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Review

Regulation effect of the intestinal flora and intervention strategies targeting the intestinal flora in alleviation of pulmonary fibrosis development

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Pulmonary fibrosis is an end-stage respiratory disease characterized by fibroblast proliferation and accumulation of extracellular matrix and collagen, which is accompanied by inflammatory damage. The disease is mainly based on pulmonary dysfunction and respiratory failure, the incidence of it is increasing year by year, and the current treatment methods for it are limited. In recent years, it has been found that gut microbes play a crucial role in the pathogenesis and development of pulmonary fibrosis. The microecological disturbance caused by changes in the composition of the intestinal flora can affect the course of pulmonary fibrosis. The regulatory network or information exchange system for gut-lung crosstalk is called the "gut-lung axis". This review focuses on the frontier research on entero-pulmonary regulation in pulmonary fibrosis and on intervention strategies for changing the gut microbiota to improve pulmonary fibrosis, including fecal microbiota transplantation, traditional Chinese medicine interventions, and supplementation with probiotics. In addition, the present problems in this field are also raised in order to provide strong theoretical and strategic support for the future exploration of regulatory mechanisms and therapeutic drug development. This paper reviews the interaction of the intestinal flora with pulmonary fibrosis, introduces the research progress for improving pulmonary fibrosis through interventions targeted at the intestinal flora, and provides new ideas for the treatment of pulmonary fibrosis.

Key words: intestinal flora, pulmonary fibrosis, gut-lung axis, intervention strategies, fecal microbiota transplantation, traditional Chinese medicines, probiotics

INTRODUCTION

Pulmonary fibrosis (PF) is a serious chronic fibrotic disease with interstitial injury. It is characterized by the proliferation of fibroblasts and the accumulation of a large amount of extracellular matrix, accompanied by inflammatory damage and tissue structure destruction, eventually resulting in pulmonary dysfunction [1]. Many lung injury diseases are often accompanied by pulmonary fibrosis and respiratory dysfunction symptoms, indicating that PF is not an independent disease. Generally speaking, diseases characterized by diffuse parenchyma, alveolar inflammation and interstitial fibrosis are referred to as interstitial lung disease (ILD) [2]. ILD is divided into two types, secondary interstitial lung disease and idiopathic interstitial lung disease [3]. The former has a relatively clear etiology, including pneumoconiosis, silicosis, asbestosis, radiation-induced PF, and drug-induced pulmonary interstitial fibrosis. In contrast, the latter has an unclear etiology, including cystic pulmonary fibrosis (CPF), interstitial pneumonia with autoimmune features (IPAF), and idiopathic pulmonary fibrosis (IPF) [2]. Due to the complex pathogenesis and short median survival after diagnosis, there is lack of effective treatments currently. If PF is not controlled promptly and effectively, it will lead to a decline in lung function and seriously affect the quality of life and life expectancy of patients [4]. Therefore, it is important to explore and determine the pathogenesis and immune regulation of PF in order to explore new therapeutic strategies and therapeutic targets for this disease.

The intestinal flora is located in the gastrointestinal tract of the host, which participates in a variety of important physiological functions, such as by affecting the nutritional metabolism of the organism, regulating the development and maturation of the immune system, and playing an antibacterial role. The human

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intestinal flora not only affects the function of intestinal system but also affects the function of remote organs, such as the brain, liver, and lungs. In recent years, it has been found that the intestinal flora plays an important role in immune regulation in various lung diseases, such as PF, and therefore plays a crucial role in maintaining lung health, which has become a new frontier in lung-related disease research [5, 6].

In this review article, the correlation and immune regulation of the intestinal flora in PF are first summarized. Then, based on the latest progress of intervention strategies for PF targeted at the intestinal flora, the current situations of research on the biological function and mechanism of intestinal flora regulation of PF disease are systematically expounded. Finally, the present problems in this field are also raised in order to provide strong theoretical and strategic support for the future exploration of regulatory mechanisms and therapeutic drug development for the treatment of PF.

RELEVANCE BETWEEN THE INTESTINAL FLORA AND PF: GUT-LUNG AXIS

The intestinal flora has a variety of physiological functions in the human gut, including fermenting non-digestible food components into absorbable metabolites, synthesizing essential vitamins, removing toxic compounds, defending against pathogens, strengthening the intestinal barrier, and stimulating and regulating the immune system [7]. These microorganisms indirectly play a role in controlling the physiological state of the host by controlling or influencing the activity of intestinal bacteria [8]. Intestinal microorganisms play an important role in maintaining host microecological homeostasis and affecting the progression of various diseases; that is, intestinal microorganisms not only affect gastrointestinal function but also affect the physiological characteristics of other important organs, such as the liver, brain, kidneys, and lungs. Among these organs, the regulatory network or information exchange of the crosstalk between the gut and the lung is designated as the "gut-lung axis" [9]. That is to say, the changes in the composition and function of the intestinal flora affect the respiratory system through the common mucosal immune system; at the same time, disturbance of the respiratory tract flora can also affect the function of the digestive tract through immune regulation. Although research on the gut-lung axis is still in its infancy, more and more evidence shows that the intestinal flora has great potential value in the treatment of lung diseases [10].

Research has proven that the richness and diversity of the intestinal flora in CPF patients are significantly decreased compared with those of healthy people [11, 12]. Specifically, the abundances of *Staphylococcus*, *Streptococcus*, and *Veillonella* in CPF patients are significantly increased, while the abundances of *Bacteroides*, *Bifidobacterium adolescentis*, and *Clostridium praxis* are significantly decreased [13]. In mouse models, it has been found that loss of the cystic fibrosis transmembrane regulator (CFTR) gene led to a decrease in the abundances of *Lactobacillus* and *Acinetobacter lwoffii* and an abnormal increase in pathogenic bacteria such as *Mycobacterium* and *Bacteroides fragilis* [14]. Multiple cross-sectional studies have shown that the gut flora in CPF patients is closely related to lung function, as well as disease deterioration [15, 16]. Progressive PF caused by silica exposure in the occupational environment is the main pathological form of silicosis. The intestinal microflora in early silicosis patients shows significant changes compared with that of healthy people [17]. Bacterial 16S rRNA gene sequencing results indicate that the number of operational taxonomic units (OTUs) and Shannon diversity index significantly decrease in silicosis patients. Meanwhile, the relative abundances of Proteobacteria and Verrucomicrobia significantly increase, and those of Firmicutes and Actinobacteria significantly decrease [17].

There have also been animal experiments to elucidate the close connection between gut and lung tissues. In mouse models of PF induced by bleomycin and silica dust, stool bacterial 16S rRNA gene sequencing results showed that 412 genera of intestinal bacteria and 26 metabolites in PF mice were significantly different from those in the control group [18]. Specifically, the abundances of *Prevotella*, *Helicobacter*, and *Rikenella* were significantly decreased, while the abundances of *Dubosiella* and *Parasutterella* were significantly increased.

In general, studies on the correlation and regulatory mechanisms between PF and the intestinal flora are still in the exploratory stage, and there are few relevant literature reports, so in-depth and systematic studies are needed. Nevertheless, the aforementioned studies demonstrate the reliability of using specific gut flora and metabolites as biomarkers for the pathological process of PF.

THE REGULATORY MECHANISM OF THE GUT-LUNG AXIS

The regulatory function between the gut and lung is reflected at three levels: the microbial flora, immune function, and metabolite regulation (Fig. 1). The microbial floras that colonize the mucosal tissues of the digestive system and respiratory system represent an important biological material basis for the gut-lung axis to play a regulatory role and strengthen the connection between the lung and intestine. Human intestinal bacteria can affect and maintain the homeostasis of the body by regulating the immune response of the digestive system and distal organs [19, 20]. Changes in the population proportions and metabolites of the intestinal flora are directly related to lung inflammation and the immune response, which in turn affect the course of lung disease [21, 22].

DIRECT EFFECT OF THE INTESTINAL FLORA AND LUNG TISSUE

Soluble microbial components in the gut carry out "information exchange" between intestinal microbes and lung tissue by means of circulation transport. In mice treated with antibiotics, the injection of lipopolysaccharide (LPS), a component of the bacterial cell wall, can induce and restore the ability of influenza virus-infected mice to produce immune effects in the rectum [23]. In an asthma model of mouse, intestinal microbiota-derived LPS significantly affects the lung's ability to respond to allergens [24]. In CPF patients, the decreases in the diversity and abundance of intestinal flora caused by antibiotics significantly worsen the symptoms of PF, which may be related to cell dysfunction caused by CFTR mutation [25, 26]. In short, changes in the population structure and colonization location of the intestinal flora can lead to lung diseases.

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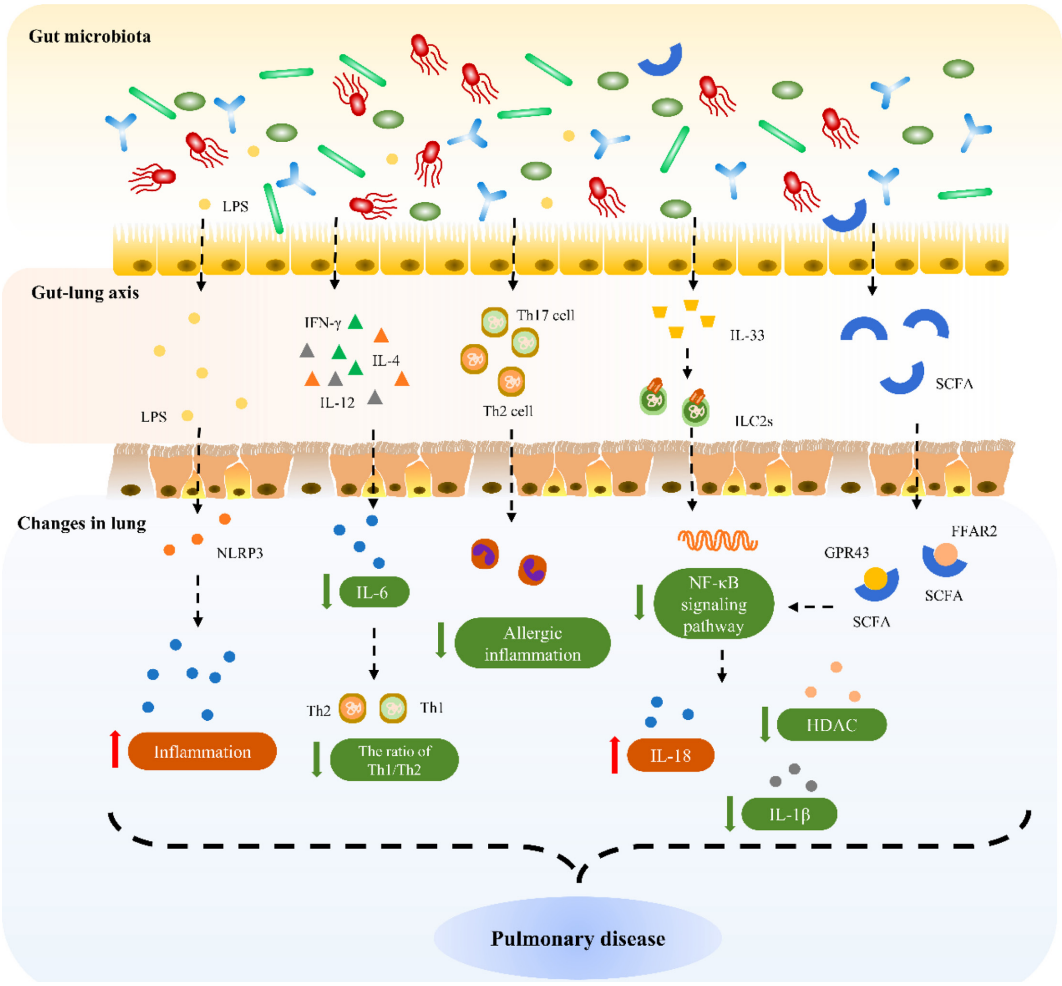

Fig. 1. Overview of the mechanism of modulation of the gut microbiota in pulmonary fibrosis.
LPS: lipopolysaccharide; SCFA: short-chain fatty acid; FFAR2: free fatty acid receptor 2; GPR43: G protein-coupled receptor 43; NLRP3: NOD-like receptor thermal protein domain associated protein 3.

The intestinal flora affects lung function through immune regulation

Studies have shown that the intestinal microbiota can affect the immune system and influence lung diseases. For example, segmented filamentous bacteria in the gut can stimulate the body to produce Th17 immune cells, which can reduce the infection rate and mortality of *Streptococcus pneumoniae* infection [27, 28]. In mouse experiments, intestinal enrichment of *Lactobacillus johnsonii*, induced by indoor dust exposure, can affect the immune response of distal lung mucosal tissue by significantly reducing Th2 cell-mediated airway allergic inflammation [29]. Disturbance of the intestinal flora also can lead to changes in immune cells. For example, treatment with ampicillin resulted in a significant increase in the abundance of Proteobacteria in the gut and the level of IL-33 [30]. The receptor of IL-33 is growth stimulation expressed gene 2 (ST2) protein. Blocking the ST2 receptor pathway can inhibit the function of natural group 2 innate lymphoid cells (ILC2s) circulating from blood to the lung. As a result, the accumulation of ILC2s in the lung is reduced, and the NF-κB signaling-mediated inflammatory damage is exacerbated in lung tissue.

The intestinal flora affects lung function through its metabolites

Microbial metabolites can also be sensed and recognized to exert a protective effect on tissues. For example, short-chain fatty acids (SCFAs), important metabolites in the gut microbiota, not only provide an energy supply for colon cells but also regulate the immune response of intestinal tissue and maintain the balance of intestinal microecology [31]. In addition, SCFAs can also bind to G protein-coupled receptor 43 (GPR43) and free fatty acid receptor 2 (FFAR2) on the cell membrane and activate downstream mitogen-activated protein kinases (MAPKs) and other cell signaling pathways, resulting in function changes in the auxiliary T cell and dendritic cell [32, 33]. Butyric acid, a metabolite of a high-fiber diet, enters cells via specific monocarboxylic acid transporters, inhibits the activity of histone deacetylase (HDAC), and causes the proliferation of anti-inflammatory macrophages in the lung after entering into cells. In addition, the secretion of chemokine CXCL1 is down-regulated, significantly improving the pulmonary allergic inflammation and symptoms of influenza virus infection [31, 34, 35]. Other metabolites produced by the intestinal flora, such as bile acids, indole derivatives, niacin, pyruvate, and lactic acid, also have anti-inflammatory and anti-infection activities, indicating that they may play regulatory roles

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in lung injury [36]. For example, *Clostridium sporogenes* can decompose flavonoids into desaminotyrosine, and the addition of desaminotyrosine enhances type I interferon immune signaling conduction and activates phagocytosis by macrophages to suppress influenza virus infection [37].

In CPF, the imbalance of various physiological and biochemical reactions caused by the dysregulation of CFTR protein expression in intestinal epithelial cells may be an important reason for the abnormal intestinal flora [38]. The physiological processes involved in CFTR-related mechanisms include the production of thick mucus caused by chloride channel obstruction, reduced carbonate secretion that changes the intestinal pH value, delayed intestinal transport, aggravated intestinal inflammation, destruction of the intestinal immune barrier function, and other factors [14, 26, 39, 40]. Different pathological states of the body are accompanied by specific changes in the intestinal flora [41]. For example, the abundance of *Coprococcus* n the gut is strongly correlated with SCFA metabolite levels in healthy people, but in the guts of CPF patients, the abundance of *Clostridium* is associated with SCFAs, and a significant increase in *Enterococcus* abundance in some patients leads to a large amount of lactic acid accumulation and a decrease in SCFA synthesis [40].

TREATMENT OF PF BY TARGETING THE INTESTINAL FLORA

Given the close relevance of the intestinal flora with respect to lung function, attempts have been made to treat PF by directly or indirectly targeting the intestinal flora. These treatments have included fecal microbiota transplantation (FMT), traditional Chinese medicines, and complementary probiotics (Table 1).

FMT

FMT is a type of treatment in which functional bacteria from normal feces are transferred to the gastrointestinal tract, and this has been applied for the treatment of PF. Studies have shown that FMT increases the number of OTUs in the pulmonary flora, and decreases the number of OTUs in the intestinal flora. Specifically, FMT down-regulates *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Sphingobium*, *Pseudomonas*, *Lactobacillus*, and *Acinetobacter*, thus maintaining the balance of the pulmonary flora [42]. In terms of therapeutic research, the pulmonary inflammation symptoms of antibiotic-treated *S. pneumoniae*-infected mice can be significantly reduced through bacteria transplantation from the feces of healthy mice [31]. However, the risk of pathogenic bacteria contamination, which can increase the occurrence of immune-related adverse events, may exist in the use of flora transplantation. Therefore, in clinical practice, we should pay attention to the safety and quality control of flora transplantation and, at the same time, prevent and reduce the occurrence of adverse events as much as possible while enhancing efficacy.

Traditional Chinese medicines

Chinese herbal prescriptions and single Chinese herbs have both been proven to improve lung tissue damage by adjusting lung-gut mucosal immune function. For example, *Amygdalus mongolica* oil therapy appears to confer protective effects against PF by affecting the level of metabolites and the abundances of related intestinal flora [43]. A *Qingwen Gupi* decoction (QGT) is a mixture of *Shengjiang* powder and a *Xiao Chaihu* decoction, which can alleviate bleomycin-induced PF by reversing gut dysbiosis in a rat model [44]. A *Qi-Long-Tian* (QLT) capsule, consisting of *San Qi* (*Notoginseng Radix* et Rhizoma), *Di Long* (*Pheretima aspergillum* (*E. Perrier*)), and *Hong Jingtian* (*Rhodiolae Crenulatae Radix* et Rhizoma) [45], intervenes in PF by regulating the differential genera of the intestinal flora, repairing the intestinal mucosal barrier, reducing LPS entry into the blood, and decreasing inflammatory factor secretion in the serum [46]. Monomers derived from Chinese medicine have also been applied for the attenuation of PF. Astragalus polysaccharide (APS) is a naturally occurring compound derived from *Astragalus membranaceus* with anti-inflammatory and antioxidant properties [47]. APS ameliorates lung tissue injury in the IPF mouse model by regulating the metabolism of gut microbiota, elevating the beneficial bacteria content, and inhibiting the TLR4/NF-κB signaling pathway [48].

Table 1. Intervention strategies for the alleviation of pulmonary fibrosis development via gut microbiota modulation

Intervention strategies		Intestinal microbiota modulation	Effects on related biomarkers	References
Fecal microbiota transplantation		↓OTU value, ↓*Firmicutes*, ↓*Proteobacteria*, ↓*Bacteroidetes*, ↓*Sphingobium*, ↓*Pseudomonas*, ↓*Lactobacillus*, ↓*Acinetobacter*	ND	[42]
Traditional Chinese medicines	*A. mongolica* oil	↑*Duncaniell*, ↑*Desulfovibrio*, ↑*Peptococcaceae_unclassified*, ↑*Dubosiella*, ↑*Tyzzerella*, ↑*Lachnospiraceae_NK4A136_group*, ↑*Lactobacillus*, ↑*Clostridiales_unclassified*	↑tetrahydrobiopterin, ↑L-serine, ↑citrulline, ↑estradiol	[43]
	Qingwen Gupi decoction	↓*Lachnospiraceae_NK4A136_group*, ↓*Escherichia-Shigella*, ↑*Alistipes*, ↑*Ruminiclostridium*, ↓*Odoribacter*, ↑*Romboutsia*, *Acetatifactor*, ↑*Ruminococcaceae_UCG-005*, ↑*Roseburia*	↓HA, ↓LN, ↓COL-IV, ↓COL-III, ↓IL-1*β*, ↓IL-6, ↑15-HETE, ↓TGF-*β*1, ↓Smad3	[44]
	Qi-Long-Tia capsule	↑*Bacteroidia*, ↓*Clostridia*	↓IL-1β, ↓IL-6, ↓TNF-α, ↓TGF-β, ↓LPS, ↑ZO-1, ↑Claudin, ↑Occludin, ↑sIgA, ↑SCFAs	[46]
	Astragalus polysaccharide	↑*Bacillus*, ↑*Lactobacillus*, ↑*Akkermansia*, ↓*Lachnoclostridium*, ↓*Faecalibaculum*, ↓*Clostridium*, ↓*Erysipelatoclostridium*	↓TNF-α, ↓IL-6, ↓IL-1β, ↑Bax, ↓Bcl-2	[48]
Probiotics extra addition	*Limosilactobacillus reuteri*	↓Proteobacteria, ↑Firmicute, ↑Bacteroidetes	↓IL-8, ↓TNFα, →IL-1β, ↑IL-12p70	[51]

↑: The abundance of the intestinal flora, the content or expression of related biomarkers increased after intervention; ↓: The abundance of the intestinal flora, the content or expression of related biomarkers decreased after intervention; →: The changes in the abundance of the intestinal flora, the content or expression of related biomarkers have no significant difference; ND: Not detected.

However, the current research on the mechanisms of traditional Chinese medicine mainly focuses on the changes in the expression levels of secreted cytokines and the number of immune cells, and in-depth studies on changes in immune cell function in mucosal systems and changes in the local microecological components of the lung-gut axis are lacking.

Supplementation with probiotics

Probiotics are kinds of active microorganisms that are beneficial to the host by changing the composition of the flora in a certain part of the host. In recent years, the regulation of lung diseases through the administration of probiotics has become a hot field. Experimentation on animals has proven that the probiotics *Bifidobacterium bifidum* and *Escherichia coli* Nissle 1917 (ECN) can reduce the inflammatory damage in lung tissue of mice. Specifically, *B. bifidum* causes changes in the balance of Th1/Th2 cells and down-regulates the production of IL-6 factor in lung [49], while ECN significantly reduces the response levels of Th2 and Th17 cells in mice with papain-induced lung injury [50]. Randomized controlled trials have also shown that regulation of the intestinal flora by administration of probiotics can significantly improve the pathological status of CPF patients [19]. For example, the addition of *Lactobacillus* can increase the diversity of the intestinal flora and decrease the total bacterial density, and this is accompanied by a significant increase in Firmicutes and a decrease in the proportion of Proteobacteria. This results in a significant decrease in the level of fecal calvetin in the intestine, which has a significant effect on the deterioration of risk control and quality of life assurance in cystic fibrosis patients [51–53].

CHALLENGES AND FUTURE PERSPECTIVES

PF is a highly fatal and progressive pulmonary interstitial disease caused by multiple environmental factors, and it can present symptoms of various respiratory diseases in its pathological process [2, 54–57]. There is growing evidence that there is an important and complex way of exchanging biological information between the intestine and lungs, that is, the gut-lung axis, similar to the relationship between the gut flora and host immunity. So far, the research on the mechanism between the gut and lung has reflected three levels, the microbial flora, immune function, and metabolite regulation; however, the regulatory mechanism still needs to be further elucidated. At present, there have been some experimental reports on strategies for targeting and regulating the intestinal flora in PF treatment by using interventions such as FMT, traditional Chinese medicines, and probiotics (Fig. 2). The intervention strategies targeting the intestinal flora for the alleviation of PF development are still in the initial stage of exploration, and future studies should focus on determining the causal relationship between PF and changes in the intestinal flora, elucidating the mechanism of bidirectional regulation between lung tissue and intestine, and exploring new therapeutic strategies for targeting and regulating the intestinal flora in PF.

The intestinal fungal microbiota as a novel therapeutic target

Beyond bacteria, the human gastrointestinal tract is also home to fungi, which also play crucial roles in human intestinal homoeostasis and disease pathogenesis [58]. Dysregulation of the intestinal fungal community is associated with many diseases, including autoimmune, metabolic, and neurological diseases and cancer [59–62]. However, gut fungi have remained largely neglected in the development of microbiome-based therapies, and the results of existing fungal studies have not been fully utilized in clinical practice. The reciprocal relationship between the gut fungal community and bacterial community is essential for human health, and in the future, it is very possible to use gut fungal communities as part of a precision medicine approach for disease diagnosis and therapeutic intervention. On the basis of the intestinal fungal flora and bacterial flora as well as human pathophysiology and clinical knowledge, future microbial targeted therapies should be directed toward the synergistic regulation of intestinal fungi and bacteria, as this will have the effect of improving clinical efficacy. Yamada *et al.* found that the intestinal overgrowth of *Candida albicans* exacerbates PF via the IL-17A-mediated endothelial–mesenchymal transition [63]. Thus, *C. albicans* might be a potential therapeutic target in the treatment of PF.

Engineered probiotics as a novel therapeutic strategy

Probiotics have been proven to alter the host gut microbiome, which has significant therapeutic effects on PF [64]. However, due to limited understanding of the complex mechanisms associated

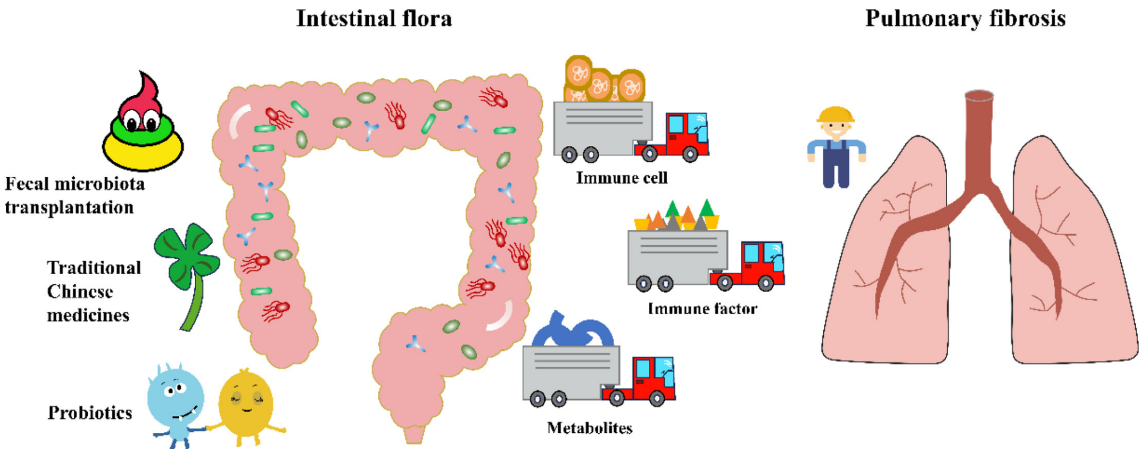

Fig. 2. Strategies targeting the intestinal flora for the treatment of pulmonary fibrosis.

with pulmonary disease, the efficacy of a single probiotic supplement strategy may be inconsistent. Synthetic biology offers a straightforward approach to the targeted design of probiotics to enable targeted drug delivery and restore homeostasis within disturbed microbial communities [65]. Engineering probiotics refers to the modification of existing probiotics through gene editing based on the theory of synthetic biology to obtain the needed probiotics. Scott *et al.* developed an engineered yeast for the treatment of inflammatory bowel disease (IBD) that expresses human P2Y2 purinergic receptors, thereby creating molecules that can sense pro-inflammatory molecules and self-regulate neutralizing pro-inflammatory molecules [66]. These self-regulating yeast probiotics inhibited intestinal inflammation in IBD mouse models and reduced intestinal fibrosis and dysbiosis. An engineered ECN was also developed to convert metabolic waste produced by tumors into L-arginine, thereby enhancing the therapeutic effect of programmed cell death protein-1 and its ligand inhibitors on mouse tumors [67]. Although research on engineering probiotics is still in the exploratory phase, the field is rapidly developing and is expected to provide more options for the prevention and treatment of PF in the future.

CONCLUSION

There is growing evidence that there is an important and complex way of exchanging biological information between the intestine and lung. PF is a highly fatal and progressive interstitial lung disease caused by multiple environmental factors. Based on the gut microbiota and its metabolites, multiple strategies for the treatment and prevention of PF have emerged, including FMT, traditional Chinese medicine interventions, and supplementation with probiotics. However, at present, it is difficult to identify key metabolites and their regulatory pathways in the regulatory mechanism of the gut flora and PF. Therefore, the specific mechanism of the gut microbiota and PF needs to be further clarified. At the same time, almost all current studies related to PF involve bacteria, while studies on the effects of fungi on PF are limited. Further exploration of fungi will expand the understanding of the relationship between the gut microbiota and PF. Engineered probiotics are a cutting-edge technology that, through gene editing, can alter specific genes in microbial communities or produce pharmacologic proteins and drugs to enhance the function of a certain probiotic, providing new ideas for the treatment and prevention of PF.

AUTHOR CONTRIBUTIONS

L.Y.Y.: Conceptualization, data collection, writing-original draft, formal analysis, funding acquisition. J.Q.G.: Writing-review & editing, supervision, funding acquisition. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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