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EXPLORING PULMONARY ALVEOLAR PROTEINOSIS DISEASE GENES THROUGH NETWORK ANALYSIS USING BIOINFORMATICS TOOLS

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Abstract:

Pulmonary alveolar proteinosis (PAP) is a rare lung disorder characterized by abnormal surfactant accumulation, primarily resulting from impaired GM-CSF signaling or mutations in surfactant-related genes, leading to alveolar macrophage dysfunction. While prior studies have focused on individual genes or isolated mechanisms, the broader gene interaction networks and regulatory pathways underlying PAP remain poorly understood. In this study, we applied an integrative bioinformatics approach to 30 PAP-associated genes, combining disease-gene association analysis (DisGeNET), protein-protein interaction and network prediction (STRING, GeneMANIA), functional enrichment (Enrichr), and pathway mapping (Reactome). Network analysis revealed strong connectivity among core genes, with key inflammatory mediators including IL6, TNF, IL1B, IL10, CCL2, CSF2, and CSF3 occupying central hub positions. Functional enrichment highlighted coordinated disruptions in surfactant metabolism, macrophage function, cytokine signaling, and immune regulation. Reactome-based mapping further demonstrated the functional interconnections of PAP genes, linking immune dysregulation with surfactant homeostasis. Overall, this integrative approach bridges the gap between gene-centric studies and a systems-level understanding of PAP, confirming known pathogenic mechanisms while uncovering novel molecular interactions and candidate therapeutic targets. These findings emphasize the utility of network-based bioinformatics in elucidating complex disease mechanisms and provide a foundation for future experimental validation and precision medicine strategies in PAP.

Keywords: *Pulmonary Alveolar Proteinosis, GM-CSF Signaling Pathway, Protein-Protein Interaction Network, Network-Based Bioinformatics, Cytokine Signaling, Surfactant Homeostasis.*

Introduction

Pulmonary alveolar proteinosis (PAP) is a rare pulmonary disorder characterized by the accumulation of surfactant-derived lipoproteinaceous material in the alveoli due to impaired clearance rather than overproduction. It is classified into congenital, secondary, and autoimmune forms, with autoimmune PAP accounting for approximately 90% of cases (1) PAP typically presents with exertional dyspnea and cough, while fever and sputum production are uncommon. Autoimmune PAP is often associated with smoking and environmental exposures, and the disease predominantly affects men. (2) 2. PAP is an ultra-rare disease with poorly defined incidence and prevalence, likely underestimated due to incidental detection, variable reporting, and diagnostic challenges. (3)

Pulmonary alveolar proteinosis results from disrupted GM-CSF signaling. Autoimmune PAP is caused by GM-CSF autoantibodies, while hereditary PAP arises from receptor mutations, both leading to impaired macrophage maturation and surfactant clearance. (28) Genetic PAP is caused by mutations in surfactant-related genes, including SFTPB, SFTPC, ABCA3, and TTF1, leading to abnormal surfactant production. (29) PAP features impaired alveolar macrophage surfactant clearance, causing foamy macrophage accumulation. PPAR-γ mitigates this by downregulating lipid-handling genes such as ABCG1, CD36, OLR1, FABP1, FABP4, and CIDEC. (30)

The nonspecific clinical and radiologic features of PAP frequently lead to misdiagnosis, most commonly as pneumonia, resulting in ineffective antibiotic treatment and a mean diagnostic delay of 1.5 years. Radiologic overlap with other interstitial lung diseases further complicates diagnosis. Bronchoscopy with bronchoalveolar lavage remains the gold standard, revealing milky fluid with foamy macrophages, while circulating anti GM-CSF antibodies confirm autoimmune PAP (4)

Currently, no pharmacologic agents are approved for the treatment of PAP (5) Management of pulmonary alveolar proteinosis (PAP) focuses on symptom severity and disease subtype. Whole-lung lavage remains the mainstay of therapy for patients with significant hypoxemia or functional impairment, providing sustained improvement in gas exchange and symptoms. Inhaled GM-CSF is an effective first-line or adjunctive treatment, particularly in autoimmune PAP, by restoring alveolar macrophage function. Refractory cases may be treated with rituximab or plasmapheresis, while lung transplantation is reserved for progressive, treatment-resistant disease. Supportive measures, including smoking cessation, avoidance of environmental exposures, supplemental oxygen, and vaccinations, are recommended for all patients. Emerging therapies targeting surfactant metabolism are under investigation and may further refine management strategies. (6)

Despite advances in understanding PAP, the mechanisms driving GM-CSF autoimmunity remain unclear. Current therapies, including whole-lung lavage, GM-CSF supplementation, and antibody-targeted treatments, are limited by invasiveness and variable efficacy. Novel pharmacologic agents are therefore needed to target underlying mechanisms and improve clinical outcomes. (7) The limitations of current therapies highlight the need for mechanism-based approaches, where bioinformatics plays a key role in drug discovery, target identification, lead optimization, safety prediction, drug repurposing, and personalized medicine, improving efficiency and reducing costs. (8)

Computational approaches, including genetic algorithms and neural networks, are employed to predict drug-likeness, helping to eliminate candidate molecules unlikely to succeed in later development stages. In the classical drug discovery pipeline- from target identification to lead compound and final drug-target discovery (9)

In this study, genes associated with Pulmonary Alveolar Proteinosis were selected and subjected to a comprehensive network-based analysis using web-based bioinformatics tools, including protein-protein interaction network construction, hub gene identification, functional and pathway enrichment analyses, and visualization to interpret the biological significance of the network.

Methodology

A structured bioinformatics approach was used to study the molecular basis of pulmonary alveolar proteinosis (PAP). Genes associated with PAP were obtained from DisGeNET using curated disease-gene association data. GeneMANIA was then used to examine functional relationships and predict gene-gene interactions based on co-expression, genetic interactions, and shared pathways. Protein-protein interaction networks were constructed using the STRING database with appropriate confidence filtering. These networks were further analyzed in NetworkAnalyst to identify key hub genes and important interaction modules. Finally, Reactome pathway enrichment analysis was carried out to identify biological pathways and processes involved in PAP.

1. DisGeNET

- DisGeNET is a comprehensive platform that integrates and annotates knowledge on the genetic basis of human diseases. It catalogs genes and variants linked to Mendelian, complex, rare, and environmentally influenced conditions, combining data from multiple databases and text-mined literature to support efficient retrieval, prioritization, and interpretation of disease-gene associations. (10)
- Evaluating the evidence supporting gene-disease and variant-disease associations is essential for genomic research. The DisGeNET Score (GDA Score) provides a quantitative measure of the strength and reliability of each association, allowing to assess not just its presence but also the robustness of the supporting evidence.
- DisGeNET was used to retrieve disease-associated genes for Pulmonary Alveolar Proteinosis (PAP) from the Gene-Disease Associations table. Genes were prioritized by GDA Score, and the top 30 high-confidence genes, including *CSF2RA, CSF2, CSF2RB, SFTPB, ABCA3, SFTPC, MUC1, SFTPD, PPARG,* and *CSF1*, were selected for further analysis. These genes play key roles in surfactant metabolism and immune function The complete list of 30 genes is provided in Table 1.

2. STRING:

- STRING is a database that compiles both experimental and predicted protein–protein interactions, encompassing physical contacts and functional relationships. Interaction evidence comes from literature mining, computational predictions, curated pathways, and experimental datasets, with confidence scores and orthology based mapping across species. (11) In STRING, proteins are nodes and predicted functional associations are edges, with edge color showing evidence type and thickness indicating confidence. Action mode displays functional details, and clicking nodes or edges reveals protein info and supporting evidence.
- Protein-protein interactions were analyzed in STRING with a medium confidence score (0.4), and functional enrichment provided GO terms and KEGG/Reactome pathways. Key GO terms were grouped into representative biological themes for interpretation.

3. **GeneMANIA**

- GeneMANIA is a web-based tool that predicts gene function and identifies related genes by expanding a query list using integrated genomics and proteomics data, assigning weights to indicate dataset relevance. It supports custom datasets and is widely used in functional genomics.(12) GeneMANIA identifies genes functionally related to an input gene set using integrated association data, including protein and genetic interactions, pathways, co-expression, co-localization, and protein domain similarity. It predicts additional genes likely involved in the same pathways or complexes and reveals functional connections within the query gene list, enabling the discovery of novel pathway members or functionally linked genes.
- The PAP gene list was analyzed in GeneMANIA using default settings. The platform added functionally related genes and constructed a network based on co-expression, physical interactions, and genetic interactions. The resulting network revealed a dense core of key genes, indicating strong functional coordination.

4. Enrichr

- Enrichr is a web-based tool for gene set enrichment analysis, offering access to more than 180,000 annotated gene sets from 102 curated libraries. It enables analysis of gene lists and provides interactive visualizations, including clustergrams, serving as a comprehensive resource for functional annotation and biological interpretation.
- Enrichr takes an input gene list and statistically tests its overlap against curated gene-set libraries to identify significantly enriched biological pathways, functional annotations, diseases, and regulatory signatures, ranking results to facilitate interpretation and hypothesis generation. (13)
- Gene set enrichment analysis was conducted using Enrichr to identify functional hub genes. Top enriched terms from pathway, disease/phenotype, regulatory, and expression libraries were examined, and recurring genes across multiple terms were recorded. A gene-term recurrence matrix was generated, and genes with high recurrence were prioritized as functional hubs.

5. Reactome

- Reactome is an open-access, manually curated and peer-reviewed database that provides comprehensive, computationally structured representations of human biological pathways. It integrates genes, proteins, small molecules, and literature to model diverse processes such as signaling, metabolism, and gene regulation, supporting pathway analysis, systems biology, and genomic research. (14) Reactome organizes biological knowledge around reactions involving genes, proteins, complexes, and small molecules, grouped into pathways such as metabolism, signaling, and disease. Pathways are expert-curated and literature-supported to ensure accuracy.
- The Reactome Pathway Browser was used to identify significantly enriched pathways in the gene list. Enriched pathways were evaluated by p-values and FDR, key pathways were examined for hub genes. Results, including pathway names, gene hits, and significance, were summarized in a table highlighting the top disease-relevant pathways.

Overall pathway

Pulmonary Alveolar Proteinosis (PAP) disease

↓

DisGeNET: Genes associated with PAP were extracted using curated disease–gene association (GDA) data

↓

STRING: Constructed protein–protein interaction (PPI) network with confidence filtering

↓

GeneMANIA: Analyzed co-expression, genetic interactions, and shared pathways; predicted related genes

↓

NetworkAnalyst: Performed network analysis to identify hub genes and key interaction modules

↓

Reactome: Conducted pathway enrichment to identify biological processes involved in PAP

↓

Final Interpretation: Identification of key genes, pathways, and molecular mechanisms of PAP

Results

Integrative bioinformatics analysis using DisGeNET, STRING, GeneMANIA, Enrichr, and Reactome identified key PAP-associated genes, their interaction networks, and enriched pathways, highlighting the disease's complex molecular regulation

1. DisGeNET

A total of 30 PAP-associated genes were retrieved from DisGeNET. For each gene, the gene symbol, GDA score, and supporting PMID were recorded

Table 1: Pulmonary alveolar proteinosis - associated genes identified from DisGeNET with their corresponding GDA scores and PMID

Sr. No.	Gene Symbol	GDA Score	PMID
1.	CSF2RA	0.95	30
2.	CSF2	0.9	100
3.	CSF2RB	0.7	11
4.	SFTPB	0.7	10
5.	ABCA3	0.65	6
6.	SFTPC	0.6	8
7.	MUC1	0.4	11
8.	SFTPD	0.35	5
9.	PPARG	0.35	4
10.	CSF1	0.35	3
11.	ABCG1	0.35	3
12.	MARS1	0.3	12
13.	SFTPA1	0.3	4
14.	CCL2	0.3	3

15.	IL1B	0.3	2
16.	TNF	0.3	2
17.	IL6	0.3	2
18.	SFTPA2	0.25	7
19.	IL3	0.25	5
20.	BCO1	0.25	4
21.	IL5	0.25	2
22.	INHBA	0.25	2
23.	GATA2	0.2	9
24.	CSF3	0.2	8
25.	SLC7A7	0.2	4
26.	STING1	0.2	3
27.	IL10	0.2	3
28.	OAS1	0.2	3
29.	COPA	0.2	2
30.	NKX2-1	0.2	2

2. STRING:

Protein-protein interaction (PPI) networks were constructed using STRING to explore the functional connectivity and interactions among the PAP-associated genes identified from DisGeNET

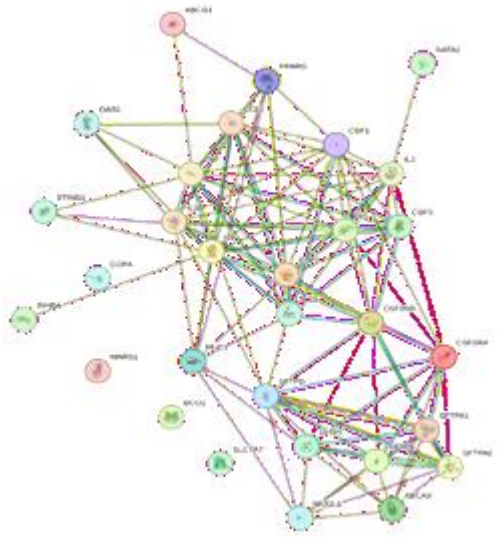

Figure 1: Protein-protein interaction (PPI) networks were constructed using STRING

Network Stats

- Number of nodes:30
- Number of edges:131
- Average node degree: 8.73
- Avg. local clustering coefficient: 0.672

- Expected number of edges: 31
- PPI enrichment p-value: < 1.0e-16

Table 2: Enriched Biological Themes with Representative GO Terms

Theme	Representative Go Terms
Surfactant homeostasis and lipid accumulation	Surfactant homeostasis, regulation of lipid localization, cellular response to lipid
Macrophage and myeloid cell differentiation	Regulation of myeloid leukocyte differentiation, macrophage derived foam cell differentiation, myeloid leukocyte migration, leukocyte differentiation
Phagocytosis and immune system	Positive regulation of phagocytosis, positive regulation of immune effector process, regulation of immune effctor process, leukocyte migration, leukocyte chemotaxis
Cytokine and GM-CSF-related signaling	Cytokine-mediated signaling pathway, regulation of cytokine production, regulation of cytokine-mediated signaling pathway, granulocyte-macrophage colony-stimulating factor signaling pathway, cellular response to cytokine stimulus
JAK-STAT and receptor signaling	Receptor signaling via JAK-STAT, Positive regulation of receptor signaling pathway via JAK-STAT, Positive regulation of tyrosine phosphorylation of STAT protein,
Inflammatory & Innate Immune Response	Immune response, regulation of immune system process, positive regulation of immune response, response to bacterium, defense response to bacterium, cellular response to lipopolysaccharide

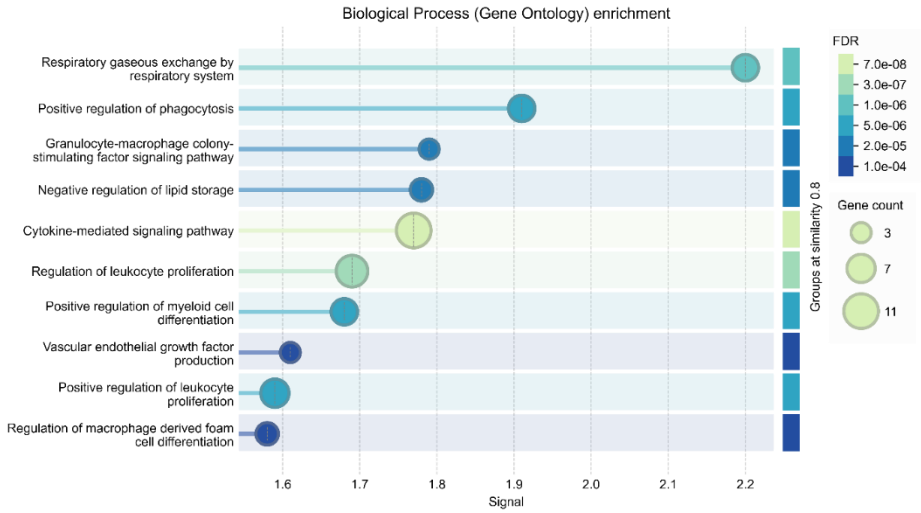

Figure 2: Functional Enrichment Plot : Bubble chart of Gene Ontology (GO) terms

Gene Ontology enrichment of the protein–protein interaction network highlighted key processes involved in pulmonary alveolar proteinosis, including surfactant homeostasis, lipid regulation, macrophage and myeloid cell differentiation, and immune functions such as phagocytosis, leukocyte migration, and chemotaxis. Signaling pathways, including cytokine-, GM-CSF, and JAK-STAT mediated signaling, were also significantly enriched, reflecting the main biological mechanisms underlying the disease.

3. Gene MANIA

These show strong interconnections and central positioning: IL6, TNF, IL1B, CSF2 (GM-CSF), CSF3 (G-CSF), CCL2, and IL10.

GeneMANIA analysis revealed a highly interconnected network of pulmonary alveolar proteinosis-associated genes. Core genes including IL6, TNF, IL1B, CSF2 (GM-CSF), CSF3 (G-CSF), CCL2, and IL10, occupied central positions, supported by multiple evidence types such as physical interactions, co-expression, and pathway co-membership, highlighting the functional coordination and biological relevance of the network.

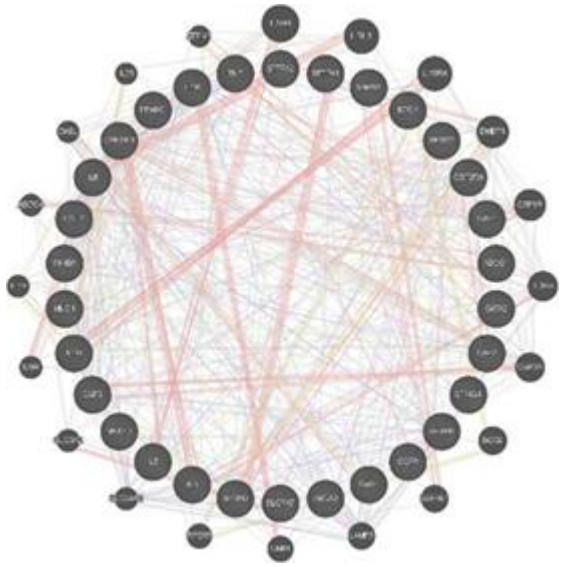

figure 3: GeneMANIA analysis

4. Enrichr

Gene set enrichment analysis was performed using Enrichr to identify significantly enriched biological pathways, functional terms, and disease associations among the PAP-associated genes.

Gene	KEGG 2021 Human	Reactome Pathways 2024	WikiPathways 2024 Human	ChEA 2022	ARCHS4 Kinases Coexp	Count
IL6	Present	Present	Present	Present	Present	5
TNF	Present	Present	Present	Present	Present	5
IL1B	Present	Present	Present	Present	Present	5
IL10	Present	Present	Present	Present	Present	5
CCL2	Present	Present	Present	Present	Present	5
CSF1	Present	Present	Present	Present	Absent	4
CSF2	Present	Present	Present	Absent	Absent	3
CSF3	Present	Present	Present	Absent	Absent	3
IL5	Present	Present	Present	Absent	Absent	3
IL3	Present	Present	Present	Absent	Absent	3

Functional enrichment using Enrichr across KEGG, Reactome, WikiPathways, ChEA, and ARCHS4 identified IL6, TNF, IL1B, IL10, and CCL2 as consistently enriched, highlighting their central role in PAP. Additional genes, including CSF1, CSF2, CSF3, IL5, and IL3, were enriched in multiple databases with lower recurrence. The repeated presence of these inflammatory mediators supports their classification as hub genes, emphasizing their key regulatory roles and potential as therapeutic targets in PAP.

5. Reactome

Pathway enrichment analysis was conducted using Reactome pathway browser to identify key biological pathways and processes associated with the PAP-related genes. Over represented pathways identified by Reactome.

Table 4: Significantly Enriched Reactome Pathways with Gene Hits and FDR Values

Pathway Name	Number of Hits	FDR Values
Cytokine signaling in immune system	28	6.22E-15
Signaling by Interleukins	25	6.22E-15
Interlukin-10 signaling	26	6.22E-15
Interleukin-4 and Intereukin-13 signaling	13	1.31E-11
Interleukin-2 family signaling	6	4.28E-7
Regulation of TLR by endogenous ligand	3	4.33E-3
CLEC7A/Inflammasome pathway	3	1.77E-5

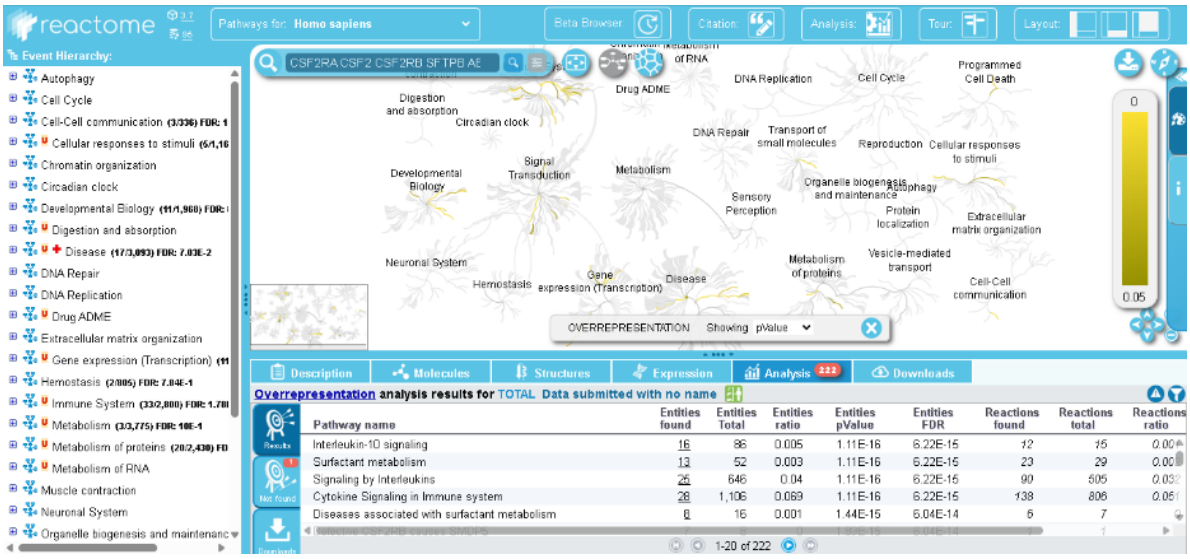

Figure 4: Hierarchical Pathway Map of Genes

The top three Reactome pathways enriched among PAP-associated genes were: Cytokine signaling in the immune system, Signaling by Interleukins, Interleukin-10 signaling. These pathways highlight the predominant involvement of cytokine-mediated immune regulation in PAP. Additionally, innate immune pathways such as the CLEC7A/inflammasome pathway and regulation of Toll-like receptors by endogenous ligands were enriched, collectively suggesting that dysregulated cytokine-mediated and innate immune signaling plays a central role in PAP pathogenesis.

Overall, Bioinformatics analysis showed that PAP-associated genes form a tightly interconnected network centered on key cytokines, including IL6, TNF, IL1B, CSF2, CSF3, CCL2, and IL10. Functional and pathway enrichment highlighted macrophage dysfunction, impaired surfactant homeostasis, and dysregulated JAK-STAT and cytokine signaling. These findings suggest that cytokine-driven immune dysregulation is central to PAP and identify key inflammatory mediators as potential therapeutic targets.

Discussion

Genetic and molecular basis of PAP

Pulmonary alveolar proteinosis (PAP) arises from mutations affecting the alpha or beta chains of the GM-CSF receptor (CSF2RA and CSF2RB), leading to impaired surfactant clearance by alveolar macrophages and accumulation of surfactant lipids and proteins. (15) Studies show that GATA2 deficiency compromises alveolar macrophage phagocytic function, reflecting qualitative rather than quantitative defects, while mutations in surfactant-related genes (SFTPB, SFTPC, ABCA3, and NKX2-1) disrupt surfactant production and function, causing interstitial lung disease with a PAP-like pattern and parenchymal fibrosis. (16) To explore the molecular mechanisms underlying PAP, 30 disease-associated genes were retrieved from DisGeNET. This gene set included key regulators of surfactant metabolism, inflammatory mediators, and genes involved in macrophage function and immune regulation.

Protein-Protein interactions

Studies have shown that Surfactant proteins (SFTPB, SFTPC, ABCA3, NKX2-1) maintain alveolar stability, while the GM-CSF receptor (CSF2RA, CSF2RB) activates macrophages via JAK/STAT, with the β-chain linking IL-3 and IL-5 pathways, showing that disruption of surfactant synthesis or GM-CSF–mediated clearance drives PAP (17) Gene Ontology enrichment of the protein-protein interaction network through STRING identified key processes, including surfactant homeostasis and lipid regulation, macrophage differentiation, phagocytosis, cytokine- and GM-CSF mediated signaling, JAK-STAT receptor signaling, and inflammatory and innate immune responses. These findings integrate experimental and computational evidence, demonstrating how coordinated disruptions in surfactant metabolism and immune signaling contribute to PAP and highlighting potential molecular targets for therapy

Gene interaction network

Studies suggest that protein co-expression networks provide a useful framework to identify key molecules in disease, generating hypotheses about functional regulation and central hub proteins. (18) Core inflammatory mediators - IL6, TNF, IL1B, CSF2, CSF3, CCL2, and IL10 form highly interconnected hubs, reflecting their key roles in PAP immune regulation. GeneMANIA analysis confirmed a tightly linked network supported by physical interactions, co-expression, and shared pathways, highlighting these cytokines as central regulators and potential therapeutic targets.

Functional enrichment and Hub genes

Identifying hub genes in bioinformatics analysis is important, as they reveal key molecular mechanisms and potential diagnostic biomarkers. Changes in hub genes or their direct interactors can have widespread effects across the network, highlighting their central regulatory role in disease. (19, 20) Enrichr analysis highlighted key PAP-associated genes and their functional roles across multiple libraries. IL6, TNF, IL1B, IL10, and CCL2 were consistently enriched, indicating central hub roles in immune regulation and surfactant homeostasis, while CSF1,

CSF2, CSF3, IL5, and IL3 showed lower recurrence. The repeated identification of these inflammatory mediators supports their central regulatory role in PAP pathogenesis and underscores their potential as therapeutic targets.

Pathway analysis

Pathway analysis is essential for interpreting high-throughput biological data by linking genes or proteins to known biomolecular functions. It helps identify key pathways driving biological processes and disease mechanisms. (21) Reactome analysis of PAP-associated genes highlighted prominent enrichment in cytokine-mediated immune pathways, including cytokine signaling, interleukin signaling, and IL-10 signaling. Innate immune pathways, such as CLEC7A/inflammasome activation and Toll-like receptor regulation, were also enriched, indicating that dysregulated cytokine and innate immune signaling plays a central role in PAP pathogenesis alongside surfactant metabolism defects.

In conclusion, this study demonstrates that network-based bioinformatics analyses provide a reliable and practical approach for elucidating the molecular mechanisms underlying pulmonary alveolar proteinosis. The consistency of these findings with previously reported studies underscores the value of systems biology approaches in PAP research and supports the use of integrative network analysis for identifying key regulatory genes and potential therapeutic targets.

Conclusion

Pulmonary alveolar proteinosis (PAP) is a rare lung disorder characterized by surfactant accumulation, often caused by impaired GM-CSF signaling from autoantibodies or mutations in CSF2RA and CSF2RB, highlighting the key role of alveolar macrophage dysfunction in disease pathogenesis. (22), most have focused on individual genes or isolated mechanisms, limiting insight into broader gene interaction networks. To address this, the present study used an integrative bioinformatics approach- including DisGeNET, STRING, GeneMANIA, Enrichr, and Reactome to analyze PAP-associated genes at a systems level. This strategy identified key interaction networks, enriched biological processes, and regulatory pathways contributing to PAP pathogenesis.

DisGeNET database captures both disease-associated genes and their interactions, integrating them into a disease-centric network (27) in this study, 30 PAP-associated genes were analyzed. PAP, including autoimmune, hereditary, secondary, and congenital forms, arises from impaired GM-CSF signaling or surfactant gene mutations, causing macrophage dysfunction and surfactant accumulation. (23) Bioinformatics network analysis using STRING revealed strong connectivity among core PAP genes, showing that these diverse causes converge on key hubs and pathways GM-CSF signaling, surfactant metabolism, and cytokine regulation highlighting coordinated molecular dysregulation as central to PAP pathogenesis.

GeneMANIA allows construction of integrated gene-gene functional networks, incorporating closely related genes and Gene Ontology annotations (24) GeneMANIA analysis of PAP-associated genes revealed a highly interconnected network, with core genes occupying central hubs and supported by physical interactions, co-expression, and shared pathways, highlighting their coordinated functional roles in disease pathogenesis.

Previous GO enrichment studies used DAVID platform. (25) Integrative analysis using Enrichr across multiple libraries revealed consistently enriched hub genes, with additional genes showing partial recurrence. Previous studies have used Reactome to link disease-associated enzymes to biological processes, providing insights into potential disease mechanisms (26). Using Reactome, mapped PAP-associated genes to reveal their functional

connections, bridging general pathway analysis with PAP-specific mechanisms and demonstrating the value of integrative bioinformatics in understanding the disease.

By combining experimental data with network-based analyses, this study bridges traditional gene-centric research and a systems-level view of PAP, identifying core hub genes and regulatory interactions that confirm known mechanisms while revealing targets for future validation and therapy.

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