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Article

Study on the mechanism of effective monomer components of Folium Isatidis indigotica in intervening pulmonary fibrosis based on TGF-β1Smad3 signaling pathway

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Abstract: Pulmonary fibrosis is a fatal interstitial lung disease characterized by abnormal fibroblast activation and overabundant extracellular matrix (ECM) accumulation without efficient clinical therapeutic interventions. The transforming growth factor-beta 1 (TGF-β1)/Smad3 signaling pathway is the fulcrum molecular mechanism of this pathologic process. Isatis indigotica Fort. (Banlangen), a traditional Chinese medicine for the elimination of heat and detoxification, contains bioactive monomeric compounds with pronounced pharmacological activities in anti-inflammation and anti-oxidation. The specific regulatory effect on the TGF-β1/Smad3 pathway and pulmonary fibrosis remains to be elucidated. The purpose of this work is to conduct extensive research on the modulatory effect of the monomeric active components of Isatis indigotica on the TGF-β1/Smad3 pathway, and elucidate their protective effect and action mechanism on a bleomycin-induced pulmonary fibrosis model, and thus to offer new natural drug leads and theoretical basis for the prevention and therapy of pulmonary fibrosis.

Keywords: Isatis indigotica; TGF-β1/Smad3 signaling pathway; Pulmonary fibrosis

1. Overview of Isatis indigotica and its active monomer components

Isatis indigotica (Isatis indigotica), the dried leaves of the cruciferous plant Isatis indigotica, has been used for clearing heat and detoxifying, cooling blood, and eliminating eczema in traditional Chinese medicine for a long time. It is used clinically to treat fever, laryngeal congestion, and eczema due to pulmonary heat and toxicity extensively (Zhou et al., 2022). Current pharmacological research has shown that the herb contains a number of structurally well-defined active components, primarily indigo, indirubin, neoindole glycosides, and epigoitrin (Wang et al., 2023; Zhou et al., 2022). Indirubin, a model indole alkaloid, was demonstrated by an enormous amount of research to possess excellent anti-inflammatory and immunomodulatory properties. Its mechanism of action involves inhibition of multiple protein kinases, particularly glycogen synthase kinase-3β (GSK-3β) and cell cycle-dependent kinases (Zhou et al., 2022). GSK-3β negatively regulates TGF-β signaling, and its activity affects the stability and nuclear entry of Smad proteins. This suggests that indirubin may have an indirect effect on Smad pathway activation and thus inhibit fibrosis-associated signaling (Zhou et al., 2022). Indigo, however, with its close structural similarity to indirubin, exhibits distinct pharmacological activities. Indigo was observed in in vitro studies to inhibit significantly the release of pro-inflammatory factors by macrophages stimulated by lipopolysaccharide and reduce the level of pro-inflammatory mediators such as TNF-α and IL-6 (Zhou et al., 2022). The pathogenesis and etiology of pulmonary fibrosis are intimately related to chronic ongoing inflammation. Aberrant macrophage polarization and overproduction of inflammatory mediators are key microenvironmental components to stimulate fibroblasts. Indigo may therefore interfere in early fibrosis by regulating inflammation (Zhou et al., 2022; Zhang et al., 2022). Neoindole glycosides are new sulfur-containing glucoside constituents in Isatis indigotica. Their hydrolysis products have strong antioxidant activity, which is able to clear free radicals and reduce oxidative stress-induced damage (Huang et al., 2023; Wang et al., 2023).

Oxidative stress not only directly damages alveolar epithelial cells but also may act as an upstream activator of TGF-β1, promoting its expression and activation.

Therefore, it is speculated that neoindole glycosides may indirectly inhibit the fibrotic process by blocking the positive feedback loop between oxidative stress and TGF-β1 (Zhou et al., 2022). Various monomeric components in Folium Isatidis exhibit clear pharmacological effects in terms of anti-inflammatory, antioxidant, and kinase inhibition, but their specific mechanisms of action in the specific pathological model of pulmonary fibrosis have not been systematically elucidated. Current research has mostly focused on the effects of whole extracts, lacking in-depth analysis of individual components at the level of key signaling pathways (Wang et al., 2023; Zhou et al., 2022). The TGF-β1/Smad3 pathway is a core driver of pulmonary fibrosis, controlling the transformation of fibroblasts to myofibroblasts, epithelial-mesenchymal transition, and abnormal accumulation of extracellular matrix (Kreuter et al., 2021). If effective monomers in Folium Isatidis can target key nodes in this pathway, inhibiting TGF-β1 receptor kinase activity, preventing Smad3 phosphorylation, or interfering with the entry of the Smad complex into the cell nucleus, it may be possible to curb the malignant progression of fibrosis at its source. There is currently no direct evidence showing that indirubin or other monomeric components in Isatis indigotica can specifically affect the phosphorylation process of Smad3. This research gap provides a key research direction for understanding how Chinese medicine monomers intervene in pulmonary fibrosis at the molecular level (Zhou et al., 2022). Based on the known inhibitory effects of Isatis indigotica monomers on kinases and the possible mutual regulatory relationships between related pathways, further research on the effects of these active ingredients on the TGF-β1/Smad3 signaling pathway is a scientifically based issue that deserves in-depth exploration (Luo et al., 2025; Zhou et al., 2022).

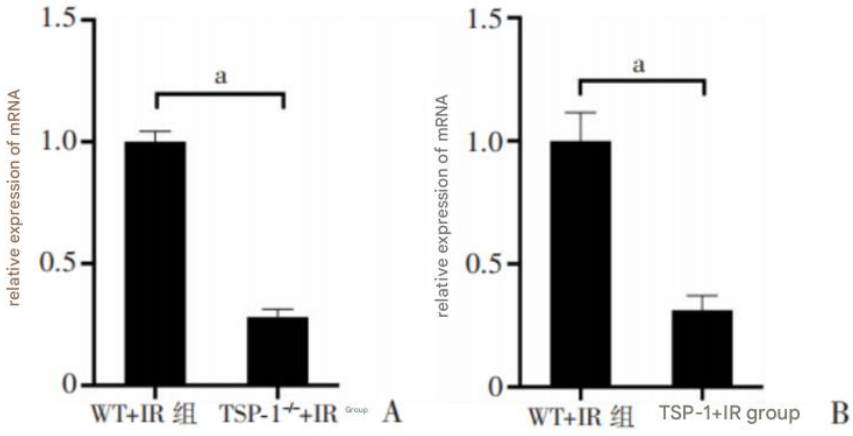
2. TGF-β1/Smad3 signaling pathway and pulmonary fibrosis mechanism

Transforming growth factor-β1 (TGF-β1) is a central cytokine in pulmonary fibrosis, and its overexpressed level is directly associated with the abnormal overaccumulation of extracellular matrix (ECM) (Kreuter et al., 2021). In experimental models of various lung injuries, TGF-β1 is secreted continuously, and it triggers its downstream canonical signaling pathway. Phosphorylation of Smad3 is

among the essential steps in the action of this pathway. Phosphorylated Smad3 (p-Smad3) and Smad4 combine as a complex and migrate into the cell nucleus. As a transcriptional co-activator, they bind to the promoter region of many pro-fibrotic genes, and directly cause the transcription and expression of key molecules such as type I collagen (Col1a1), fibronectin and α-smooth muscle actin (α-SMA) (Kreuter et al., 2021). This series of molecular reactions promotes the transformation of lung fibroblasts into myofibroblasts and the abnormal accumulation of ECM. Researchers can use quantitative molecular biology methods to accurately detect the activation of this pathway. In a study on the mechanism of pulmonary fibrosis, quantitative real-time fluorescence PCR (qRT-PCR) was used to analyze mouse lung tissue and found that the mRNA expression levels of TGF-β1 and Col1a1 in the model group were significantly higher than those in the control group, which was statistically significant (Jiao et al., 2023).

Figure 1

Differences in mRNA expression of TGF-β1 (A) and Collagen I (B) in lung tissues of mice in different groups (n = 6)



a：$P<0.01$

Figure 1 clearly demonstrates the positive correlation between activation of the TGF-β1 signaling pathway and the synthesis of major ECM components. As a major building block of collagen fibers, upregulation of Col1a1 mRNA levels is a molecular event occurring early in fibrosis and serves as the material basis for subsequent histopathological changes. The profibrotic effects of the TGF-β1/Smad3 signaling pathway are not limited to directly promoting ECM synthesis. This pathway also inhibits normal ECM degradation by inhibiting the activity of matrix metalloproteinases (MMPs) and upregulating the expression of their tissue inhibitors

(TIMPs), leading to increased net ECM deposition (Kreuter et al., 2021). This dual imbalance between synthesis and degradation accelerates scarring in the lung interstitium. Furthermore, this signaling axis can induce epithelial-mesenchymal transition (EMT) in alveolar epithelial cells, causing them to lose polarity and acquire the migratory and secretory properties of mesenchymal cells, further expanding the myofibroblast pool (Kreuter et al., 2021). In fibrotic lung tissue, nuclear localization of p-Smad3 often colocalizes with expression of EMT markers such as vimentin, suggesting a close regulatory link between the two. TGF-β, a major pro-fibrotic stimulus, is directly amplified by the nucleocapsid protein of SARS-CoV-1. Since the nucleocapsid proteins of SARS-CoV-2 have a 90% similarity to SARS-CoV-1, it can be hypothesized as one of the possible mechanisms for lung fibrosis. TGF-β, along with connective tissue growth factor, is also upregulated by angiotensin II, which gets accumulated in the lungs due to the downregulation of ACE-2 caused by the virus (Mohammadi et al., 2022). A more complex regulatory network involves the interaction between this pathway and cellular autophagy. As an important mechanism for maintaining intracellular homeostasis, impaired autophagy can lead to the accumulation of damaged organelles and persistent inflammation, thereby promoting fibrosis. Studies have shown that TGF-β1 signaling can inhibit autophagic flux, while inhibition of the TGF-β1/Smad3 pathway can restore autophagic activity, manifested as an increase in the LC3-II/LC3-I ratio and accelerated degradation of p62 protein (Jiao et al., 2023; Zhou et al., 2022). This mechanism of indirectly affecting the process of fibrosis by regulating cellular autophagy further highlights the multi-effects of TGF-β1/Smad3 as a core hub. Therefore, this pathway drives the irreversible progression of pulmonary fibrosis through multiple pathways such as directly regulating ECM metabolism, inducing cell phenotypic transformation, and interfering with cell protective mechanisms, making it an extremely attractive target for therapeutic intervention (Karampitsakos et al., 2023; Kreuter et al., 2021, Pitre et al., 2022).

3. Conclusion

The active monomer components of Folium Isatidis can alleviate the progression of pulmonary fibrosis by inhibiting the overactivation of the TGF-β1/Smad3 signaling pathway. This mechanism of action provides a theoretical basis and potential

candidate molecules for the development of new anti-pulmonary fibrosis drugs based on natural products (Luo et al., 2025; Wang et al., 2023; Zhou et al., 2022).

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