A ferroptosis-related gene signature for lung function and quality of life in patients with idiopathic pulmonary fibrosis

Yupeng Li (✉ 954203558@qq.com)
Second Affiliated Hospital of Harbin Medical University
https://orcid.org/0000-0001-5082-5914

Shangwei Ning
Harbin Medical University Institute of Biological Information Science and Technology

Yi Yang
Respiratory and Critical Care Medicine of Second Affiliated Hospital of Harbin Medical University

Hong Chen
Respiratory and critical care medicine of Second Affiliated Hospital of Harbin Medical University

Chen Wang
Chinese Academy of Medical Sciences and Peking Union Medical College

Huaping Dai
Pulmonary and Critical Care, center of respiratory medicine, China-Japan friendship hospital

Research article

Keywords: idiopathic pulmonary fibrosis, gene, ACSL1, network, lung function

DOI: https://doi.org/10.21203/rs.3.rs-201670/v2

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background: Rapid advances in genetic and genomic technologies have begun to reshape our understanding of idiopathic pulmonary fibrosis (IPF). Ferroptosis, an iron-dependent form of regulated cell death, play an important role in the development of IPF. Therefore, our study aimed to explore the role of ferroptosis-related genes (FRGs) and their correlation with lung dysfunction and quality of life in patients with IPF.

Methods: Datasets were acquired by researching the Gene Expression Omnibus. FRGs were acquired by researching GeneCard database and PubMed. Ferroptosis-related differentially expressed genes (FRDEGs) were identified according to integrating FRGs and the DEGs identified in the GSE110147 dataset. Candidate key genes were identified from the miRNA-target FRDEGs network and protein-protein interactions (PPI) network. The relationship between key genes and lung function or quality of life was calculated using the GSE32537 datasets.

Results: 293 FRGs were obtained, and 71 FRDEGs were identified. According to enrichment analysis, cell growth and death and pathways associated cancer were the important pathways, and significant biological processes were mainly consisted of cellular responses to stimulus and various situations. In addition, this study constructed an PPI network and a miRNA-target network based on the 71 FRDEGs, determined 19 candidate key genes. Furthermore, acyl-CoA synthetase long chain family member 1 (ACSL1), integrin subunit beta 8 (ITGB8) and ceruloplasmin (CP) were identified as the key genes. The expression level of ACSL1 was the strongest predictor for lung function (negatively) including percent predicted forced vital capacity (FVC% predicted) and percent predicted diffusion capacity of the lung for carbon monoxide (Dlco% predicted) and quality of life (negatively). In addition, ITGB8 and CP were negatively associated with FVC% predicted. According to DrugBank and PubMed, 4 drugs and 16 drugs have been found to act on ACSL1 and CP, respectively.

Conclusion: These results imply that FRGs may shed new understanding on disease mechanism and provide potential biomarkers and therapy target to predict IPF progression.

Introduction

Idiopathic pulmonary fibrosis (IPF), a common interstitial lung disease (ILD) of unknown etiology with repeated acute lung injury, causes worsening dyspnea and deteriorating lung function [1]. The incidence of IPF among people aged 18–64 years between 2005 and 2010 according to a study in the United States was 6.1 new cases per 100000 person-years [2]. Currently, two drugs (Pirfenidone and Nintedanib) have been identified to be moderately effective in treating IPF [3, 4]. However, the prognosis of IPF remains severe, with death usually occurring within 2–3 years after diagnosis [5, 6], and the 5-year survival rate is only 20% [7]. Through the past decades, rapid advances in genetic and genomic technologies have begun to reshape our understanding of IPF. Studies have uncovered some genes that are linked to IPF, including telomerase reverse transcriptase, TERT [8, 9]; transforming growth factor beta 1, TGFB1 [10]; and mucin 5B, MUC5B [11] et al. However, the pathophysiologic mechanisms of IPF are complex and remain incompletely understood.

Ferroptosis is a new type of regulated cell death (RCD) which is dependent on iron, and different from apoptosis, cell necrosis and autophagy [12]. Previous study had confirmed that iron overload may cause lung...
fibrosis according to increased lipid peroxidation and decreased glutathione peroxidase 4 (GPX4) activity in lung tissues [13]. Furthermore, studies have verified that ferroptosis plays an important role in the development of pulmonary fibrosis, and ferroptosis inhibitor may attenuate pulmonary fibrosis progression [14, 15]. Many genes such as GPX4, solute carrier family 7 member 11 (SLC7A11), transforming growth factor beta receptor 1 (TGFBR1) and so on have also been identified as regulators or markers of ferroptosis, and were associated with the development of pulmonary fibrosis [14–16]. However, the systematic exploration of role of ferroptosis-related genes (FRGs) as well as their values for lung function and quality of life are absent in patients with IPF.

Therefore, the purposes of the study are to analyzed the characteristics of ferroptosis-related differentially expressed genes (FRDEGs) in IPF based on the Gene Expression Omnibus (GEO) or other databases, such as miRDB, GeneCards etc., and construct miRNA-target interactions network to explore a novel approach for the determination of gene functions and the pathogenesis of IPF. Furthermore, we summarized the information derived from miRNA-target interactions, gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, protein-protein interaction (PPI) data, and then screened out useful potential biomarkers for lung function and quality of life and therapeutic targets for IPF.

**Materials And Methods**

**Acquisition of datasets.**

Figure 1 shows the workflow of our study. On the GEO database (http://www.ncbi.nlm.nih.gov/geo/), we selected datasets must meet the following items: (1) the gene expression profile was measured using same platform; (2) the detected samples came from the lung tissues of patients with IPF or healthy donors; (3) raw data or a gene expression matrix should be provided. Finally, two datasets were identified, GSE110147 and GSE32537 (platform: GPL6244). Approval of the Ethics Committee was not required because the information of patients was obtained from the GEO.

Human miRNA-target interactions data were downloaded from miRDB [17]. FRGs were obtained from GeneCards database (https://www.genecards.org/) by searching the terms “ferroptosis” and PubMed by searching the terms “Ferroptosis [MeSH] OR Ferroptosis* [tiab]”. Consequently, 103 FRGs and 190 FRGs were respectively collected from GeneCards and PubMed in the study as shown in Supplementary Table 1.

**Datasets preprocessing**

The raw data (CEL format) of GSE110147 and GSE32537 were downloaded from GEO. “Affy” package (http://bioconductor.org/packages/release/bioc/html/affy.html, v.1.68.0) was used to normalize the array data according to the robust multi-array average (RMA) method. We defined IPF differentially expressed genes (DEGs) as expression levels of genes were significantly diverse in IPF patients compared with the controls (|log Fold Change|>1 and adjusted p-value < 0.05). “Limma” package (v.3.46.0) [18] was used for the analysis of DEGs. In addition, St. George's Respiratory Questionnaire (SGRQ) score and lung function [percent predicted forced vital capacity (FVC% predicted) and percent predicted diffusion capacity of the lung for carbon monoxide (Dlco% predicted)] were extracted from the GSE32537 dataset (Table 1).
Table 1
Demographic data for subjects used in this study.

| Characters               | GSE32537 (119 IPF) |
|--------------------------|--------------------|
| Age (years)              | 62 ± 8             |
| Sex (%)                  |                    |
| Male                     | 77 (64.7)          |
| Female                   | 42 (35.3)          |
| Smoking history (%)      |                    |
| Yes                      | 70 (58.8)          |
| No                       | 41 (34.5)          |
| NA                       | 8 (6.7)            |
| FVC% predicted           | 61.25 ± 17.02      |
| DLco% predicted          | 45.13 ± 20.297     |
| SGRQ score               | 47.43 ± 21.45      |
| Data are presented as mean ± SD or n(%) |

Analysis of data

GO and KEGG enrichment analyses of the FRDEGs of IPF were analyzed and visualized by R package “clusterProfiler” [19]. Heatmap was constructed according to R packages “gplots” (v.3.1.1) and “RColorBrewer (v.1.1-2)”. STRING (http://www.string.embl.de/, version: 11.0b) was used to analyze the protein-protein interactions (PPI) [20]. Cytoscape (version 3.7.1) [21] was used to visualize miRNA-target network and PPI network, and its MCODE were used to make the visualization of PPI network and identify the modules in the network [parameters: Degree cutoff ≥ 2 (degrees of each nodes in module were larger than 2 at least), K-core ≥ 2 (subgraphs of each node in module were more than 2 at least )].

Drug discovery

DrugBank [22] and PubMed were used to screen drugs associated with related gene that was predicted to be an important gene in this study.

Statistical analysis

Continuous variables were compared between two groups by applying the non-parametric t test. Associations between the expression levels of genes and lung function and SGRQ score were determined by Spearman correlation coefficient. All statistical analyses were carried out with GraphPad Prism 7.0, and P < 0.05 was considered statistically significant.
Results

FRDEGs of IPF

After integrating 293 FRGs and the DEGs identified in the GSE110147, 47 up-regulated and 24 down-regulated FRDEGs were identified (Fig. 2A-2B).

PPI network

The PPI network was constructed based on the 71 FRDEGs according to the STRING database (average node degree: 5.6, PPI enrichment p-value: < 1.0e-16), which was visualized by Cytoscape [20, 21]. We removed the nodes with no connections, Therefore, the final network contained 66 nodes and 196 edges (Fig. 2C). Ceruloplasmin (CP) was the highest up-regulated gene, and angiopoietin like 4 (ANGPTL4) was the highest down-regulated gene in the PPI network. We calculated the connectivity degree of each node, and selected those with degrees ≥ 15, as follows: mitogen-activated protein kinase 3 (MAPK3, down-regulated), heme oxygenase 1 (HMOX1, down-regulated), KRAS proto-oncogene, GTPase (KRAS, up-regulated), heat shock protein family A member 5 (HSPA5, up-regulated) and ATM serine/threonine kinase (ATM, up-regulated). In addition, one module (Figure S1) were selected after MCODE analysis of the whole network, and the results of enrichment analysis of FRDEGs within the module were showed in Figure S2 by R package “clusterProfiler” [19], which revealed the important pathways: cell growth and death, and pathways associated cancer.

Key gene ontology and pathways enriched in IPF

In order to reveal the biological significance of 71 FRDEGs regulating IPF at a single level, we used R package “clusterProfiler” [19] to conduct biological pathway enrichment and biological process annotation for the 71 genes mentioned above. The 20 most significantly KEGG pathways were selected (Supplementary Table 2, Fig. 3A, 3F). More importantly, cell growth and death, pathways associated cancer and signal transduction were the main pathways, implying that FRDGEs may participate in the process of IPF according to these pathways (Figure S3A). Hsa04216 (Ferroptosis, including 11 FRDEGs) was the first significantly enriched pathway (Figure S3B). FRDEGs-related top 20 biological processes (BP), cellular component (CC) and molecular function (MF) were showed in Fig. 3B-3D respectively. The top 20 GOs were showed in Supplementary Table 3 and Fig. 3E, which were consisted of cellular responses to stimulus and various situations.

Some potential biomarkers had been found in IPF

A total of 1638 miRNA-target interactions associated with 68 of 71 FRDEGs and 463 related miRNAs were derived from miRDB [17] and visualized by Cytoscape (Fig. 4). The related nodes with degrees ≥ 25 were shown in Table 2. The more interactions with miRNAs, the more degree is. Therefore, integrin subunit beta 8 (ITGB8) was considered the hub node. In addition, for miRNA, the related nodes with degrees ≥ 9 were shown in Table 3. The top 5 hub nodes with higher degrees were hsa-miR-513a-3p, hsa-miR-513c-3p, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-3065-5p.
Table 2
The node with degrees ≥ 25 were shown according to the miRNA-target network

| Target Gene | Degrees | Target Gene | Degrees | Target Gene | Degrees |
|-------------|---------|-------------|---------|-------------|---------|
| ITGB8       | 99      | HIF1A       | 36      | DLD         | 27      |
| ACSL4       | 87      | TFRC        | 36      | RNF20       | 27      |
| PIK3CA      | 81      | ACSL3       | 35      | TFAM        | 27      |
| TGFBR1      | 77      | G3BP1       | 34      | NCOA4       | 26      |
| KRAS        | 69      | CAV1        | 33      | HMGB1       | 25      |
| FBXW7       | 65      | IREB2       | 33      | NFE2L2      | 25      |
| PRKAA1      | 60      | SNX4        | 32      |             |         |
| ITGA6       | 51      | ATM         | 31      |             |         |
| MYB         | 45      | GCLC        | 31      |             |         |
| ACSL1       | 40      | MAP3K5      | 31      |             |         |
| TP63        | 40      | HMGCR       | 30      |             |         |

Table 3
The node with degrees ≥ 9 were shown according to miRNA-target network.

| miRNA         | Degrees | miRNA         | Degrees | miRNA         | Degrees |
|---------------|---------|---------------|---------|---------------|---------|
| hsa-miR-513a-3p | 17      | hsa-miR-374b-5p | 9       | hsa-miR-548e-3p | 9       |
| hsa-miR-513c-3p | 17      | hsa-miR-493-5p | 9       | hsa-miR-548f-3p | 9       |
| hsa-miR-19a-3p | 12      | hsa-miR-506-3p | 9       | hsa-miR-548h-5p | 9       |
| hsa-miR-19b-3p | 12      | hsa-miR-548a-3p | 9       | hsa-miR-548j-5p | 9       |
| hsa-miR-3065-5p | 10     | hsa-miR-548am-5p | 9      | hsa-miR-548o-5p | 9       |
| hsa-miR-124-3p | 9       | hsa-miR-548b-5p | 9       | hsa-miR-582-5p | 9       |
| hsa-miR-1297  | 9       | hsa-miR-548c-5p | 9       | hsa-miR-664b-3p | 9       |
| hsa-miR-374a-5p | 9      | hsa-miR-548d-5p | 9       | hsa-miR-450b-5p | 9       |

Identify of key genes

The top 12 genes with high degrees in Table 2 and the top 7 genes with high degrees or high DEG level as mentioned above in the PPI network were selected as the candidate key genes. Subsequently, the expression levels of 19 candidate key genes were compared between IPF patients and healthy control in the GSE32537 dataset. Finally, acyl-CoA synthetase long chain family member 1 (ACSL1, down-regulated), CP (up-regulated),
tumor protein p63 (TP63, up-regulated), ITGB8 (up-regulated) and MYB proto-oncogene, and transcription factor (MYB, up-regulated) were selected as the key genes (Fig. 5A-5E). According to linear regression, ASCL1 was negatively associated with FVC% predicted, DLco% predicted, and positively associated with SGRQ score, and the Spearman correlation coefficients were calculated as -0.4132, -0.3609 and 0.2964, respectively (Fig. 6A-6C). In addition, CP and ITGB8 were only negatively associated with FVC% predicted, the Spearman correlation coefficients were calculated as -0.2095 and -0.2345, respectively (Fig. 6D, 6G). However, the correlations between CP and DLco% predicted or SGRQ score were not significant (Fig. 6E-6F), ITGB8 showed the same result (Fig. 6H-6I).

Drug discovery
According to DrugBank [22], 16 drugs have been found to be acted on CP, and 2 drugs have been found to be acted on ACSL1. According to searching from PubMed, 2 drugs [Benzimidazole series (compound 13) [23] and Aspirin [24]] were found to be acted on ACSL1, however, no additional drugs associated with CP or ITGB8 were found. These drugs and related papers were listed in Table 4.
Table 4
The drugs acting on ACSL1 and CP in DrugBank and PubMed.

| Targeted gene | Drug               | DrugBank ID | Drug group                      | Pharmacological action? | Action   | PubMed IDs |
|---------------|--------------------|-------------|---------------------------------|--------------------------|----------|------------|
| ACSL1         | Adenosine phosphate| DB00131     | Approved, investigational, nutraceutical | Unknown                  | Product of | 16981708, 17350930 |
|               | ATP                | DB00171     | Investigational, nutraceutical   | Unknown                  |          | 17139284, 17016423 |
|               | Benzinidazole series (compound 13) | NA | Investigational | Unknown | Inhibitor | 33285268 |
|               | Aspirin            | NA          | Investigational                | Unknown                  | Inhibitor | 28359761 |
| CP            | Copper             | DB09130     | Approved, investigational       | No                       | Binder    | 14652164 |
|               | Calcium            | DB01373     | Nutraceutical                   | Unknown                  |          | 17242517 |
|               | Iron               | DB01592     | Approved                       | Unknown                  |          | 21049900 |
|               | Zinc               | DB01593     | Approved, investigational       | Unknown                  |          | 23896426 |
|               | Cupric sulfate     | DB06778     | Approved                       | Unknown                  |          | NA         |
|               | Ferrous sulfate anhydrous | DB13257 | Approved                      | Yes                      | Substrate | 775938 |
|               | Cupric oxide       | DB11134     | Approved                       | Unknown                  | Binder    | NA         |
|               | Silver             | DB12965     | Approved, investigational       | Unknown                  | Binder    | NA         |
|               | Zinc acetate       | DB14487     | Approved, investigational       | Unknown                  |          | 23896426 |
|               | Ferrous gluconate  | DB14488     | Approved                       | Unknown                  |          | 21049900 |
|               | Ferrous succinate  | DB14489     | Approved                       | Unknown                  |          | 21049900 |
|               | Ferrous ascorbate  | DB14490     | Approved                       | Unknown                  |          | 21049900 |
|               | Ferrous fumarate   | DB14491     | Approved                       | Unknown                  |          | 21049900 |
|               | Ferrous glycine sulfate | DB14501 | Approved                      | Unknown                  |          | 21049900 |
| Targeted gene | Drug                  | DrugBank ID | Drug group          | Pharmacological action? | Action | PubMed IDs |
|--------------|-----------------------|-------------|---------------------|-------------------------|--------|------------|
|              | Zinc chloride         | DB14533     | Approved, investigational | Unknown                 | Ligand | 23896426   |
|              | Zinc sulfate, unspecified form | DB14548     | Approved, experimental          | Unknown                 | Ligand | 23896426   |

**Discussion**

IPF is a serious lung disease, and until today, there is no effective way to treat it. In this study, 71 FRDEGs from 293 FRGs were identified in disease samples compared to normal control in the GSE110147 dataset. The bioprocess enrichment analysis showed that the 71 FRDEGs mentioned above were significantly correlated with a series of biological processes: cellular responses to stimulus and various situations. Persistent alveolar epithelial injury and the abnormal repair are the important causes of lung fibrosis [25]. Therefore, cellular responses to the persistent injury are important in the development of IPF. Abnormal cellular responses may lead to epithelial-mesenchymal transition (EMT), which may promote the development of lung fibrosis [15]. Therefore, FRGs may participate in the development of IPF according to these biological processes.

Furthermore, KEGG pathways analysis of 71 FRDEGs and the module identified from the PPI network showed that cell growth and death, pathways associated cancer and signal transduction were significant enriched pathways. Similar to cancer, IPF affects susceptible individuals and shares common risk factors for cancer such as smoking, environmental or professional exposure, viral infections, and chronic tissue injury [26]. The incidence of cancer in IPF patients is higher compared with matched controls, especially for lung cancer [27]. Ferroptosis, FoxO signaling pathway, HIF-1 signaling pathway and so on play key roles in the development and prognosis of cancer [28–31]. In addition, the programmed death ligand-1/programmed cell death 1 (PD-L1/PD-1) axis can promote cancer cells to escape the surveillance of the immune system. And studies showed that PD-L1 was overexpressed in the lung tissues [32], lung fibroblasts [33] and CD4 T cells [34] in IPF. Therefore, we speculated that FRDEGs may participate in the development of cancer in patients with IPF according to these pathways.

MicroRNAs (miRNAs), a kind of small non-coding regulatory rna, are composed of 18–25 nucleotides that inhibit the translation or degradation of RNA transcripts in a sequence-specific manner, thus controlling the expression of protein-coding/non-protein-coding genes [35, 36]. To date, several studies have suggested that differently expressed miRNAs, DEGs, and microRNA-controlled differential gene expression represent key topics in the field of biomedical research into pulmonary fibrosis [37–39]. In this study, we constructed a miRNA-target FRDEGs network, and found that ITGB8 has the highest degree in the network, followed by ACSL4 and PIK3CA, which may be important biomarkers for regulating IPF. According to searching in the ILDGB database [40] (a manually curated database of genomics, transcriptomics, proteomics and drug information for interstitial lung diseases), no related study was found for the three genes in patients with IPF.
However, studies have verified the important role of ITGB8 in renal fibrosis [41], ACSL4 in liver fibrosis [42] and PIK3CA in myocardial fibrosis [43]. Therefore, further study is needed.

Subsequently, we verified the expression of 19 candidate key genes derived from the miRNA-target network and the PPI network in the GSE32537 dataset, then, 5 key genes were found. According to linear regression, ACSL1 was the strongest predictor for lung function and quality of life. ACSL1 plays a key role in fatty acid metabolism. Studies have found that lipid metabolism dysregulation play an important role in the pathogenesis of IPF [44, 45]. In addition, the levels of stearic acid (the one of fatty acid) is down-regulated in IPF lung tissues than in control lung tissues, and further study found that stearic acid had antifibrotic activity [45]. Therefore, ACSL1 may play a key role in the development of IPF according to regulating the fatty acid metabolism. Interestingly, ACSL1 is up-regulated in the GSE110147 dataset, however, it is down-regulated in the GSE32537 dataset. The expression level of ACSL1 may need further study to confirm.

The drugs were also screened in DrugBank and PubMed for ACSL1, ITGB8 and CP. Four drugs and sixteen drugs have been found to act on ACSL1 and CP, respectively. For example, representative compound 13 was remarkable inhibitor against not only ACSL1 (IC50 = 0.042 µM) but also other ACSL isoforms [23]. However, more experimental verifications are still needed to prove this hypothesis.

Conclusion

These results suggest that FRDEGs may provide new clues to potential biomarkers and therapeutic targets for predicting the lung function and quality of life of patients with IPF. However, the results need further study to verify.

Abbreviations

idiopathic pulmonary fibrosis, IPF; gene ontology, GO; interstitial lung disease, ILD; telomerase reverse transcriptase, TERT; transforming growth factor beta 1, TGFB1; mucin 5B, MUC5B; regulated cell death, RCD; glutathione peroxidase 4, GPX4; ferroptosis-related genes, FRGs; solute carrier family 7 member 11, SLC7A11; transforming growth factor beta receptor 1, TGFR1; ferroptosis-related differentially expressed genes, FRDEGs; Gene Expression Omnibus, GEO; protein-protein interaction, PPI; differentially expressed genes, DEGs; Kyoto Encyclopedia of Genes and Genomes, KEGG; St. George’s Respiratory Questionnaire, SGRQ; percent predicted forced vital capacity, FVC%; predicted; percent predicted diffusion capacity of the lung for carbon monoxide, Dlco%; Ceruloplasmin, CP; angiopoietin like 4, ANGPTL4; mitogen-activated protein kinase 3, MAPK3; heme oxygenase 1, HMOX1; KRAS proto-oncogene, GTPase, KRAS; heat shock protein family A member 5, HSPA5; ATM serine/threonine kinase, ATM; biological processes, BP; cellular component, CC; molecular function, MF; integrin subunit beta 8, ITGB8; acyl-CoA synthetase long chain family member 1, ACSL1; tumor protein p63, TP63; MYB proto-oncogene, transcription factor, MYB; death ligand-1/programmed cell death 1, PD-L1/PD-1; MicroRNAs, miRNAs.

Declarations
Acknowledgements

Not applicable.

Author contributions

Yang Y performed data collection. Li YP and Ning SW performed data collection, prepared the first manuscript draft, validated data collection, refined the research idea, performed data analysis and edited manuscripts. designed the study and wrote the manuscript. Wang C and Chen H developed the research idea, refined the research idea, validated data collection and edited manuscripts. All authors read and approved the final manuscript. Wang C and Chen H are the guarantor of the manuscript.

Financial/Non-financial disclosures: None

Conflicts of Interest

The authors have no conflicts of interest.

Ethics approval and consent to participate

Not applicable.

Competing interests

Not applicable.

Funding

None

Availability of data and materials

The datasets used and/or analyzed during the current study are available in the GEO repository, https://doi.org/10.1186/s12931-018-0857-1 [46] and https://thorax.bmj.com/content/68/12/1114 [47].

References

1. Richeldi L, Collard HR, Jones MG: Idiopathic pulmonary fibrosis. Lancet 2017, 389(10082):1941-1952.
2. Raghu G, Chen SY, Hou Q, Yeh WS, Collard HR: Incidence and prevalence of idiopathic pulmonary fibrosis in US adults 18-64 years old. The European respiratory journal 2016, 48(1):179-186.
3. King TE, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, Gorina E, Hopkins PM, Kardatzke D, Lancaster L et al: A Phase 3 Trial of Pirfenidone in Patients with Idiopathic Pulmonary Fibrosis. New England Journal of Medicine 2014, 370(22):2083-2092.
4. Lederer DJ, Martinez FJ: Idiopathic Pulmonary Fibrosis. N Engl J Med 2018, 378(19):1811-1823.
5. King TE, Jr., Albera C, Bradford WZ, Costabel U, du Bois RM, Leff JA, Nathan SD, Sahn SA, Valeyre D, Noble PW: All-cause mortality rate in patients with idiopathic pulmonary fibrosis. Implications for the
design and execution of clinical trials. *American journal of respiratory and critical care medicine* 2014, 189(7):825-831.

6. King TE, Jr., Tooze JA, Schwarz MI, Brown KR, Cherniack RM: *Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model.* *American journal of respiratory and critical care medicine* 2001, 164(7):1171-1181.

7. Navaratnam V, Fleming KM, West J, Smith CJ, Jenkins RG, Fogarty A, Hubbard RB: *The rising incidence of idiopathic pulmonary fibrosis in the U.K.* *Thorax* 2011, 66(6):462-467.

8. Petrovski S, Todd JL, Durheim MT, Wang Q, Chien JW, Kelly FL, Frankel C, Mebane CM, Ren Z, Bridgers J *et al.*: *An Exome Sequencing Study to Assess the Role of Rare Genetic Variation in Pulmonary Fibrosis.* *American journal of respiratory and critical care medicine* 2017, 196(1):82-93.

9. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA, 3rd *et al.*: *Telomerase mutations in families with idiopathic pulmonary fibrosis.* *N Engl J Med* 2007, 356(13):1317-1326.

10. Lu J, Liu Q, Wang L, Tu W, Chu H, Ding W, Jiang S, Ma Y, Shi X, Pu W *et al.*: *Increased expression of latent TGF-beta-binding protein 4 affects the fibrotic process in scleroderma by TGF-beta/SMAD signaling.* *Lab Invest* 2017, 97(5):591-601.

11. Seibold MA, Wise AL, Speer MC, Steele MP, Brown KK, Loyd JE, Fingerlin TE, Zhang W, Gudmundsson G, Groshong SD *et al.*: *A common MUC5B promoter polymorphism and pulmonary fibrosis.* *N Engl J Med* 2011, 364(16):1503-1512.

12. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS *et al.*: *Ferroptosis: an iron-dependent form of nonapoptotic cell death.* *Cell* 2012, 149(5):1060-1072.

13. Yatmark P, Morales NP, Chaisri U, Wichaiyo S, Hemstapat W, Srichairatanakool S, Svasti S, Fucharoen S: *Effects of Iron Chelators on Pulmonary Iron Overload and Oxidative Stress in beta-Thalassemic Mice.* *Pharmacology* 2015, 96(3-4):192-199.

14. Gong Y, Wang N, Liu N, Dong H: *Lipid Peroxidation and GPX4 Inhibition Are Common Causes for Myofibroblast Differentiation and Ferroptosis.* *DNA Cell Biol* 2019, 38(7):725-733.

15. Sun L, Dong H, Zhang W, Wang N, Ni N, Bai X, Liu N: *Lipid Peroxidation, GSH Depletion, and SLC7A11 Inhibition are Common Causes of EMT and Ferroptosis in A549 Cells, but Different in Specific Mechanisms.* *DNA Cell Biol* 2020.

16. Murray LA, Argentieri RL, Farrell FX, Bracht M, Sheng H, Whitaker B, Beck H, Tsui P, Cochlin K, Evanoff HL *et al.*: *Hyper-responsiveness of IPF/UIP fibroblasts: interplay between TGFbeta1, IL-13 and CCL2.* *Int J Biochem Cell Biol* 2008, 40(10):2174-2182.

17. Chen Y, Wang X: *miRDB: an online database for prediction of functional microRNA targets.* *Nucleic Acids Res* 2020, 48(D1):D127-D131.

18. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK: *limma powers differential expression analyses for RNA-sequencing and microarray studies.* *Nucleic Acids Res* 2015, 43(7):e47.

19. Yu G, Wang LG, Han Y, He QY: *clusterProfiler: an R package for comparing biological themes among gene clusters.* *OMICS* 2012, 16(5):284-287.
20. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P et al: The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017, 45(D1):D362-D368.

21. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T: Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003, 13(11):2498-2504.

22. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson D, Li C, Sayeeda Z et al: DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res 2018, 46(D1):D1074-D1082.

23. Hayashi K, Kondo N, Omori N, Yoshimoto R, Hato M, Shigaki S, Nagasawa A, Ito M, Okuno T: Discovery of a benzimidazole series as the first highly potent and selective ACSL1 inhibitors. Bioorg Med Chem Lett 2020, 33:127722.

24. Yang G, Wang Y, Feng J, Liu Y, Wang T, Zhao M, Ye L, Zhang X: Aspirin suppresses the abnormal lipid metabolism in liver cancer cells via disrupting an NFkappaB-ACSL1 signaling. Biochem Biophys Res Commun 2017, 486(3):827-832.

25. Meyer KC: Pulmonary fibrosis, part I: epidemiology, pathogenesis, and diagnosis. Expert Rev Respir Med 2017, 11(5):343-359.

26. Selman M, King TE, Pardo A, American Thoracic S, European Respiratory S, American College of Chest P: Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med 2001, 134(2):136-151.

27. Lee HY, Lee J, Lee CH, Han K, Choi SM: Risk of cancer incidence in patients with idiopathic pulmonary fibrosis: A nationwide cohort study. Respirology 2021, 26(2):180-187.

28. Gao X, Tang M, Tian S, Li J, Liu W: A ferroptosis-related gene signature predicts overall survival in patients with lung adenocarcinoma. Future Oncol 2021.

29. Farhan M, Silva M, Li S, Yan F, Fang J, Peng T, Hu J, Tsao MS, Little P, Zheng W: The role of FOXOs and autophagy in cancer and metastasis-Implications in therapeutic development. Med Res Rev 2020, 40(6):2089-2113.

30. Samec M, Liskova A, Koklesova L, Mersakova S, Strnadel J, Kajo K, Pec M, Zhai K, Smejkal K, Mirzaei S et al: Flavonoids Targeting HIF-1: Implications on Cancer Metabolism. Cancers (Basel) 2021, 13(1).

31. Vancheri C: Common pathways in idiopathic pulmonary fibrosis and cancer. Eur Respir Rev 2013, 22(129):265-272.

32. Jovanovic D, Roksandic Milenkovic M, Kotur Stevuljevic J, Markovic J, Ceriman V, Kontic M, Skodric Trifunovic V: Membrane PD-L1 expression and soluble PD-L1 plasma levels in idiopathic pulmonary fibrosis-a pilot study. Journal of thoracic disease 2018, 10(12):6660-6669.

33. Geng Y, Liu X, Liang J, Habel DM, Kulur V, Coelho AL, Deng N, Xie T, Wang Y, Liu N et al: PD-L1 on invasive fibroblasts drives fibrosis in a humanized model of idiopathic pulmonary fibrosis. JCI Insight 2019, 4(6).

34. Celada LJ, Kropski JA, Herazo-May JD, Luo W, Creecy A, Abad AT, Chioma OS, Lee G, Hassell NE, Shaginurova GI et al: PD-1 up-regulation on CD4(+) T cells promotes pulmonary fibrosis through STAT3-mediated IL-17A and TGF-beta1 production. Sci Transl Med 2018, 10(460).
35. Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004, 116(2):281-297.
36. Filipowicz W, Bhattacharyya SN, Sonenberg N: Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008, 9(2):102-114.
37. Rajasekaran S, Rajaguru P, Sudhakar Gandhi PS: MicroRNAs as potential targets for progressive pulmonary fibrosis. *Front Pharmacol* 2015, 6:254.
38. Huang R, Bai C, Liu X, Zhou Y, Hu S, Li D, Xiang J, Chen J, Zhou P: The p53/RMRP/miR122 signaling loop promotes epithelial-mesenchymal transition during the development of silica-induced lung fibrosis by activating the notch pathway. *Chemosphere* 2021, 263:128133.
39. Bagnato G, Roberts WN, Roman J, Gangemi S: A systematic review of overlapping microRNA patterns in systemic sclerosis and idiopathic pulmonary fibrosis. *Eur Respir Rev* 2017, 26(144).
40. Li Y, Wu G, Shang Y, Qi Y, Wang X, Ning S, Chen H: ILDGD: a manually curated database of genomics, transcriptomics, proteomics and drug information for interstitial lung diseases. *BMC Pulm Med* 2020, 20(1):323.
41. Yu J, Yu C, Feng B, Zhan X, Luo N, Yu X, Zhou Q: Intrarenal microRNA signature related to the fibrosis process in chronic kidney disease: identification and functional validation of key miRNAs. *BMC Nephrol* 2019, 20(1):336.
42. Macias-Rodriguez RU, Inzaugarat ME, Ruiz-Margain A, Nelson LJ, Trautwein C, Cubero FJ: Reclassifying Hepatic Cell Death during Liver Damage: Ferroptosis-A Novel Form of Non-Apoptotic Cell Death? *Int J Mol Sci* 2020, 21(5).
43. Yang X, Li X, Lin Q, Xu Q: Up-regulation of microRNA-203 inhibits myocardial fibrosis and oxidative stress in mice with diabetic cardiomyopathy through the inhibition of PI3K/Akt signaling pathway via PIK3CA. *Gene* 2019, 715:143995.
44. Suryadevara V, Ramchandran R, Kamp DW, Natrajan V: Lipid Mediators Regulate Pulmonary Fibrosis: Potential Mechanisms and Signaling Pathways. *Int J Mol Sci* 2020, 21(12).
45. Kim HS, Yoo HJ, Lee KM, Song HE, Kim SJ, Lee JO, Hwang JJ, Song JW: Stearic acid attenuates profibrotic signalling in idiopathic pulmonary fibrosis. *Respirology* 2020.
46. Cecchini MJ, Hosein K, Howlett CJ, Joseph M, Mura M: Comprehensive gene expression profiling identifies distinct and overlapping transcriptional profiles in non-specific interstitial pneumonia and idiopathic pulmonary fibrosis. *Respir Res* 2018, 19(1):153.
47. Yang IV, Coldren CD, Leach SM, Seibold MA, Murphy E, Lin J, Rosen R, Neidermyer AJ, Mckean DF, Groshong SD et al: Expression of cilium-associated genes defines novel molecular subtypes of idiopathic pulmonary fibrosis. *Thorax* 2013, 68(12):1114-1121.