconvenience caused.

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