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Original Research

The effects of miR-27a-3p-mediated Smurf2 on bleomycin A5-induced pulmonary fibrosis in rats

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Abstract: This study aimed to explore the effects of miR-27a-3p-mediated Smurf2 on bleomycin A5-induced pulmonary fibrosis in rats. Sixty clean-grade SD rats were made into models of pulmonary fibrosis induced by bleomycin A5. They were randomly divided into the control group (fed as usual), the bleomycin A5 group, and the miR-27a-3p group according to the modeling. Pathological sections and morphological observations were performed on the lung tissues of all rats, and the expression of miR-27a-3p, Smurf2 mRNA, Smurf2 protein, collagen type I (Col I), collagen type III (Col III), and related inflammatory factors in lung tissues were measured. Dual fluorescein detection was performed for miR-27a-3p and Smurf2 in lung tissues. The lung tissue of rats in the bleomycin A5 group showed obvious pathological changes. The degree of pulmonary fibrosis in the miR-27a-3p group was significantly lower than that in the bleomycin A5 group. The expression levels of Smurf2 mRNA, Smurf2 protein, Col I, Col III, and related inflammatory factors in the lung tissue of rats in the control group were notably lower than rats in the bleomycin A5 group and the miR-27a-3p group (levels of those factors in the miR-27a-3p group were lower than the bleomycin A5 group). The expression level of miR-27a-3p in the lung tissue of rats in the control group was significantly higher than that in the bleomycin A5 group and the miR-27a-3p group (miR-27a-3p level in the miR-27a-3p group was significantly higher than in the bleomycin A5 group). Results of dual fluorescein detection demonstrated that Smurf2 was a direct target gene of miR-27a-3p, and the expression of miR-27a-3p negatively associated with Smurf2. Up-regulation of miR-27a-3p expression can effectively improve the disease degree and inflammatory response in rats with pulmonary fibrosis. Its mechanism may be achieved by regulating Smurf2.

Key words: miR-27a-3p; Smurf2; Pulmonary fibrosis rats; Effects.

Introduction

Pulmonary fibrosis has been known as a chronic progressive disease, and its pathological changes mainly involve the pulmonary blood vessels and the pulmonary interstitial (1). The pathological process of pulmonary fibrosis mainly includes alveolitis and pulmonary fibrosis. Pulmonary fibrosis occurs if alveolitis is not treated effectively and timely, leading to declining lung function and even respiratory failure or death (2, 3). Current treatment methods for pulmonary fibrosis are not effective enough. Lung transplantation, despite its good efficacy for pulmonary fibrosis, the huge difficulty of donor collection and the postoperative immune rejection make its application a hard thing (4). Therefore, the search for a treatment that can effectively reduce the severity of pulmonary fibrosis is of great clinical significance.

Lately, a growing number of researches on the relationship between miRNA and pulmonary fibrosis believe that the deep exploration of the relationship between miRNA and pulmonary fibrosis may spark new ideas on the targets for the treatment and prevent pulmonary fibrosis (5, 6). As a member of the miR-27 family, miR-27a-3p is recently found to be abnormally expressed in various malignant tumors such as liver cancer (7) and osteosarcoma (8). In recent years, the close relationship between miR-27a-3p and the occurrence of pulmonary fibrosis has been noted. In the report by Cui (9), miR-27a-3p was able to inhibit the differentiation from lung fibroblasts into myofibroblast, thus inhibiting pulmonary fibrosis. Smad ubiquitin regulatory factor 2 (Smurf2) has been known as an E3 ubiquitin ligase and the most critical factor in ubiquitination. It has been found to affect pulmonary fibrosis by regulating the TGF-β1/Smad signaling pathway (10, 11). This study discovered that Smurf2 is one of the target genes of miR-27a-3p according to TargetScan, suggesting that the regulation of Smurf2 by miR-27a-3p is also one of the mechanisms of miR-27a-3p's effect on pulmonary fibrosis.

This study established rat models of pulmonary fibrosis to investigate the effect of miR-27a-3p on pulmonary fibrosis in rats. Also, this study established a lung fibroblast model to explore the action mechanism of miR-27a-3p in pulmonary fibrosis, aiming to provide a more experimental basis for the target treatment of pulmonary fibrosis.

Materials and Methods

Experimental animals and materials

Sixty clean-grade SD rats obtained from Laboratory Animal Center of Sun Yat-sen University were toge-

ther, with a body mass of about 205-245 g. They were raised in an environment with a temperature of 20-25 °C and relative humidity of 40% to 70% in normal day/night cycles, free to eat and drink. Bolezyme A5 was purchased from Shanghai Hengfei Biotechnology Co., Ltd. Human embryonic lung fibroblast MRC-5 was purchased from Shanghai Cell Bank of the Chinese Academy of Sciences. IL-6 and IL-4 ELISA kits were prepared from Shanghai Enzyme-linked Biotechnology Co., Ltd. Col I, Col III, Smurf2, and GAPDH primary antibodies were purchased from Abcam (USA). Horseradish peroxidase-labeled goat anti-rabbit IgG secondary antibody was purchased from Wuhan Boster Bioengineering Co., Ltd. Lipofectamine 3000 was purchased from Invitrogen, Thermo Fisher Scientific, Inc., USA).

Animal modeling

All rats were randomly and equally divided into the control group, the bleomycin A5 group, and the miR-27a-3p group. Bleomycin A5 was used to induce pulmonary fibrosis in rat models (12), and the procedures were as follows: Firstly, rats in the bleomycin A5 group and the miR-27a-3p group were anesthetized by an intraperitoneal injection of 10% chloral hydrate at a dose of 3 ml/kg. After anesthesia, the rats underwent a tracheotomy and 0.2 mL of bleomycin A5 solution at a concentration of 5 mg/kg was injected once in the trachea of rats in the bleomycin A5 group and the miR-27a-3p group. On the next day after modeling, 0.2 ml of normal saline containing 40 nmoL of miR-27a-3p agomir was injected into the tail vein of rats in the miR-27a-3p group. The same dose of normal saline was injected into rats in the bleating A5 group and the control group once every 3 days. After 15 days, the detection of factors was performed on rat models. All animal experiments were agreed by the ethics committee of the Gaomi Hospital of Traditional Chinese Medicine and followed the guidelines for the care and usage of laboratory animals. Great effort was made to reduce the suffering of animals.

Collection of specimens

Twenty-four hours after the modeling, the blood sample was collected from all rats through the abdominal aorta and then centrifuged at 3000r/min for 5minutes to obtain the serum for detections. Both lungs of the rat were exacted after cervical dislocation. The left lung was paraffin-embedded, sliced, and stained for histopathological analysis. The right lung was ground to prepare a tissue suspension for index detection.

Indicator detection

Observation of lung tissue lesions

HE and Masson staining were performed on the section of the left lung according to the literature (11-13). The degree of alveolitis was assessed by HE staining, while the degree of pulmonary fibrosis by Masson staining.

RT-PCR detection of miR-27a-3p and Smurf2 expression in lung tissue of rats in each group

Total RNA was extracted by adding Trizol reagent to the prepared tissue suspension, and the purity and concentration of total RNA were examined using an ultraviolet spectrophotometer. The total RNA of miR-27a-3p and Smurf2 was reverse transcribed using the SYBR-Green Realtime PCR Master mix in strict accordance with the instruction of kits. The PCR amplification was then performed. PCR reaction conditions were as follows: 40 cycles of pre-denaturation at 95 °C for 35 minutes, annealing at 94 °C for 30 seconds and 60 °C for 40 seconds, and a final extension at 72 °C for 30 seconds. Smurf2 reaction conditions were as follows: 40 cycles of pre-denaturation at 95 °C for 10 seconds, annealing at 95 °C for 5 seconds and 60 °C for 30 seconds, and a final extension at 60 °C for 30 seconds. Primers were synthesized by Shanghai Bioengineering Co., Ltd. More details are shown in Table 1. U6 was used as the internal reference of miR-27a-3p, GAPDH was the internal reference of Smurf2. The relative mRNA expression was measured by $2^{-\Delta\Delta Ct}$.

Western blot analysis of Smurf2, Col I, Col III protein expression in rat lung tissue

RIPA lysis method was applied to purification the total protein from the lung tissue suspension. Then the BCA method was used for protein quantitative detection, and the protein concentration was adjusted to 5 μg/μL after the detection. The protein was then electrophoretically separated by 12% SDS-PAGE and transferred to a PVDF membrane, and then blocked with 5% skimmed milk powder for 2 hours at room temperature. Then, rat monoclonal antibodies of Smurf2 primary antibody (1:1000), Col I primary antibody (1:1000), Col III primary antibody (1:1000) and GAPDH (1:1000) were added and blocked overnight at 4 °C. After that, the horseradish peroxidase-labeled goat anti-rabbit IgG secondary antibody (dilution ratio of 1:3000) was added and the liquid was incubated at 37 ° C for 1 hour. Finally, ECL color development was performed. The experiment was repeated three times.

Detection of related inflammatory factors in serum by ELISA

The expression of inflammatory factors TGF-β1 and IL-4 in rat lung tissue was examined in strict accordance with the instructions of the ELISA kit.

Dual-luciferase reporter assay

A dual-luciferase reporter assay was performed to determine if Smurf2 is a direct target gene for miR-27a-

Table 1. Related primers for amplification of miR-27a-3p, U6, Smurf2 and GAPDH.

Factors	Forward primer	Reverse primer
miR-27a-3p	5'-TGCGGTTCACAGTGGCTAAG-3'	5'-CTCAACTGGTGTCGTGGA-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
Smurf2	5'-GATCCAAAGTGGAATCAGCA-3'	5'-TGGCATTGGAAAGAAGACG-3'
GAPDH	5'-CCATTTGCAGTGGCAAAG-3'	5'-CACCCCATTTGATGTTAGTG-3'

3p. The Smurf2 3'UTR dual-luciferase reporter plasmid (WT and MUT) was constructed by RiboBio. They were cotransfected into human embryonic lung fibroblast MRC-5 with miR-27a-3p mimetic or miR-NC through Lipofectamine 3000. After 48 hours of incubation, luciferase activity was identified by a dual-luciferase reporter assay system (Promega Corp., Madison, WI, USA).

Statistical analysis

The experimental data were analyzed by SPSS19.0 and visualized by GraphPad Prism 6. The measurement data were expressed by the mean ± standard deviation (SD ± means) and compared between the two groups using the independent sample t-test. One-way ANOVA was used for comparison between multiple groups. The LSD t-test was used post-hoc pairwise comparison. A statistical difference was recognized if $P< 0.05$.

Results

The lung tissue lesions of rats in each group

After 15 days of modeling, the lung tissue morphology of the rats in each group was observed. The lung tissue of the control group had a clear structure and intact alveolar wall, and no inflammatory cell infiltration was observed. The lung tissue of the Bolezyme A5 group showed severely damaged structure, edema, shedding or even necrosis in the epithelial cells of the bronchioles, fibrotic wall, hyperplasia of the local type II alveolar epithelial cells, congestion and widened alveolar septum with infiltration of a large number of inflammatory cells and hyperplasia of fibroblast, and hyperplasia of pulmonary interstitial fibrous tissue. Compared with the bleating A5 group, the lung tissue of the miR-27a-3p group had improved conditions, with reduced fibroblasts, collagen deposition, and inflammatory infiltration. The alveolitis score and pulmonary fibrosis score of the control group were significantly lower than those of the bleating A5 group and the miR-27a-3p group (the miR-27a-3p group had lower alveolitis score and pulmonary fibrosis score than the bleating A5 group), and the differences were statistically significant ($P< 0.05$) based on Table 2.

Expression and correlation of miR-27a-3p and Smurf2 RNA in lung tissues of rats in each group

The control group had markedly higher miR-27a-3p level and lower Smurf2 RNA level in the lung tissue than the bleating A5 group and the miR-27a-3p group, the miR-27a-3p group had significantly higher miR-27a-3p level and lower Smurf2 RNA level than the bleating A5 group, and the differences were statistically significant ($P< 0.05$). The expression of miR-27a-3p and Smurf2 RNA in lung tissue of rats with pulmonary fibrosis was negatively correlated ($r=-0.661$, $P< 0.05$) based on Figure 1.

Expressions of Smurf2, Col I, Col III protein in rat lung tissue of each group

The control group had markedly lower levels of Smurf2, Col I, Col III protein than the bleating A5 group and the miR-27a-3p group, the miR-27a-3p group had significantly lower Smurf2, Col I, Col III protein than the bleating A5 group, and the differences were significant ($P< 0.05$) based on Figure 2.

Serum expressions of related inflammatory factors in rats of each group

The control group had markedly lower levels of IL-6 and IL-4 than the bleating A5 group and the miR-27a-3p group, the miR-27a-3p group had significantly lower IL-6 and IL-4 levels than the bleating A5 group, and the differences were significant ($P< 0.05$) according to Figure 3.

Smurf2 is a direct target gene of miR-27a-3p

This study performed a bioinformatics analysis to predict target genes for miR-27a-3p. Smurf2 was identified as the target gene of miR-27a-3p (Figure 4A). To examine whether the 3'UTR of Smurf2 can be directly targeted by miR-27a-3p, a luciferase reporter assay was

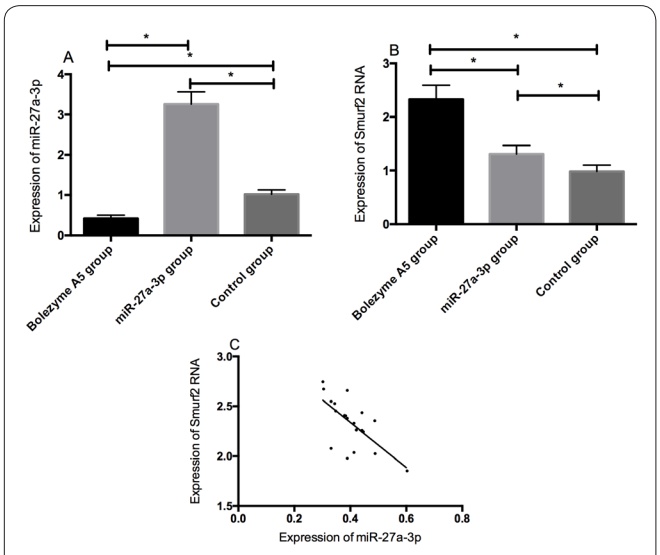

Figure 1. Expression and correlation of miR-27a-3p and Smurf2 RNA in lung tissues of rats in each group. **(A)** The control group had a markedly higher miR-27a-3p level in the lung tissue than the bleating A5 group and the miR-27a-3p group, and the miR-27a-3p level in the lung tissue of the miR-27a-3p group was significantly higher than in the bleating A5 group. **(B)** The control group had markedly lower Smurf2 RNA level in the lung tissue than the bleating A5 group and the miR-27a-3p group, and the Smurf2 RNA level in the lung tissue of the miR-27a-3p group was significantly lower than in the bleating A5 group. **(C)** The expression of miR-27a-3p and Smurf2 RNA in lung tissue of rats with pulmonary fibrosis was negatively correlated ($r=-0.661$, $P< 0.05$). * indicates $P < 0.05$.

Table 2. Alveolitis score and pulmonary fibrosis score of all groups.

Score	Control group (n=20)	Bleating A5 group (n=20)	miR-27a-3p group (n=20)	F	P
Alveolitis score	0.06±0.01*	2.46±0.26**	1.01±0.12#	1068	<0.001
Pulmonary fibrosis score	0.05±0.02*	2.78±0.21**	1.22±0.15#	1680	<0.001

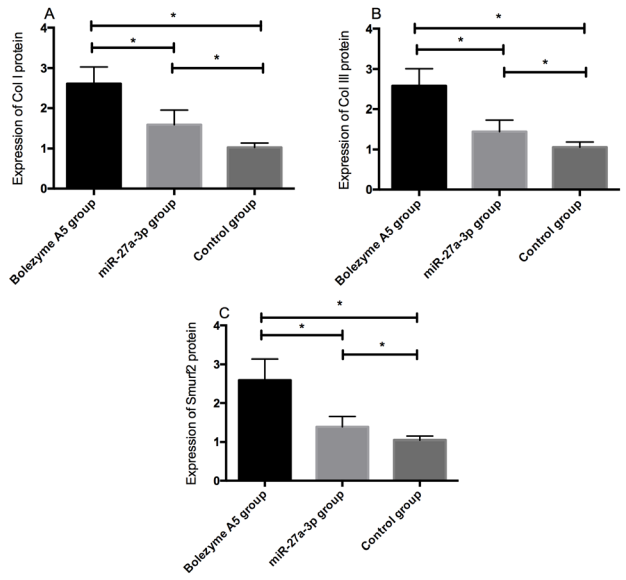

Figure 2. Expressions of Smurf2, Col I, Col III protein in rat lung tissue of each group**. (A)** The control group had markedly lower Col I level in the lung tissue than the bleating A5 group and the miR-27a-3p group, and the Col I level in the lung tissue of the miR-27a-3p group was significantly lower than in the bleating A5 group. **(B)** The control group had markedly lower Col III level in the lung tissue than the bleating A5 group and the miR-27a-3p group, and the Col III level in the lung tissue of the miR-27a-3p group was significantly lower than in the bleating A5 group. **(C)** The control group had markedly lower Smurf2 level in the lung tissue than the bleating A5 group and the miR-27a-3p group, and the Smurf2 level in the lung tissue of the miR-27a-3p group was significantly lower than in the bleating A5 group. * indicates $P < 0.05$.

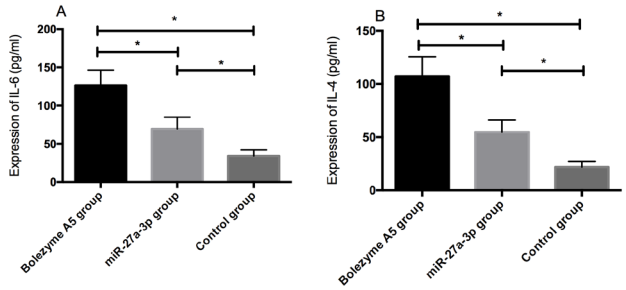

Figure 3. Serum expressions of related inflammatory factors in rats of each group. **(A)** The control group had markedly lower IL-6 level in the lung tissue than the bleating A5 group and the miR-27a-3p group, and the IL-6 level in the lung tissue of the miR-27a-3p group was significantly lower than in the bleating A5 group. **(B)** The control group had markedly lower IL-4 level in the lung tissue than the bleating A5 group and the miR-27a-3p group, and the IL-4 level in the lung tissue of the miR-27a-3p group was significantly lower than in the bleating A5 group. * indicates P< 0.05.

performed. Results of the luciferase reporter assay displayed that (Figure 4B) the overexpression of miR-27a-3p inhibited the luciferase activity of Position 144-150 of Smurf23' UTR Wt (P< 0.05), but did not influence the position 144-150 of Smurf2 3' UTR Mut. Western blot showed that the expression of Smurf2 protein in human embryonic lung fibroblast MRC-5 transfected with miR-27a-3p mimetic (Figure 4C, P< 0.05).

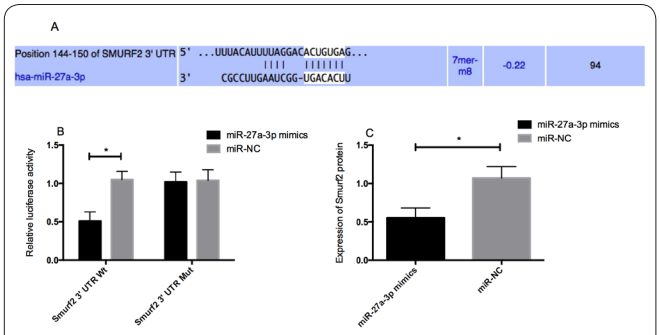

Figure 4. Target gene prediction of miR-27a-3p**. (A)** Sequence alignment of putative binding sites in miR-27a-3p and Smurf2 wild type regions. **(B)** Dual-luciferase reporter assay indicated that the miR-27a-3p mimetic reduced the fluorescence intensity of MRC-5 cells transfected with Smurf2-Wt, but did not affect the Smurf2-mut vector. **(C)** The expression of Smurf2 protein in miR-27a-3p mimetic-transfected MRC-5 cells was down-regulated. * indicates P< 0.05.

Discussion

As an interstitial disease, pulmonary fibrosis is considered by inflammation of the alveoli and extensive derivation of collagen fibers. Many patients with pulmonary fibrosis die from respiratory failure (14, 15). In recent years, a growing number of miRNAs are found to be closely related to the occurrence and development of pulmonary fibrosis (16). As a pleiotropic mRNA, miR-27a-3p is also one of the most studied factors recently (17). A study (18) found that overexpression of miR-27a-3p can effectively inhibit the formation of Col I in renal fibroblasts, thereby alleviating renal fibrosis in mice. In this study, the expression of miR-27a-3p in lung tissue of rats with pulmonary fibrosis induced by bleomycin A5 was significantly reduced, and the degree of pulmonary fibrosis in rats with up-regulated miR-27a-3p expression was significantly lower than rats without miR-27a-3p up-regulation. However, few efforts have been made to study the effects of miR-27a-3p on pulmonary fibrosis and its mechanism.

The pathogenesis of pulmonary fibrosis involves a very wide range of signaling pathways. Recently the transforming growth factor β1 (TGF-β1) signaling pathway has been frequently studied (19). As a key factor in the ubiquitin-proteasome pathway, Smurf2 is highly expressed in pulmonary fibrosis tissue. Through the selective degradation of key parts of the TGF-β1/Smad signaling pathway, Smurf2 can degrade Smad7 protein ubiquitination and regulates TGF-β1 signaling, causing abnormalities in the conduction of the TGF-β1 signaling pathway and ultimately leading to fibrosis (20, 21). However, little is known on the way to regulate Smurf2. One research (22) found that TGF-β1 can promote the expression of Smurf2. But this is a negative feedback regulation, which is considered as a result of the relationship between Smurf2 and TGF-β1/Smad signaling pathways. This study discovered that Smurf2 is one of the target genes of miR-27a-3p according to TargetScan, which led us to speculate whether miR-27a-3p can affect lung fibrosis by regulating Smurf2. In this study, the expression of Smurf2 in lung tissue of rats with pulmonary fibrosis was significantly increased, but the expression of Smurf2 was significantly decreased after

the up-regulation of miR-27a-3p in rats with pulmonary fibrosis. According to the dual-luciferase reporter assay, the expression of Smurf2 protein was down-regulated after the transfection of miR-27a-3p in human lung fibroblasts, which further supports our conjecture.

During the pathogenesis of pulmonary fibrosis, the massive formation of extracellular matrices such as Col I and Col III leads to massive deposition in lung tissue, which is one of the major pathological changes in pulmonary fibrosis (23). Alveolitis, one of the main causes of pulmonary fibrosis, is mainly characterized by the infiltration of inflammatory cells (24). Subsequently, in order to further explore the effect of miR-27a-3p on pulmonary fibrosis in rats, this study also quantified Col I, Col III protein, and related serum inflammatory factors in each group of rats. The results demonstrated that the up-regulation of miR-27a-3p in rats with pulmonary fibrosis can effectively reduce the expression of Col I, Col III protein, and related inflammatory factors, suggesting that miR-27a-3p can not only perform a negative regulation on the synthesis of the extracellular matrix but also alleviate the inflammatory response. So far few studies have been conducted on the regulation of the extracellular matrix by miR-27a-3p. One literature (25) reported that the expression of Col I protein is decreased when the expression of Smurf2 in human lung fibroblasts is silenced, and speculated that the regulation of Col I protein by Smurf2 is based on the regulation of TGF-β1/Smad signaling pathway. The aforementioned studies lead to speculation that miR-27a-3p can inhibit the synthesis of the extracellular matrix by regulating Smurf2. This speculation should be verified through in vitro cell experiments, but it enlightens a new direction for the study of the relationship between miR-27a-3p and Smurf2 in pulmonary fibrosis (25-39).

In summary, the up-regulation of miR-27a-3p expression can effectively improve the disease degree and inflammatory response in rats with pulmonary fibrosis. Its mechanism may be achieved by regulating Smurf2. However, this study is defective. For example, we did not explore the effects of miR-27a-3p on the biological functions of human lung fibers and failed to figure out the specific mechanism of miR-27a-3p's influences on pulmonary fibrosis though its regulation of Smurf2, which will be one of the focus of our future researches.

Authors' contributions

Jin Z wrote the manuscript. Jing Z performed PCR, Western blot, ELISA and Dual-luciferase reporter assay. QZ and Jin Z contributed to the observation indexes analysis. The final version was read and adopted by all the authors. All authors read and approved the final manuscript.

References

1. Liu YM, Nepali K, Liou JP. Idopathic Pulmonary Fibrosis:current status, recent progress and emerging targets. J Med Chem 2017; 60: 527-553.

2. Bahmer T, Kirsten AM, Waschki B, Rabe KF, Magnussen H, Kirsten D, Gramm M, Hummler S, Brunnemer E, Kreuter M, Wtaz H. Prognosis and longitudinal changes of physical activity in idiopathic pulmonary fibrosis. BMC Pulm Med 2017; 17: 104-111.

3. Dowman LM, Mcdonald CF, Bozinovski S, Vlahos R, Gillies R, Pouniotis D, Hill CJ, Goh NSL, Holland AE. Greater endurance capacity and improved dyspnoea with acute oxygen supplementation in idiopathic pulmonary fibrosis patients without resting hypoxaemia. Respirology 2017; 22: 957-964.

4. Kaur A, Mathai SK, Schwartz DA. Genetics in Idiopathic Pulmonary Fibrosis Pathogenesis, Prognosis, and Treatment. Front Med (Lausanne) 2017; 4: 154-162.

5. Liu LJ, Qian H. Up-regulation of miR-21 promotes cell proliferation and collagen synthesis in pulmonary fibroblasts. Chin J Cell Mol Immunol 2015; 31: 918 -922.

6. Díazpiña G, Ordoñezrazo RM, Montes E, Páramo I, Becerril C, Salgado A, Santibañez-Salgado JA, Maldonado M, Ruiz V, The Role of ADAR1 and ADAR2 in the Regulation of miRNA-21 in Idiopathic Pulmonary Fibrosis. Lung 2018; 196 201: 1-8.

7. Zhao N, Sun H, Sun B, Zhu D, Zhao X, Wang Y, Gu Q, Dong X, Liu F, Zhang Y, Li X. miR-27a-3p suppresses tumor metastasis and VM by down-regulating VE-cadherin expression and inhibiting EMT:an essential role for Twist-1 in HCC. Sci Rep 2016; 6: 23091-23106.

8. Salah Z, Arafeh R, Maximov V, Galasso M, Khawaled S, Abou-Sharieha S, Volinia S, Jones KB, Croce CM, Aqeilan RI. miR-27a and miR-27a* contribute to metastatic properties of osteosarcoma cells. Oncotarget 2015; 6: 4920 - 4935.

9. Cui H, Banerjee S, Xie N, Ge J, Liu RM, Matalon S, Thannickal VJ, Liu G. miR-27a-3p is a negative regu- lator of lung fibrosis by targeting myofibroblast differentiation. Am J Respir Cell Mol Biol 2016; 54: 843 -852.

10.Cai Y, Zhou CH, Fu D, Shen XZ. Overexpression of Smad ubiquitin regu- latory factor 2 suppresses transforming growth factor-β mediated liv- er fibrosis. J Dig Dis 2012; 13: 327-334.

11.Robinson PA, Ardley HC. Ubiquitin- protein ligases. J Cell Sci 117: 5191-5194, 2004.

12.Wang L, Lin Q, Yang T, Liang Y, Nie Y, Luo Y, Shen J, Fu X, Tang Y, Luo F. Oryzanol Modifies High Fat Diet-Induced Obesity, Liver Gene Expression Profile, and Inflammation Response in Mice. J Agri Food Chem 2017; 65(38): 8374-8385.

13.Ren Y, Jiao X, Zhang L. Expression level of fibroblast growth factor 5 (FGF5) in the peripheral blood of primary hypertension and its clinical significance. Saudi J Biol Sci 2018; 25(3): 469-473.

14.Martinez FJ, Chisholm A, Collard HR, Flaherty KR, Myers J, Raghu G, Walsh SL, White ES, Richeldi L. The diagnosis of idiopathic pulmonary fibrosis:current and future approaches. Lancet Respir Med 5: 61-71, 2017.

15.King CS, Nathan SD. Idiopathic pulmonary fibrosis:effects and optimal management of comorbidities. Lancet Respir Med 2017; 5: 61-71.

16.Zhang Q, Ye H, Xiang F, Song LJ, Zhou LL, Cai PC, Zhang JC, Yu F, Shi HZ, Su Y, Xin Jb, Ma WL. miR-18a-5p Inhibits Sub-pleural Pulmonary Fibrosis by Targeting TGF-β Receptor II. Mol Ther 2017; 25: 728-738.

17.Zhou L, Liang X, Zhang L, Yang L, Nagao N, Wu H, Liu C, Lin S, Cai G, Liu J. miR-27a-3p functions as an oncogene in gastric cancer by targeting BTG2 [J/OL]. Oncotarget 2016; 7: 51943 - 51954.

18.Knudsen L, Ruppert C, Ochs M. Tissue remodelling in pulmonary fibrosis. Cell Tissue Res 2017; 367: 607 - 626.

19.Guan S, Zhou J. Frizzled-7 mediates TGF-β-induced pulmonary fibrosis by transmitting non-canonical Wnt signaling. Exp Cell Res 2017; 359: 226-234.

20.Wu B, Guo B, Kang J, Deng X, Fan Y, Zhang X, Ai K. Downregulation of Smurf2 ubiquitin ligase in pancreatic cancer cells reversed TGF-β-induced tumor formation.Tumour Biol 2016; 4: 1-15.

21.Ganji A, Mosayebzadeh H, Varasteh A, Moghadam M, Sankian M. The effects of WW2/WW3 domains of Smurf2 molecule on

TGF-βsignaling and arginase I gene expression. Cell Biol Int 2015; 39: 690-695.
22. Mutlu GM, Budinger GS, Wu M, Lam AP, Zirk A, Rivera S, Urich D, Chiarella SE, Go LH, Ghosh AK, Selman M. Proteasomal inhibition after injury prevents fibrosis by modulating TGF-β(1) signalling. Thorax 2012; 67: 139-146.
23. Li LC, Xu L, Hu Y, Cui WJ, Cui WH, Zhou WC, Kan LD. Astragaloside IV Improves Bleomycin-Induced Pulmonary Fibrosis in Rats by Attenuating Extracellular Matrix Deposition. Front Pharmacol 2017; 8: 513.
24. Beach TA, Johnston CJ, Groves AM, Williams JP, Finkelstein JN. Radiation induced pulmonary fibrosis as a model of progressive fibrosis: Contributions of DNA damage, inflammatory response and cellular senescence genes. Exp Lung Res 2017; 43: 1.
25. Yang J, Cao S, Zhang M. Effect of Smad ubiquitination regulatory factor 2 on TGF-β1-induced activation in lung fibroblasts and its molecular mechanism. J. Jilin Univ 2015; 30: 129-151.
26. Zhang T, Wu X, Shaheen SM, Zhao Q, Liu X, Rinklebe J, Ren H. Ammonium nitrogen recovery from digestate by hydrothermal pretreatment followed by activated hydrochar sorption. Chem Eng J 2020; 379: 1-54.
27. Zhu B, Pang R, Chevallier J, Wei YM, Vo DT. Including intangible costs into the cost-of-illness approach: a method refinement illustrated based on the PM2.5 economic burden in China. Europ J Health Econ 2019; 20(4): 501-511.
28. Chen H, Chen Y, Yang L. Intelligent early structural health prognosis with nonlinear system identification for RFID signal analysis. Comput Commun 2020; 157: 150-161.
29. Chen HX, Huang L, Yang L, Chen YT, Huang JM. Model-based method with nonlinear ultrasonic system identification for mechanical structural health assessment. Trans Emerge Telecommun Technol 2020; 1-15.
30. Chen X, Xu Y, Meng L, Chen X, Yuan L, Cai Q, Shi W, Huang G. Non-parametric partial least squares-discriminant analysis model based on sum of ranking difference algorithm for tea grade identification using electronic tongue data. Sens Actuators B Chem 2020; 311:127924-127931.
31. Guo T, Lin Q, Li X, Nie Y, Wang L, Shi L, Xu W, Hu T, Guo T, Luo F. Octacosanol Attenuates Inflammation in Both RAW264.7 Macrophages and a Mouse Model of Colitis. J Agri Food Chem 2017; 65(18): 3647-3658.
32. Jiang X, Zhu B, Chevallier J, Xie R. Allocating provincial CO2 quotas for the Chinese national carbon program. Australian J Agri Res Econ 2018; 62(3): 457-479.
33. Li W, Jia MX, Wang JH, Lu JL, Deng J, Tang JX, Liu, C. Association of MMP9-1562C/T and MMP13-77A/G Polymorphisms with Non-Small Cell Lung Cancer in Southern Chinese Population. Biomolecules 2019; 9(3): 107-119.
34. Liang Y, Lin Q, Huang P, Wang Y, Li J, Zhang L, Cao J. Rice Bioactive Peptide Binding with TLR4 To Overcome H2O2-Induced Injury in Human Umbilical Vein Endothelial Cells through NF-kappa B Signaling. J Agri Food Chem 2018; 66(2): 440-448.
35. Lou Y, Guo D, Zhang H, Song L. Effectiveness of mesenchymal stems cells cultured by hanging drop vs. conventional culturing on the repair of hypoxic-ischemic-damaged mouse brains, measured by stemness gene expression. Open Life Sci 2016; 11(1): 519-523.
36. Lou Y, Shi J, Guo D, Qureshi AK, Song L. Function of PD-L1 in antitumor immunity of glioma cells. Saudi J Biol Sci 2017; 24(4): 803-807.
37. Lou Y, Yang J, Wang L, Chen X, Xin X, Liu Y. The clinical efficacy study of treatment to Chiari malformation type I with syringomyelia under the minimally invasive surgery of resection of Submeningeal cerebellar Tonsillar Herniation and reconstruction of Cisterna magna. Saudi J Biol Sci 2019; 26(8): 1927-1931.
38. Nie Y, Luo F, Lin Q. Dietary nutrition and gut microflora: A promising target for treating diseases. Trends Food Sci Technol 2018; 75: 72-80.
39. Nie Y, Luo F, Wang L, Yang T, Shi L, Li X, Shen J, Xu W, Guo T, Lin Q. Anti-hyperlipidemic effect of rice bran polysaccharide and its potential mechanism in high-fat diet mice. Food Func 2017; 8(11): 4028-4041.