

Qilongtian Ameliorate Bleomycin-induced Pulmonary Fibrosis in Mice via Inhibiting IL-17 signal pathway

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Abstract

Pulmonary fibrosis (PF) is a special type of pulmonary parenchymal disease, with chronic, progressive, fibrosis, high mortality. There is a lack of safe, effective, and affordable treatment methods. Qilongtian (QLT) is a traditional Chinese prescription that is composed of Panax notoginseng, Earthworm, and Rhodiola, and shows the remarkable clinical curative effect of PF. However, the mechanism of QLT remains to be clarified. Therefore, we studied the effectivity of QLT in treating Bleomycin (BLM) induced PF mice. Our studies have shown that QLT significantly reduced the inflammatory injury, hydroxyproline content and collagen deposition of lung tissue in BLM induced PF mice and downregulated the cytokine related to inflammation and fibrosis and PF expression on the mRNA and protein level in PF mice. To identify the mechanism of QLT, the Transcriptome was measured, and the IL-17 signal pathway was screened out for further research. Further studies indicated that QLT reduced the mRNA level of *CCL12*, *CXCL5*, *FOSL1*, *MMP9*, and *AREG*, which are inflammation and fibrosis-related genes in the IL-17 signal pathway. In summary, the results indicated that the potential mechanism of QLT in preventing PF progress was by inhibiting the inflammation resulting in IL-17 signal pathway. Our study provides the novel scientific basis of QLT and represents new therapeutics for PF in clinical.

1. Introduction

COVID-19 examination of chest CT in severe patients shows more PF changes, and this change may persist [1]. The pathogenesis of PF includes the theories of imbalance of inflammatory response, extracellular matrix deposition, epithelial mesenchymal transformation and oxidative stress. Proliferation of interstitial fibroblasts and fibrotic changes of interstitial tissue are characteristics of PF [2–5]. The Repeating of inflammation lead to the abnormal expression of downstream gene and differentiation of fibroblasts, resulting in excessively fibrous tissue repairing [6–8]. The treatment methods like glucocorticoids, immunosuppressants, antioxidants, anti-fibrosis drugs, etc., delay the disease progression, result in the low life quality of the patients [9–14]. Qilongtian (QLT) is a traditional Chinese medicine prescription composed of panax notoginseng, earthworm and rhodiola. Clinical efficiency observation study showed that QLT reduced inflammatory factor and fiber factor TGF- β 1 and MMP9 to improve anti-infection ability of respiratory tract in chronic obstructive pulmonary disease (COPD) patients [15]. The animal experimental research indicated that QLT reduced the inflammatory factor IL-1 β and TNF- α to inhibit the inflammatory reaction of airway in Pulmonary Hypertension rat model [16–17]. In recent years, Pro. Yi Fu finds that QLT delays the process of PF, improves lung function and life quality of PF patients. IL-17 is a cytokine mainly derived from Th17 cells. Its main biological effect is to promote inflammatory response. After IL-17 binding to the receptor, it exerts biological activity by activating mitogen activated protein kinase [18–19]. The stimulation of IL-17 signaling pathway promotes the inflammation and fibrosis in lung [20–24]. In the current study, we used QLT to intervene the mouse PF model, observed the treatment effect and explore the possible anti-fibrosis mechanism, so as to provide the experimental basis for QLT in clinical treatment of PF.

2. Materials And Methods

2.1 Experimental animals and feeding

36 male C57BL/6J mice, SPF grade, 4 weeks old, body weight (20 $\pm$ 2) g, (Chengdu Dashuo experimental animal Co., Ltd., Chengdu, China, regular animal No.: SCXK (Sichuan) 2020-030). All methods were carried out in accordance with the care and use of experimental animals published by the National Institutes of Health and the guidelines of the Animal Care & Welfare Committee of Yunnan University of Chinese Medicine. According to the guidelines for, the mouse experiments have been approved and implemented (Approval Institution: Animal Experiment Ethics Review Committee of Yunnan University of Traditional Chinese, Medicineanimal experiment license No.: SYXK (Dian) K2013-0007, animal ethics license No.: R-06202023). All methods are reported in accordance with ARRIVE guidelines.

2.2 Experimental drugs

QLT capsule: Panax notoginseng, Earthworm, Rhodiola; 0.4g per capsule, equivalent to 1.53g of decoction pieces, Hydrochloride Bleomycin (Hanhui Pharmaceutical Co., Ltd. Hangzhou, China) for injection: 15000 bleomycin units (equivalent to 15mg)/bottle, purchased from, national medicine permission number (NMPN) H20055883. Before use, add 7.5ml of normal saline to each bottle to obtain a solution with a concentration of 2mg/ml.

2.3 Modeling and Administration

36 mice were randomly divided into control group, model group, QLT low, medium, high administration group and pirfenidone group. Modeling method: the model was made by endotracheal intubation and injection of bleomycin (5mg/kg) [25-26], the control group and model group were gavaged with normal saline, and the QLT low, medium and high dose groups were gavaged with 0.39g/kg, 0.78g/kg and 1.56g/kg respectively, and Pirfenidone group was gavaged with 0.78g/kg. The drug was administered at 14 days after modeling, and the materials were taken 21 days after administration.

2.4 Invasive pulmonary function test

After administration, mice were anesthetized intraperitoneally with 1% pentobarbital sodium (0.4g/kg). After fixation, skin preparation and disinfection, the exposed trachea was gently and passively separated. The trachea was cut horizontally between the tracheal cartilage rings. The trachea was lifted after threading, the intubation needle was inserted, and the pulmonary function instrument (model: crfm100, EMMS, UK) was connected, the related indexes of pulmonary function were recorded.

2.5 Specimen collection

Three weeks after administration, the middle lobe of the right lung was taken and fixed in tissue fixation solution (Wuhan Servicebio Technology Co., Ltd., Wuhan, PR China) for HE and Masson staining. The left lung was placed in 1.5ml EP tube and frozen at -80 °C.

2.6 HE staining

Paraffin sections were used for dewaxing, hematoxylin staining, eosin staining and dehydration sealing. After the above operations, the sections were dried, sealed with neutral gum, observed under the microscope, photographed, collected images and analyzed.

2.7 Masson staining

Paraffin sections were dewaxed to water, stained with potassium dichromate, iron hematoxylin, Ponceau red, acid fuchsin, phosphocupric acid, aniline blue, differentiated, transparent sealed with neutral gum, observed under the microscope, photographed, collected images, and analyzed the results.

2.8 Hydroxyproline (HYP) detection

The HYP assay was measured with hydroxyproline determination kit (Cat#: A030-2-1, Nanjing Jiancheng Bioengineering Institute), following the product instruction book. The absorbance of each tube was measured with a microplate reader (BioTek SynergyH1, Agilent Technologies, Inc., USA) at a wavelength of 550nm.

2.9 Transcriptome sequencing

Detected by Metabo-Profile Biotechnology (Shanghai) Co., Ltd., the mRNA with PolyA structure in total RNA was enriched by oligo (DT) magnetic beads, and after RNA extraction, purification and library construction, these libraries were sequenced by next-generation sequencing (NGS) based on Illumina sequencing platform.

2.10 Elisa

Samples were tested according to the Elisa Kit instructions. The kits were Mouse CCL12 Elisa Kit (SEKM-0164, Solarbio), Mouse FOSL1 Elisa Kit (E03C0735, BlueGene Biotech Co., Ltd.), Mouse MMP9 Elisa Kit (ARG81241, Arigo Biolaboratories Corp.), Mouse CXCL5 Elisa Kit (EMCXCL5, Thermo Fisher), Mouse AREG Elisa Kit (EMAREG, Thermo Fisher). The absorbance was measured, and the concentration was calculated using an enzyme marker (Synergy, BioTek, USA) at 550 nm.

2.11 qRT-PCR

The total tissue mRNA was extracted with Invitrogen™ TRIzol™ Reagent (Thermo Fisher Scientific Inc.). The primer sequences were listed in Table1 and were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The qPCR assay was measured by using Applied Biosystems QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific Inc.). The relative mRNA levels of each gene were calculated by using the $2^{-\Delta\Delta C_t}$ method.

Table 1. Sequences of qRT-PCR primers

GeneNames	Primer Sequences
TGF- β	Forward primer: 5'-AGCAACAATTCCTGGCGATACCTC-3' Reverse primer: 5'-TCAACCACTGCCGCACAACTC-3'
TNF- α	Forward primer: 5'-CAGGCGGTGCCTATGTCTC-3' Reverse primer: 5'-CGATCACCCCGAAGTTCAGTAG-3'
COL-I	Forward primer: 5'-GCTCCTCTTAGGGGCCACT-3' Reverse primer: 5'-CCACGTCTCACCATTGGGG-3'
COL-III	Forward primer: 5'-TGCTGCTGGTACTCCTGGTCTG-3' Reverse primer: 5'-ACCTGGACCGCCTGGTTCAC-3'
α -SMA	Forward primer: 5'-TGCTGGACTCTGGAGATGGTGTG-3' Reverse primer: 5'-CGGCAGTAGTCACGAAGGAATAGC-3'
CCL12	Forward primer: 5'-TCATAGCTACCACCATCAGTCCTCAG-3' Reverse primer: 5'-CTGGCTGCTTGATTCTCCTGTAG-3'
FOSL1	Forward primer: 5'-ACCACACCCTCCCTAACTCCTTC-3' Reverse primer: 5'-TGCTGCTGCTACTCTTGCGATG-3'
MMP9	Forward primer: 5'-GGACTAGCCAGGAGGGAGAACAG-3' Reverse primer: 5'-GCCAGTGAGTGAAAGGGACAGAAC-3'
CXCL5	Forward primer: 5'-TGCCTTGCTTTGCTTAACCGTAAC-3' Reverse primer: 5'-TGAATTCCACCGTAGGGCACTG -3'
AREG	Forward primer: 5'-GAAGACTCACAGCGAGGATGACAAG-3' Reverse primer: 5'-CGAGGATGATGGCAGAGACAAAGATAG-3'
β -actin	Forward primer: 5'-GTCGTACCACAGGCATTGTGATGG-3' Reverse primer: 5'-GCAATGCCTGGGTACATGGTGG-3'

2.12 Immunofluorescence assay

The experimental operation steps of immunofluorescence were as follows: dewaxing paraffin sections to water, circle, serum blocking, added COL-I antibody: add Goat anti rabbit IgG HRP, After the sections were slightly dried and incubated for 50 minutes, added Cy3 fluorescence enhancer, added COL-III antibody: add Goat anti rabbit IgG HRP, added FITC fluorescence enhancer, added dropwise into the slice circle and incubated. DAPI staining, autofluorescence quenching, sealing slide, microscopic photography. DAPI staining was blue, COL-I was red, and COL-III was green.

2.13 Western blot

The primary antibody was used as follows: TGF- β antibody (EPR18163, Cat#: ab215715, Abcam), α -SMA antibody (Cat#: af1032, Affinity), COL-I antibody (EPR22894-89, Cat#: ab260043, Abcam), COL-III antibody (RM1028, Cat#: ab283694, Abcam), TNF- α antibody (EPR20972, Cat#: ab215188, Abcam), β -actin antibody (Cat#:GB12001, Affinity). Goat anti-rabbit IgG (Cat#: b1033a, Biosharp) and Goat anti-mouse IgG (Cat#: b1001a, Biosharp) were used as secondary antibodies. The images were obtained by chemiluminescence systems chemiluminescence systems (GeneGnome XRQ, Syngene, A Division of Synoptics Ltd.), with exposure time of 30s~90s. Use Image J 18.0 software to analyze the gray value of each strip.

2.14 Statistical analysis

The experimental data were statistically processed by graphpad prism 10.0 software. The measurement data were expressed by mean $\pm$ Standard error of mean (SEM). The difference was statistically significant when $P < 0.05$.

3. Results

3.1 QLT improved the morphology of lung tissue and reduce collagen deposition in PF mice

Bleomycin induced PF in mice is a widely recognized PF model, which lead to pulmonary fibrosis in mice. Low, medium and high doses of QLT were given to PF mice with intragastric administration within 14 to 35 days. The morphology of lung tissue was observed by HE staining and Masson staining. Compared with model group, QLT significantly inhibited the infiltration of inflammatory cells and the expansion of alveolar space. At the same time, QLT inhibited the accumulation of collagen in the lung in a dose-dependent manner, in which the high dose is more effective (Fig. 1a). QLT also administration reduced the area of pulmonary collagen deposition (Fig. 1b, Fig. 1c). The level of hydroxyproline in the lung tissue of the model group was significantly higher than that of the

normal group. The content of hydroxyproline was decreased when treated with QLT and was dose-dependent (Fig. 1d). The above findings illustrate that QLT could alleviate PF efficiently (Fig. 1).

3.2 QLT improved the pulmonary function of PF mice

As a necessary examination method for respiratory diseases, pulmonary function could be used as one of the diagnostic methods of PF. Cchord, MMEF, PEF, FEV50 / FVC, FRC and TLC reflect the pulmonary function severity of PF. The results show that compared with model group, QLT significantly improved Cchord, MMEF, PEF, FEV50 / FVC, FRC and TLC value with dose-dependent, indicated that QLT improved pulmonary function in PF mice. (Fig. 2).

3.3 QLT reduced the inflammation and collagen deposition in the lung tissue of PF mice

Collagen I(COL-I) and collagen III(COL-III) are markers of extracellular matrix deposition. Compared with the control group, the mRNA relative expression levels of COL-I and COL-III in the lung tissue was up-regulated in that of the model group). In the lung tissue of QLT administration PF mice, the mRNA level of COL-I and COL- was decreased, indicating that QLT reduced the extracellular matrix deposition of PF mice. We further tested the α -SMA, TGF- β and TNF- α mRNA level in lung tissue. α -SMA is a marker protein for the transformation of fibroblasts into myofibroblasts, TGF- β is considered to be a key promoter of fibrosis, TNF- α is an important factor in promoting inflammation and fibrosis. The results confirmed that after treating with QLT, the mRNA level of α -SMA, TGF- β and TNF- α was significantly down-regulated in lung, comparing with that of the model group (Fig. 3a).

To further confirm the influence of inflammation and collagen deposition level of QLT, the protein level of COL-I, COL-III, α -SMA, TGF- β and TNF- α was evaluated. The results showed that the protein level of COL-I and COL-III in the lung tissue of the model group was increased when compared with control group and was decreased in QLT treatment group when compared with model group (Fig. 3b, 3d). Meanwhile, the protein level of α -SMA, TGF- β and TNF- α was determined by ELISA and western blot assay. The results demonstrated that QLT decreased the protein level of α -SMA, TGF- β and TNF- α in lung tissue of PF mice, and the high dose was more effective (Fig. 3c, 3d). These results suggested that QLT efficiently in down-regulation inflammation and collagen deposition of PF.

3.4 QLT interfere in inflammation and collagen deposition in PF mice through IL-17 signal pathway

By sequencing the transcriptome of lung tissue in the model and control group, 94 meaningful Differential Expressed Genes (DEGs) were obtained after the comparison the data of two groups. 72 DEGs were up-regulated and 22 were down-regulated in the model group (Fig. 4a and 4b). Through analyzing in database Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), we found that IL-17 signal pathway was important to PF (Fig. 4c). And in these two pathways, *CCL12*, *FOSL1*, *MMP9*, *CXCL5* and *AREG* were enriched with fold change (Fig. 4d).

To confirm the role of IL-17 pathway in PF mice and the influence of the related genes of QLT, the mRNA level of *CCL12*, *FOSL1*, *MMP9*, *CXCL5* and *AREG* in lung tissue was determined. The results showed that the relative mRNA expression level of *CCL12*, *FOSL1*, *MMP9*, *CXCL5* and *AREG* were significantly up regulated in the model group compared with the control group. The relative mRNA expression level of *CCL12*, *FOSL1*, *MMP9*, *CXCL5* and *AREG* in QLT group was significantly down-regulated when compared with model group, indicated that QLT reduced the expression of genes that related with IL-17 signal pathway (Fig. 5a). To further study, the protein level CCL12, FOSL1, MMP9, CXCL5 and AREG was measured. In model group, the expression of CCL12, FOSL1, MMP9, CXCL5 and AREG is much higher than that in control group. While the protein level of CCL12, FOSL1, MMP9, CXCL5 and AREG was significantly decreased in QLT group (Fig. 5b), suggested that QLT efficiently inhibited the IL-17 signal pathway (Fig. 4, Fig. 5).

4. Discussion

The main pathological feature of PF is the acute inflammatory reaction of the lower respiratory tract at the early stage, accompanied by pathological proliferation and transformation of a large number of fibroblasts, resulting in the deposition of extracellular matrix (ECM) components in alveoli and stroma at the later stage. The excessive repair of fibrous tissue leads to the disorder of lung tissue structure, alveolar injury and collagen deposition, which eventually leads to PF [3, 27]. The incidence rate of PF is high in the middle-aged and elderly people. PF is mostly the pathological result of respiratory diseases, and also one of the common complications of COVID-19 patients. The accumulation of inflammatory cells and the release of cytokines increase collagen fibers, resulting in lung tissue remodeling, reduction of alveolar number, deformation, atresia and loss of pulmonary function, which are the main pathological changes of PF [28]. Previous research found that Yifei Sanjie Recipe alleviated pulmonary fibrosis by reducing the phosphorylation level of PI3K and AKT, upregulating the expression of autophagy marker protein, enhancing autophagy, reducing the content of HYP and the expression of COL-I and COL- in lung tissue [29]. Further study indicated that curcumin and curcuminol reduced fibroblasts α -SMA expression and decreased the contents of HYP and COL-I / , which could reduce collagen synthesis. Curcumin and curcuminol increased the expression levels of Beclin1, ATG7 and Lc3b- , promoted the formation of autophagy bodies, and activate fibroblast autophagy [30]. The previous work also found that Panax notoginseng saponins down regulated the expression of fibrosis related factors, reduced collagen fiber deposition and alleviate bleomycin induced pulmonary fibrosis in mice [31]. Through HPLC experiments, we found that the main chemical components of QLT include Salidroside and Rg1 play an important role in anti-inflammatory and anti-fibrosis [32] (Figure S1). In this experimental study, the results of pathological sections showed that a large number of inflammatory cell infiltration in the lung tissue of PF model group, and the alveolar space is widened. At the same time, the expression level of hydroxyproline, as one of the main components of collagen deposition, increased significantly. After QLT administration, the lung tissue was repaired and the expression level of hydroxyproline decreased significantly, indicating that QLT could reduce collagen deposition and inhibit the expression of hydroxyproline. These results suggest that QLT can repair the pathological changes of PF.

Pulmonary function examination is one of the necessary examinations for respiratory diseases and is mainly used to detect the patency of respiratory tract and the size of lung volume. It is of great value for detecting lung diseases, evaluating the severity and prognosis of diseases, and evaluating the efficacy of drugs or other treatment methods. This study showed that compared with PF model group, taking QLT could improve Cchord, MMEF, PEF, FEV50 / FVC, FRC and TLC ($P < 0.05$), and improved pulmonary function, which is consistent with relevant research reports on pulmonary function [33].

Inflammatory factors can repeatedly mediate the injury and repair of alveolar cells. In this process, fibroblasts migrate go through the damaged basement membrane, proliferate and transform into myofibroblasts. A large amount of inflammatory and fibrogenic factors that produced by myofibroblasts is released to ECM. These factors lead to the pathological processes such as cell structure remodeling, airway wall structure destruction, basement membrane thickening and interstitial hyperplasia, and resulting in pulmonary fibrosis, finally [34]. In process of ECM formation, TGF- β has the activity to induce fibroblasts divided to myofibroblasts. TGF- β specifically promotes the secretion of α -SMA and plays an important role in formation of pulmonary fibrosis. In addition, collagen I/ III secreted by myofibroblasts also promote ECM deposition and accelerate the progress of pulmonary fibrosis [35–36]. In this study, the expression of fibrosis related factors was detected. The results showed that QLT administration group significantly reduced the mRNA and protein expression level of TGF- β , α -SMA, COL-I, COL-III and TNF- α in lung tissue of model mice. It indicated that QLT inhibited the activation of TGF- β , α -SMA and TNF- α , reduced pulmonary inflammatory response and collagen deposition, to delay pulmonary fibrosis.

Transcriptome sequencing is a high-throughput sequencing of the sum of all RNA that could be transcribed by a specific cell in a certain functional state [37]. In this study, the high-throughput sequencing of the transcriptome of the lung tissue of mice in control group and model group was used to explore the relevant mechanism of PF. Through transcriptome sequencing, the differentially expressed genes were found, and the KEGG signal pathway were enriched and analyzed. We found that the differentially expressed genes were involved in a variety of biological processes of the body, especially the regulation of the immune system, signal transduction pathway. Through the calculation of *FDR* and *P* value, we ranked the pathways with significant differences. In these pathways, we found that IL-17 signal pathway was important to PF.

IL-17 is an early promoter of T cell and induces inflammatory response. After IL-17 binds to IL-17 receptor, IL-17 play its biological activity through pathway, which effectively mediates the inflammatory response in tissue cells. Literature research on the enrichment gene of IL-17 signaling pathway in the first place showed that CCL12 chemokine produced by macrophages and epithelial cells is a fibrogenic medium, and CCL12 could promote the development of fibrosis [38]. FOSL1 is involved in regulating biological behaviors such as proliferation, apoptosis and migration of a variety of cells, which is closely related to inflammatory signal pathway [39]. MMPs family members MMP2 and MMP9 play an important role in the process of collagen hydrolysis, and they can promote the hydrolysis of collagen in myocardial interstitium, TIMP1 and TIMP2 are the specific inhibitory molecules of MMPs and can be combined with MMP molecules to reduce their hydrolysis activity, which is conducive to the deposition of collagen, and indicates that the IL-17A gene can break the balance of MMPs/TIMPs [40–42]. CXCL5 chemokine is a member of CXC chemokine family. It is produced by a variety of cells and has strong granulocyte chemotaxis. As an important component of inflammatory environment, CXCL5 plays an important role in tumorigenesis, invasion, metastasis and progression [43]. AREG is secreted by a variety of cells, which can cause the proliferation and differentiation of fibroblasts and inhibit TNF- α by activating EGFR signaling pathway, mediate apoptosis signaling pathway and reduce the damage of alveolar epithelial cells, to protect lung tissue [44–45]. Our studies indicated that QLT reduced the level of *CCL12*, *CXCL5*, *FOSL1*, *MMP9*, and *AREG*, which are inflammation and fibrosis-related genes in the IL-17 signal pathway.

5. Conclusion

In this study, we have confirmed the effect and revealed the potential mechanism of QLT in prevention and treatment of pulmonary fibrosis. The results indicated that QLT ameliorated pulmonary fibrosis in multi-ways, and the IL-17 signaling pathway might be the most potential target of QLT. Our study provides a novel strategy for PF clinical therapy.

Declarations

Supplementary data

Supplementary data related to this article can be found at <http://doi.org/XXX/j.s1>, Figure S1: The HPLC analyze of QLT.

Authorship

Q. Z. and T. L. did the conception and designed the study. D. -Z. Y. and J. L. drafted the article and revised it critically for important intellectual content. Y. F. and J. -L. Y. approval of the version to be submitted. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest The authors declare no conflict of interest.

Institutional Review Board Statement

The animal study protocol was approved by the Animal Care & Welfare Committee of Yunnan University of Chinese Medicine (protocol code: R-06202023, June 15th, 2020).

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Figures

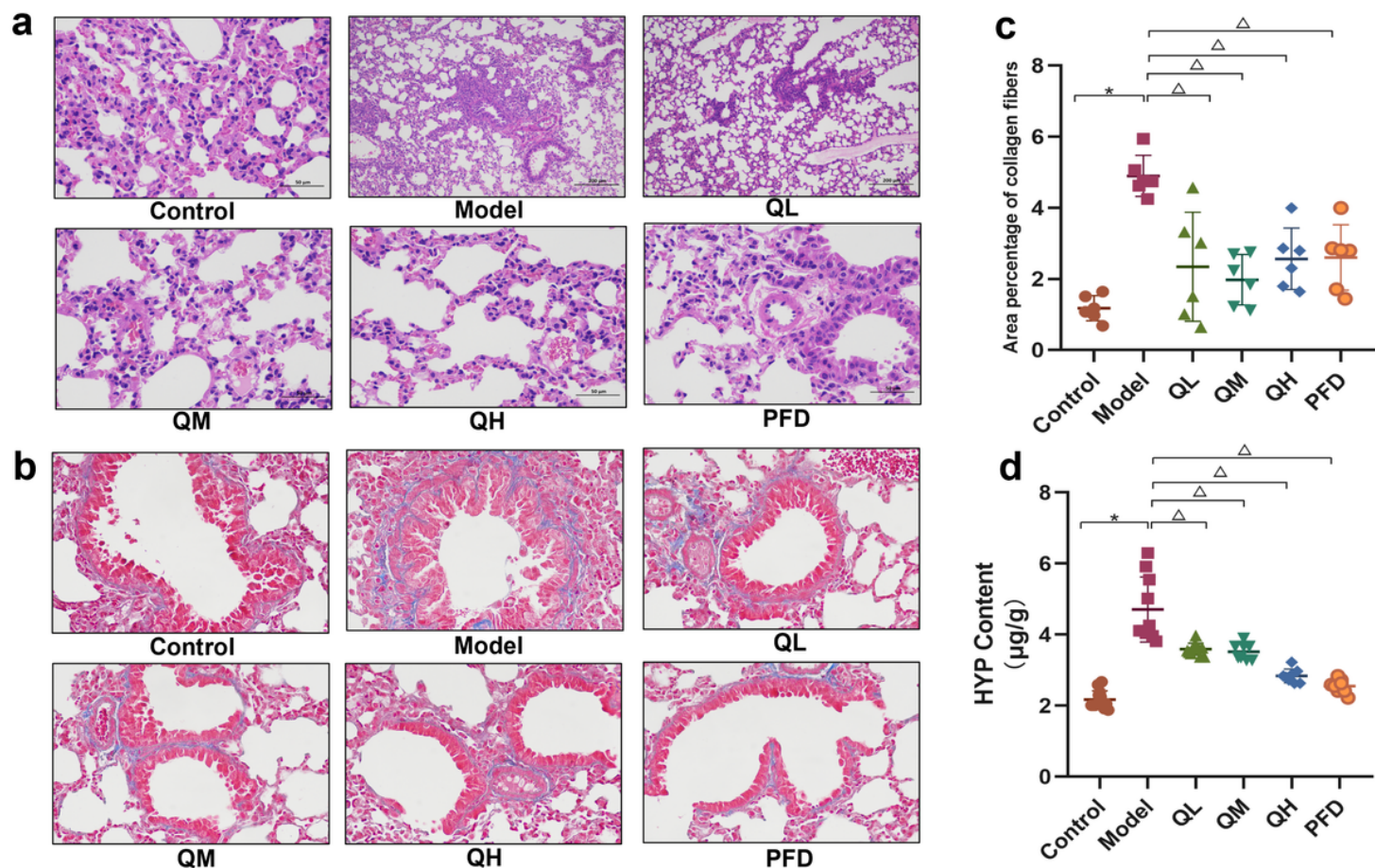


Figure 1

Therapeutic effect of QLT in PF mice. (a) HE staining shows the morphology of lung tissue (400 times magnification). (b) Masson staining was used to indicate collagen deposition in lung tissue (400 magnification). (c) The area percentage of blue collagen fibers in Masson pathological sections was stained. (d) The level of hydroxyproline in lung tissue reflects the degree of PF. (n=6; $P<0.05$, significant difference; * $P<0.05$, Control group compared with the model group; $\Delta P<0.05$, drug treated group compared with the model group).

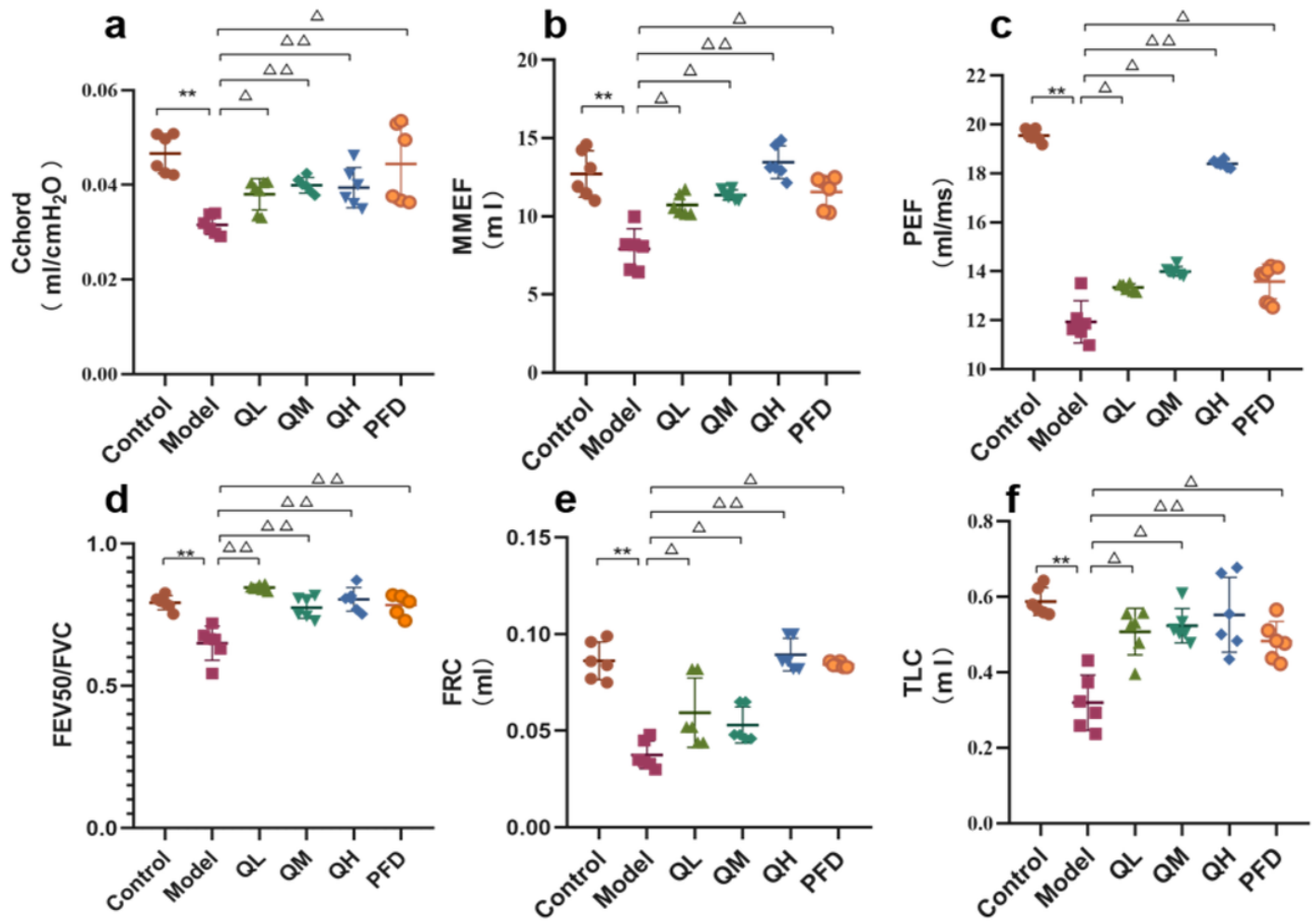


Figure 2

Effects of pulmonary function in each group. (a) Cchord: An index reflecting lung compliance and expansibility, representing the effect of changes in thoracic pressure on lung volume. It reflects lung tissue elasticity and airway resistance. (b) MMEF: It reflects the detection index of pulmonary diffusion function. (c) PEF: It mainly reflects whether the large airway is blocked. (d) FEV₅₀ / FVC: it is the volume of exhaled volume after maximum deep inhalation and maximum exhalation for 50ms in mice, Obstructive or mixed type is slightly reduced to significantly reduced. (e) FRC: The decrease indicates PF. (f) TLC: The decrease is mostly related to restrictive ventilation disorder, suggesting that it is related to PF. (n=6; P<0.05, significant difference; *P<0.05, Control group compared with the model group; ΔP<0.05, ΔΔP<0.01, drug treated group compared with the model group).

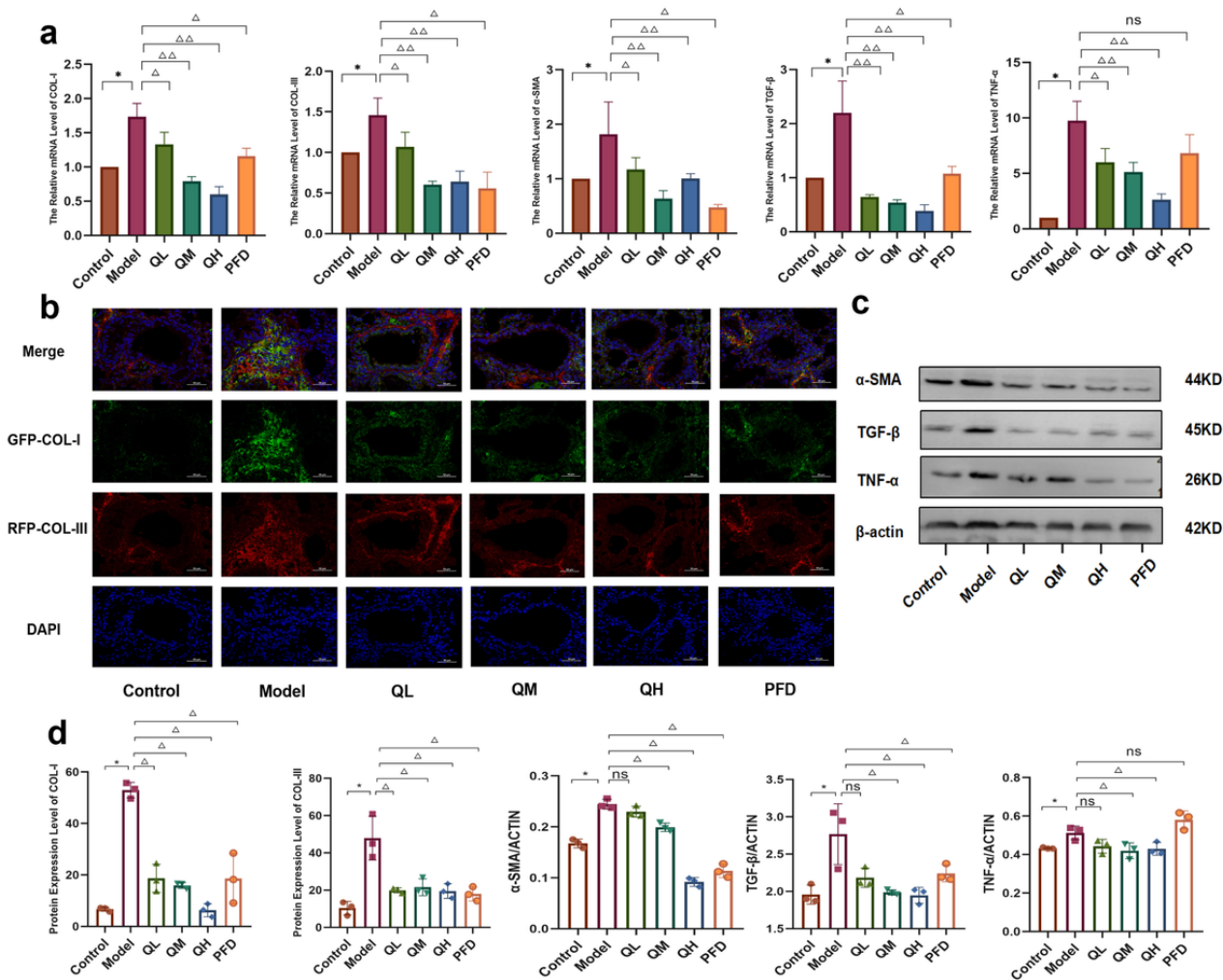


Figure 3

Effects of inflammation and collagen related indexes in each group. (a) The relative expression level of COL-I, COL-III, α-SMA, TGF-β and TNF-α mRNA level in lung tissue samples. (b) The location and expression level of COL-I and COL-III in lung tissue by using immunofluorescence assay. (c) The protein level of α-SMA, TGF-β and TNF-α was determined by western blot. (d) The quantification and statistical analysis of b-d. (n=3; ns, not significant; $P<0.05$, significant difference; * $P<0.05$, ** $P<0.01$, Control group compared with the model group; $\Delta P<0.05$, $\Delta\Delta P<0.01$, drug treated group compared with the model group).

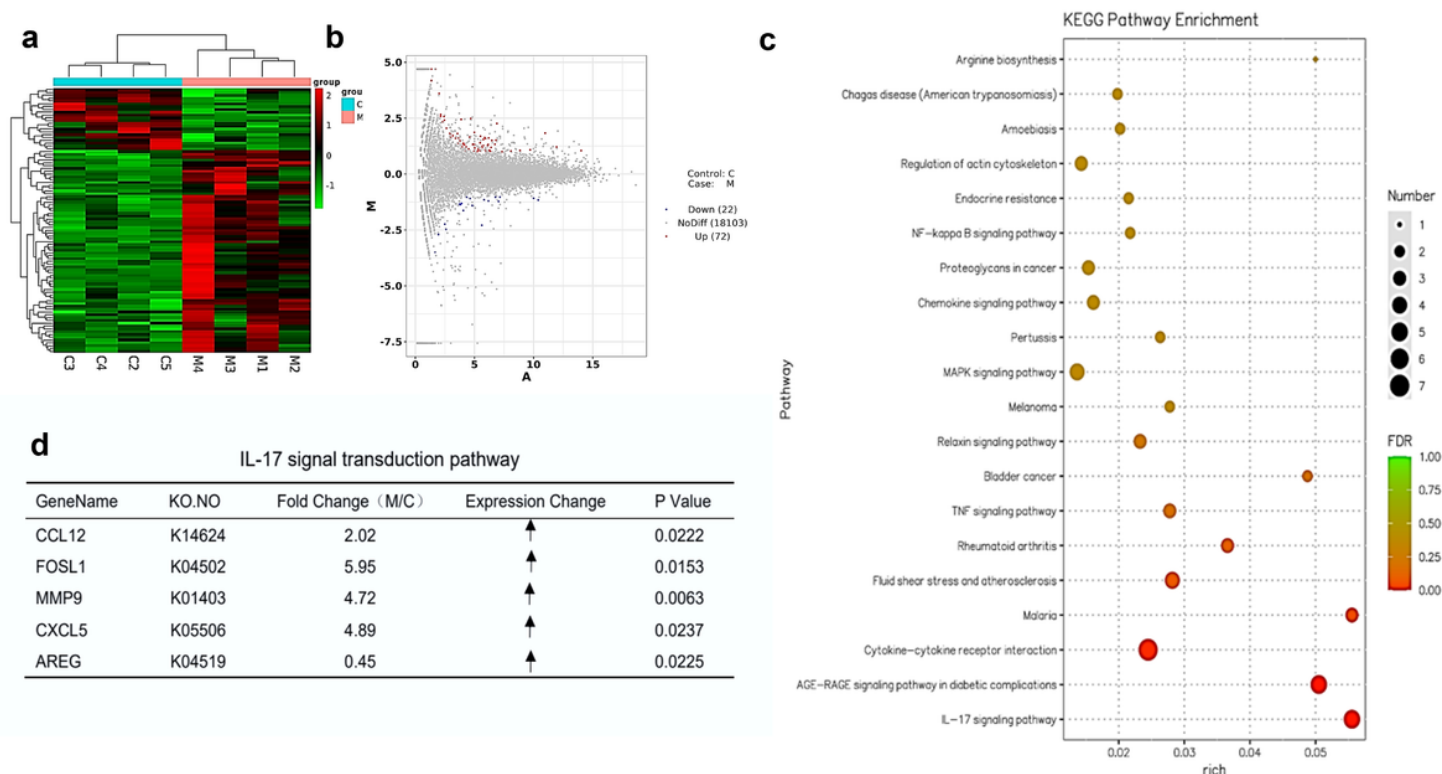


Figure 4

IL-17 signal pathway is prominent in the development of pulmonary fibrosis and the influence of QLT on IL-17 signal pathway. (a)Heat map of differential genes clustering in lung tissue samples. (b)Volcano plot distribution of PM2.5 differential genes cluster heat map in lung tissue. (c)KEGG analysis bubble diagram of differential genes in lung tissue (X-axis represents the *P*-value of the enrichment factor, and the Y-axis represents the names of the 12 pathways with significant differences. The size of the dots represents the number of target genes, and the color of the dots represents the range of the FDR, which represents the ratio of target genes of a specific pathway to the number of all annotated genes in that pathway). (d)The 5 differential genes in lung tissue were enriched into the genes table of IL-17signal pathway.

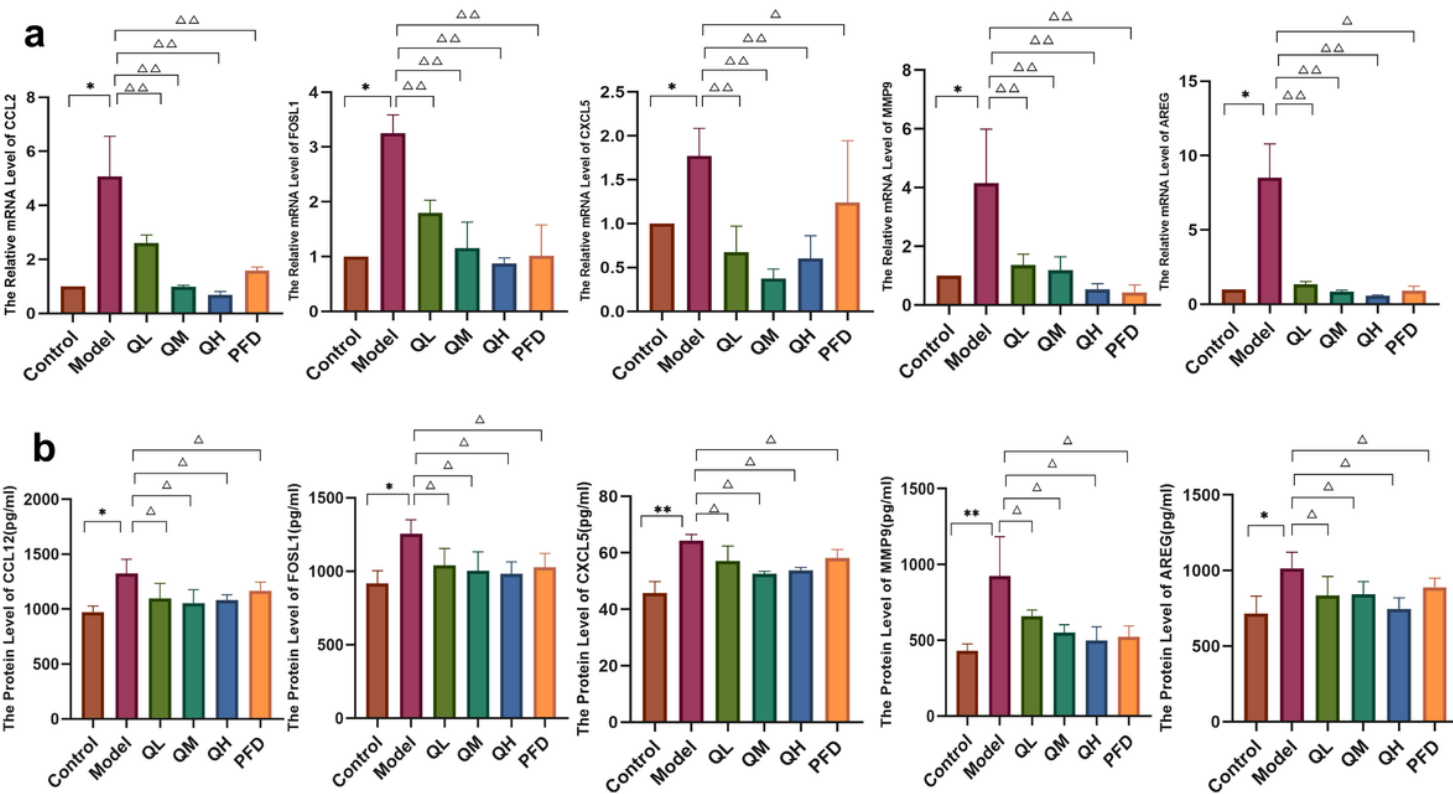


Figure 5

The influence of QLT on IL-17 signal transduction pathway. (a) qRT-PCR was used to verify the mRNA level of *CCL12*, *FOSL1*, *MMP9*, *CXCL5* and *AREG* in mouse lung tissue. (b) Elisa was used to verify the protein level of CCL12, FOSL1, MMP9, CXCL5 and AREG in mouse lung tissue. The data shown are Mean $\pm$ SEM of 3 independent experiments. (n=3; ns, not significant, $P<0.05$, significant difference; * $P<0.05$, ** $P<0.01$, Control group compared with the model group; $\Delta P<0.05$, $\Delta\Delta P<0.01$, drug treated group compared with the model group).

Supplementary Files

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