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Application of Proteomics and Metabolomics in Interstitial Lung Disease Research

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Abstract:

Interstitial lung disease (ILD) is a heterogeneous group of disorders characterized by pathological changes such as inflammatory infiltration, fibrosis, and cell proliferation involving the lung interstitium. The clinical etiology of this disease is not yet fully understood, especially during acute exacerbations, where patients often have a poor prognosis. The combined use of proteomics and metabolomics holds promise as a powerful tool for screening novel biomarkers of ILD. This article reviews the application of proteomics and metabolomics techniques in the study of ILD, aiming to clarify the current research status and provide an outlook for future research directions.

Keywords:

Interstitial lung disease
Proteomics
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Disease biomarkers
Pathogenesis
Metabolic pathways

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1. Introduction

Interstitial lung disease (ILD) is a heterogeneous group of disorders characterized by pathological changes in the lung interstitium, including inflammatory infiltration, fibrosis, and cell proliferation [1]. When the etiology of ILD remains unknown, patients are diagnosed with idiopathic interstitial pneumonia (IIP), the most common type being idiopathic pulmonary fibrosis (IPF). IPF is a chronic, progressive, and idiopathic form of ILD with significant variations in its natural history, ranging from chronic stability to progressive respiratory failure or acute exacerbations [2]. The specific cause of acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) remains unclear, but it is defined by an international working group as a new, widespread, acute respiratory deterioration characterized by alveolar abnormalities [3]. Clinical studies have shown that the annual incidence of AE-IPF among ILD patients is 5% to 15% [4], and these patients often have a poor prognosis and high mortality rate [5]. Therefore, there is an urgent need for new methods to improve diagnostic accuracy, evaluate prognosis, and monitor the effectiveness of treatment strategies.

In recent years, systems biology, particularly "omics" research, has provided powerful technical support for the discovery of disease markers and the study of pathogenesis. These techniques, including genomics,

transcriptomics, proteomics, and metabolomics, are based on the analysis of group indicators and are complemented by high-throughput detection and data processing methods. They have been widely used to explore abnormal cellular and molecular mechanisms in the process of pulmonary fibrosis [6]. Among them, proteomics combined with metabolomics analysis can identify differentially expressed proteins and metabolites with synchronous changes. Supported by bioinformatics analysis, this approach can rapidly determine changes in key pathways, screen for relevant biomarkers, and explore the pathogenesis of diseases [7]. Therefore, multi-omics research combining proteomics and metabolomics has the potential to become a powerful tool for studying ILD [8,9]. This article reviews the application of proteomics and metabolomics in ILD research, aiming to clarify the current research status and provide insights into future research directions.

2. Proteomics

2.1. Overview of proteomics

Proteomics is a high-throughput detection technique that allows for the large-scale and simultaneous quantification of numerous proteins. It enables the exploration of potential relationships between protein biomarkers and disease-specific parameters [10]. There are two main approaches in proteomics research: one is to reveal the core proteins involved in specific symptoms by comparing differences in protein expression across different diseases with similar symptoms; the other is to study the various symptoms exhibited by a particular disease in different patient populations or at different stages of disease progression, and to analyze the relationship between these symptoms and differences in gene and protein expression, thereby revealing the connection between symptom changes and gene/protein expression. Experimental procedures in proteomics include sample collection and preprocessing, protein separation and identification, and bioinformatics analysis. The results of these studies can be validated through specific experimental methods such as enzyme-linked immunosorbent assay (ELISA) and Western blotting (WB). Currently, proteomics techniques have been widely applied in ILD research [11-25], with specific examples presented in **Table 1**.

2.2. Proteomics research in stable idiopathic pulmonary fibrosis

At the beginning of the 20th century, proteomics technology was proposed to be applied in the medical field to explore protein changes in diseases [26]. Among ILD studies, IPF research started earliest and currently has the broadest scope. To screen for biomarkers associated with IPF, Foster *et al.* [11] used bronchoalveolar lavage fluid as a detection sample to compare protein expression differences between IPF patients and healthy controls. They found that proteins such as osteopontin, non-secretory ribonuclease, and platelet basic protein were significantly upregulated in IPF patients, suggesting that these proteins may be related to the progression of pulmonary fibrosis. Another study performed enrichment analysis on differentially expressed proteins in peripheral blood. The results showed that compared to normal human plasma, proteins related to immune defense response, wound healing, and protein phosphorylation were significantly upregulated in the plasma of IPF patients [14]. Based on these findings, researchers believe that impaired host defense is a key hallmark of IPF disease biology, indicating that pulmonary fibrosis is continuously developing.

Additionally, Moodley *et al.* [16] discovered that compared to healthy controls, platelet basic protein expression was upregulated in IPF patients, while the expression of actin, cytoplasmic protein 2, antithrombin III, extracellular matrix protein 1, and fibronectin was downregulated. These differentially expressed proteins are primarily involved in biological processes such as immune defense, inflammation, and platelet activation, further validating the association between the pulmonary fibrosis process and immune response. Tian *et al.* [17] used lung tissue from IPF patients as research samples to explore the pathogenesis of pulmonary fibrosis. Their findings revealed that proteins with larger fold changes were mainly enriched in the PI3K-Akt signaling pathway, focal adhesion pathway, extracellular matrix-receptor interaction pathway, and carbon metabolism pathway.

Furthermore, Saraswat *et al.* [27] identified abnormalities in complement activation and oxidative damage in IPF patients. They proposed haptoglobin-related protein as a novel candidate biomarker for IPF, as this protein has a higher coefficient of variation compared

Table 1. Selected proteomics studies on interstitial lung disease (ILD)

Researchers	Samples	Subjects	Techniques	Validation methods	Key proteins or pathways
Foster *et al.* [11]	BALF	4 IPF patients, 5 healthy controls	2D-LC-MS/MS	MRM	Upregulated: CCL24
Häggmerk *et al.* [12]	BALF, serum	40 patients with sarcoidosis, 17 patients with asthma, 16 healthy controls	IgG reactivity screening	immunohistochemistry	Upregulated in sarcoidosis patients: zinc finger protein 688, mitochondrial ribosomal protein L43.
O'Dwyer *et al.* [14]	Peripheral blood	60 patients with IPF, 21 healthy controls	SomaScan analysis	None	Pathways: immune defense response, wound healing, and protein phosphorylation.
Åhrman *et al.* [15]	Lung tissue	6 patients with IPF, 5 patients with COPD, 5 healthy controls	LC-MS/MS	None	Downregulated type III collagen and laminin
Moodley *et al.* [16]	Plasma		iTRAQ, MIM	None	Upregulated: platelet basic protein; Downregulated: actin, cytoplasmic protein 2, antithrombin III, extracellular matrix protein 1, and fibronectin.
Tian *et al.* [17]	Lung tissue	20 patients with IPF, 20 healthy controls	iTRAQ, LC-MS/MS	WB, immunohistochemistry	Upregulated: galectin, intracellular proteases heat shock protein AA1, and heat shock protein AB1.
Todd *et al.* [18]	Plasma	300 patients with IPF, 100 healthy controls	Aptamer-based platform including 1,305 proteins	None	Pathways: platelet and hemostatic responses, vascular or platelet-derived growth factor signaling, immune activation, and extracellular matrix organization.
Carleo *et al.* [19]	BALF	9 patients with AE-IPF, 18 patients with stable IPF	2DE, PMF	None	
Wu *et al.* [25]	Serum	39 patients with RA-ILD, 36 patients with RA, 42 patients with IPF, 42 healthy controls	SomaScan analysis	ELISA	Pathways: proteolytic enzyme/antiproteolytic enzyme imbalance, clathrin-mediated endocytosis signaling. Upregulated in RA-ILD patients: CCL18, IL-17A, FGF4, and FGF7.

Abbreviations: BALF: bronchoalveolar lavage fluid; 2D-LC-MS/MS: two-dimensional liquid chromatography-tandem mass spectrometry; MIM: multiple reaction monitoring; CCL: chemokine CC subfamily ligand; SomaScan: a new generation proteomics detection platform launched by SomaLogic; COPD: chronic obstructive pulmonary disease; LC-MS/MS: liquid chromatography-tandem mass spectrometry; iTRAQ: isobaric tag for relative and absolute quantitation; 2DE: two-dimensional gel electrophoresis; PMF: peptide mass fingerprinting; RA-ILD: rheumatoid arthritis-associated interstitial lung disease; RA: rheumatoid arthritis; IL: interleukin; FGF: fibroblast growth factor.

to other proteins and demonstrates a two-fold or greater change in a larger healthy population. Finally, a combined proteomics and transcriptomics analysis study found that IPF samples exhibited strong activation of chemotaxis, tumor invasion, and mast cell migration pathways, and downregulation of extracellular matrix degradation [22]. In summary, the progression of IPF is primarily associated with extracellular matrix secretion, immune response, coagulation cascade, and other related processes. Key proteins include osteopontin, platelet basic protein, and extracellular matrix proteins.

2.3. Proteomics research in acute exacerbations of idiopathic pulmonary fibrosis

Compared to the extensive research conducted on IPF during its stable phase, proteomics research on acute exacerbations of IPF (AE-IPF) is relatively limited. Carleo *et al.* [19] proposed that the pathogenesis of AE-IPF involves multiple signaling pathways, including acute-phase response signaling, clathrin-mediated endocytosis signaling, atherosclerosis signaling, macrophage interleukin (IL)-12 signaling, and the production of nitric oxide and reactive oxygen species by macrophages. Furthermore, they discovered that macrophages and their fine-tuned regulation play a central role in the development and progression of AE-IPF. Additionally, receptors involved in lipid metabolism, such as liver X receptors (LXRs) and farnesoid X receptors (FXRs), as well as proteins involved in clathrin-mediated endocytosis signaling, may contribute to the promotion of AE-IPF development. On the other hand, Yamaguchi *et al.* [28] found that high levels of serum high mobility group box 1 protein (HMGB1) are associated with the onset and severity of AE-IPF. As a nuclear chromatin protein, HMGB1 promotes the binding of transcription factors to chromatin and plays a crucial role in the transcription process. Studies have indicated that HMGB1 is released during cell necrosis and by inflammatory cells. Compared to the stable phase of IPF, plasma HMGB1 levels in AE-IPF patients are significantly elevated and associated with reduced survival. Currently, research on differential proteins in AE-IPF is insufficient, and future studies will focus on screening for differential proteins that can serve as biomarkers, exploring their mechanisms, and validating them.

2.4. Proteomics research in other types of interstitial lung disease

Beyond IPF, proteomics techniques have also been widely applied in other types of ILDs. Kjellin *et al.* [13] identified the upregulation of two phagocytic pathways in alveolar macrophages from patients with sarcoidosis: Fcγ receptor-mediated phagocytosis and clathrin-mediated endocytosis signaling. This discovery suggests that an imbalance in oxidative homeostasis may be associated with the increased risk of fibrosis observed in this sarcoidosis phenotype. Other studies have found that proteins involved in intercellular signaling and extracellular matrix proteins are abundant in rheumatoid arthritis-associated ILD. Differential proteins, including cytokines (such as CCL18 and IL-17), chemokines (such as CXCL12 and CCL5), fibroblast growth factors (FGF4 and FGF7), and galectin-3, play roles in fibroblast proliferation, fibrosis formation, and tissue repair [24,25]. Chen *et al.* [29] discovered that patients with ILD induced by idiopathic arthritis are characterized by elevated levels of intercellular adhesion molecule 5 (ICAM-5), matrix metalloproteinase 7 (MMP-7), and eosinophil chemotactic factors, which are all associated with lung injury. Additionally, progressive ILD has been studied. Bowman *et al.* [23] examined plasma proteins from 385 patients with different subtypes of ILD and found that proteins such as urokinase receptor, integrin, smooth muscle cell growth-promoting factor, hepatocyte growth factor, serine protease, and keratin have the strongest correlation with progressive ILD. Furthermore, the pathways associated with progressive ILD primarily involve immune and host responses (such as IL-17 signaling, pattern recognition receptors for bacteria and viruses, granulocyte adhesion, and extravasation) and fibrosis formation (such as liver cholestasis, cardiac hypertrophy signaling, HMGB1 signaling, liver fibrosis pathways, and regulation of epithelial-mesenchymal transition by growth factor pathways). With over 200 subtypes, ILD exhibits variations in protein expression and signaling pathway changes among different subtypes. However, they all share biological processes such as immune-mediated lung injury and fibrosis formation, indicating similar pathogenic mechanisms across different ILD subtypes. It is foreseeable that applying proteomics technology to the study of ILD subtypes holds promising prospects.

3. Metabolomics

3.1. Overview of metabolomics

Metabolomics is the science that studies how the metabolome of biological systems (including cells, tissues, or entire organisms) changes in response to stimuli or perturbations. It focuses on the downstream stage of the central dogma of molecular biology, namely, the characterization of biological functions through metabolites [30]. Metabolomics encompasses all endogenous small molecules (with a relative molecular mass of less than 1,000) involved in metabolism and maintaining the growth, function, and development of organisms, such as amino acids, peptides, carbohydrates, lipids, nucleic acids, and others. Since metabolites are at the terminus of the central dogma, they are widely regarded as dynamic and sensitive measures of disease phenotypes at the molecular level. Therefore, metabolomics is also applied to the exploration and study of biomarkers and mechanisms related to pathophysiological processes [31].

The research process of metabolomics can be roughly divided into three steps: sample pretreatment, metabolite determination, and data processing and analysis. From the perspective of metabolism, we can gain a deeper understanding of the relationship between metabolite changes, metabolic pathways, and metabolic capabilities, and ILD. This helps to explore significantly changed metabolites, potentially involved signaling pathways, and related targets, providing a basis for elucidating the pathogenesis of ILD and identifying new therapeutic targets in later stages. Currently, a series of studies have employed metabolomics techniques to explore the pathogenesis of ILD, screen for biomarkers, and assess prognosis [32-40]. Specific examples are shown in **Table 2**.

3.2. Metabolomics research in the stable phase of idiopathic pulmonary fibrosis

Initial studies found that during lung structural remodeling, metabolic pathways related to energy consumption undergo changes, suggesting that metabolites may be involved in the progression of pulmonary fibrosis in IPF [35]. Further research revealed that the reprogramming of glycolysis plays a critical role in the formation and development of pulmonary fibrosis, promoting fibrosis progression by activating fibroblasts [32,33].

Pyruvate kinase M2 (PKM2) is a key enzyme that

Table 2. Selected metabolomics studies on ILD

Researchers	Samples	Methods	Metabolic pathways	Biomarkers
Xie *et al.* [32]	Lung tissue	Real-time cellular metabolism monitoring	Glycolysis	
Kang *et al.* [33]	Lung tissue	GC-MS	Glycolysis/Mitochondrial β-oxidation	
Yan *et al.* [34]	Plasma	LC-MS	Lipid metabolism	Six lipids
Zhao *et al.* [35]	Lung tissue	GC/LC-MS	Lipid metabolism/Glycolysis/ Tricarboxylic acid cycle	
Bindlisbacher *et al.* [36]	Serum	LC-MS	Lipid metabolism	Lysophosphatidylcholine
Gaugg *et al.* [37]	Exhaled breath	UHPLC-MS/MS	Amino acid metabolism	Six amino acids including proline
Kim *et al.* [38]		LC-MS/MS	Lipid metabolism	Stearic acid
Nambiar *et al.* [39]	Lung tissue	GC-MS	Lipid metabolism	Triglycerides, Phosphatidylcholine
	Plasma	LC-MS	Lipid metabolism	
Nambiar *et al.* [40]	Plasma	LC-MS	Lipid metabolism	Four lipids including palmitoleic acid

Abbreviations: GC-MS: Gas Chromatography-Mass Spectrometry; GC/LC-MS: Gas Chromatography/Liquid Chromatography-Mass Spectrometry; UHPLC-MS/MS: Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry.

catalyzes the final step of glycolysis. Its tetrameric form is a highly active pyruvate kinase that regulates glycolysis and oxidative phosphorylation, while its dimeric form is a protein kinase that initiates gene transcription through nuclear translocation. This dual functionality allows PKM2 to promote cell proliferation through both metabolic and non-metabolic pathways. Gao *et al.* [41] reported that PKM2 enhances the transforming growth factor-β1 (TGF-β1) signaling pathway by directly interacting with SMAD7, thereby promoting fibrosis progression.

Besides glucose metabolism, molecules involved in lipid metabolism have also been found to participate in the process of pulmonary fibrosis. Kim *et al.* [38] observed systemic changes in lipid components, particularly significant alterations in free fatty acid levels, with a notable decrease in stearic acid levels, in IPF patients. Researchers hypothesize that stearic acid may exert an anti-fibrotic effect by downregulating the secretion of profibrotic factors.

Serum amyloid A (SAA) is an apolipoprotein primarily produced by activated monocytes and the liver, which is considered an important marker of systemic inflammatory response [42]. SAA promotes fibrosis by regulating inflammatory responses and lipid metabolism, potentially offering clinical value for the early diagnosis and prognostic evaluation of IPF [43].

Significant differences in amino acid metabolism exist between IPF patients and healthy individuals. Studies have identified six amino acids, represented by proline, that show the most pronounced differences [37]. As one of the main components of collagen and elastin, proline may promote the fibrosis process by influencing collagen secretion.

3.3. Metabolomics research in the acute exacerbation phase of idiopathic pulmonary fibrosis

Currently, metabolomics research on ILD metabolites mainly focuses on the stable phase of ILD, while research on metabolic changes during acute exacerbations of ILD remains inadequate. Nambiar *et al.* [39] explored changes in blood lipids in the plasma of patients with stable and progressive IPF, finding differences in triglycerides and phosphatidylcholine between progressive and stable IPF patients. These differential metabolites are associated with mitochondrial β-oxidation pathways and abnormal lipid metabolism.

Yang *et al.* [44] employed a combined proteomics and metabolomics analysis to compare the serum of patients in the stable and progressive phases of pulmonary fibrosis following novel coronavirus infection. The results revealed associations between differential proteins and metabolites, involving key signaling pathways such as Fcγ receptor-mediated phagocytosis, peroxisome proliferator-activated receptor (PPAR) signaling, tryptophan-inflammation pathway, and urea cycle. Notably, a link was discovered between pulmonary fibrosis and dysfunction of the urea cycle metabolic pathway, identifying D-arginine and D-ornithine as potential biomarkers for early disease warning and progression assessment. However, this study did not include *in vitro* or *in vivo* validation. The integrated proteomics and metabolomics analysis of the acute exacerbation phase of IPF remains an area of research with broad prospects.

3.4. Metabolomics research in other types of interstitial lung disease

Metabolomics techniques have also been applied to other types of ILD. Mirsaeidi *et al.* [45] investigated metabolite changes in patients with fibrotic pulmonary nodule disease, finding significant differences in metabolites between the experimental group and the control group, primarily in the collagen pathway, especially the arginine-proline pathway. Among these, β-coumaroylguanidine butylamine and palmitoyl carnitine may serve as biomarkers for fibrotic pulmonary nodule disease.

Another multi-omics study combining metabolomics and transcriptomics revealed significant changes in the expression of metabolites such as lactic acid, pyruvic acid, and proline in the serum, exhaled breath condensate, and bronchoalveolar lavage fluid of patients with hypersensitivity pneumonitis. These changes may be related to the pathogenesis of the disease [46]. Different subtypes of non-IPF ILD have diverse etiologies, and there are significant differences in metabolic states among patients with different subtypes of ILD. Therefore, future research should focus on specific subtypes of ILD.

4. Conclusion and prospects

The core of proteomics research lies in the large-scale investigation of protein expression differences at the overall level, while metabolomics focuses on exploring the characteristics and inherent laws of each metabolic component. The combination of these two omics technologies provides a comprehensive approach to studying changes in proteins and metabolic pathways. By systematically describing the characteristic changes of proteins and metabolites in disease states, these two technologies play a crucial role in identifying biomarkers and elucidating pathological mechanisms. Previous studies have revealed, to some extent, a series of biomarkers and signaling pathways related to the occurrence and development of ILD. However, the pathogenesis of ILD is not yet fully understood, and the development of therapeutic drugs is still in its infancy, requiring further in-depth research.

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