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Metformin as Potential Treatment for Pulmonary Fibrosis Through AMPK Activation and Fibroblast Reprogramming

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ABSTRACT

Pulmonary Fibrosis (PF) is a subtype of ILD (interstitial lung disease) characterized by a chronic and progressive disease causing lung tissue to become thick and stiff due to the overactivation of fibroblasts in the lung tissues. Relating to pulmonary fibrosis, metformin has shown to have antifibrotic effects and in the best cases, a reversal of existing fibrosis unlike any other approved treatments for PF. The aim of study was to conclude how metformin could work to reverse fibrosis in PF through existing datasets. Analyzing miRNAs heavily upregulated in Systematic-Sclerosis associated ILD (SSc-ILD) patents in data processing programs like DAVID and Enrichr had revealed significant activity in the signalling pathway of AMPK enzymes. Metformin revealed a formidable AMPK activator. Upregulation of the AMPK enzyme has shown the lead the the decrease in fibroblast differential into myofibroblast, indicating a decrease in fibrosis. This paper explores the molecular mechanism and potentials of metformin as a drug for pulmonary fibrosis patients. Future research could build on this by testing metformin's antifibrotic effects in clinical trials. This work also points to AMPK as a promising target for treating fibrosis more broadly in other organs as well.

INTRODUCTION

Interstitial Lung Disease is a broad term encompassing the inflammation and fibrosis occurring in the interstitium of the lungs. It causes gases in the lungs to diffuse between cells at a lower rate due to accumulated fibroids in the alveoli. Seen in Figure 1, the blue circle depicts the alveoli at the molecular level. It shows the weakened oxygen and carbon dioxide transfer at the alveoli to capillary interface. The alveolar space (light purple) contains inhaled oxygen. The alveolar epithelium is shown as a thick blue curved barrier. Fibroblasts (spiky purple cells within the wall and airspace) release too much ECM which stiffens and scars the alveolus. Immune cells (dark red and light pink circular cells within the alveolar space) show the inflammatory macrophages and neutrophils, which accumulate during fibrosis overall decreasing the amount of space available for gas to come in. At the alveolar capillary interface, a capillary cross section (red circular structure) contains red blood cells (red ovals) that normally take up oxygen

from the alveolar space and release carbon dioxide. The fibroblasts (beige structures between the blue and red structures) between the capillary and the alveolar inhibit the free exchange of gas. Fibrosis is subtype of ILD including the following diseases: Asbestosis, Coal workers' pneumoconiosis, Post COVID-19 Interstitial Lung Disease, "Hot tub lung" hypersensitivity pneumonitis, Hypersensitivity pneumonitis, Idiopathic non-specific, interstitial pneumonia, Idiopathic pulmonary fibrosis, Interstitial pneumonia with autoimmune features, Mold-related hypersensitivity pneumonitis, Myositis-associated interstitial lung disease, Progressive Pulmonary Fibrosis and Progressive Fibrotic Interstitial Lung Disease, Rheumatoid arthritis-associated interstitial lung disease (RA-ILD), and Scleroderma-associated interstitial lung disease (SSc-ILD) all fall under (Pulmonary Fibrosis Foundation, 2025). This paper addresses all of these diseases under the overarching term Pulmonary Fibrosis. AMP- activated protein kinase (AMPK), a heterotrimeric protein with multiple subunits, “is an energy sensor that regulates cellular metabolism” (Long, 2006). Metformin, a common drug used for controlling the glucose production in the liver for Type 2 diabetes, is an AMPK activator and has shown novel effects in reducing fibrosis in organs. In diabetes, it works by blocking the liver's mitochondrial respiratory chain, which activates the adenosine monophosphate-activated protein kinase (AMPK) pathway (Rena et al., 2017). Since metformin has not had any clinical studies yet, not much is known about its efficacy in different classifications of PF. There is no approved cure for pulmonary fibrosis. Only treatments will help prevent further incoming fibrosis rather than reverse present fibrosis.

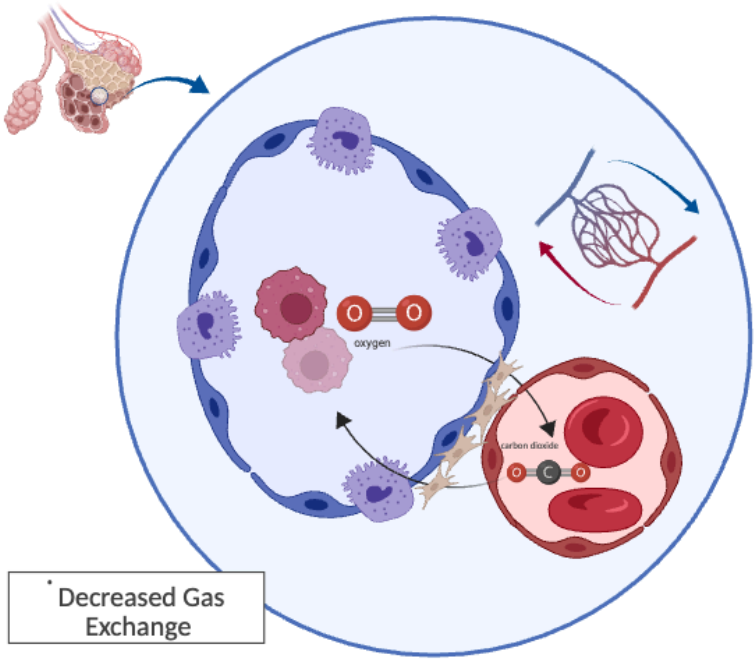

Figure 1: Detailed model of decreased gas exchange in alveoli cells

Epidemiology

ILD and PF prevalence rates are ”on the rise with more than 50,000 new cases diagnosed annually” (Pulmonary Fibrosis Foundation, 2025). The incidence levels annually in IPF specially “is estimated to be 0.57 - 4.51 cases per 100,000 in Asia, 0.33 - 2.51 cases in Europe, and 2.4 - 2.98 cases in North America” (Wang et al., 2024). The high prevalence rates are mainly from “Japan, some Western European countries, and North America is also accompanied by high rates observed in Peru, Bolivia, and Chile, likely associated with mining, smoking and biomass exposure, genetic factors, or their combination” (Sauleda et al., 2018).

Etiology

Currently, Idiopathic Pulmonary Fibrosis (IPF) is the most prevalent form of PF and it is characterized mainly by its unknown etiology. For all forms of ILD, smoking and old age are the number one risk factors for any impairment in the lungs. Fibrosis is, many times, an immune response to any repair after organ damage due to injuries. Currently known, many forms of ILDs including PF are triggered by either a lung injury (caused by smoking or even physical injury to lung), infectious agent (after chronic virus like COVID causing lung impairment), or environmental agent (triggered by exposure to certain molds, animals, or other triggers). Autoimmune disease may also result in the onset of PF due to its connective tissue relations. In Figure 2 below, the top most identified causes of non-idopathic ILDs include inhaled agents; inorganic dust, gases or fumes; organic material like hypersensitivity; pneumonitis; drugs like antimicrobial, chemotherapeutic agents Infections: bacterial, fungal, viral, protozoal; neoplasia like lymphangitis carcinomatosis; Radiation; systemic toxic agents like paraquat; transplantation rejection; other organ disorders; hepatitis, cirrhosis; Left heart failure; Chronic uremia; and Inflammatory bowel disease (Crystal et al., 1981).

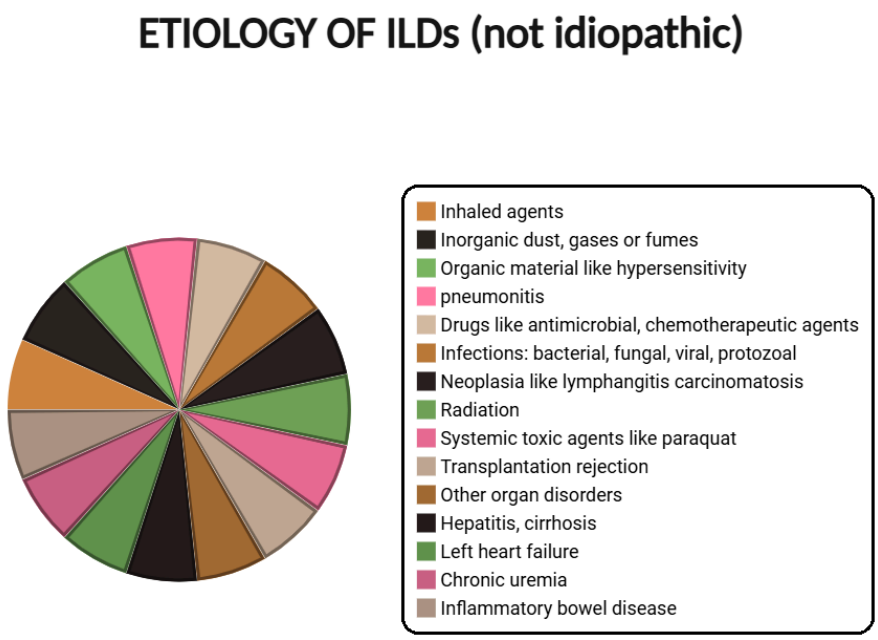

Figure 2: Pie chart displaying etiologies of interstitial lung diseases (ILDs) other than idiopathic forms.

Pathophysiology

The pathogenesis of PF is not understood greatly but the general outline is as follows. Usually with an environmental trigger, infectious agent, or even lung injury, the onset of PF starts. An inflammatory response first, leads to "the hyperactivation of resident cells, such as epithelial or endothelial cells" (Kang & Song, 2023). When the alveoli in the lungs are constantly impaired due to some injury, and the immune system is backloaded with inflammation, fibroblasts are released due to certain signaling molecules like the TGF-β/SMAD3 and the AMPK pathways. Once that happens, they start multiplying and turning into myofibroblasts, which dispose of an excess of extracellular matrix (ECM). Over time, this buildup changes the lung structure (causing honeycombing, a prominent feature of IPF) and makes it stiffer. This stiffness and thickness built up in the lung tissue can cause severe issues with the respiratory system of the patient with very strenuous efforts to breathe. This transition is seen in Figure 3. The top healthy lungs contain alveoli with thin walls that allow for easy gas exchange. The bottom fibrotic lungs show thick and stiff alveolar walls with little alveolar space and increased fibroblasts causing gas exchange to be strenuous. This is why antifibrotic drugs can help slow down lung function loss in people with IPF while normal antiinflammatory drugs are usually only useful in tandem with other antifibrotic drugs.

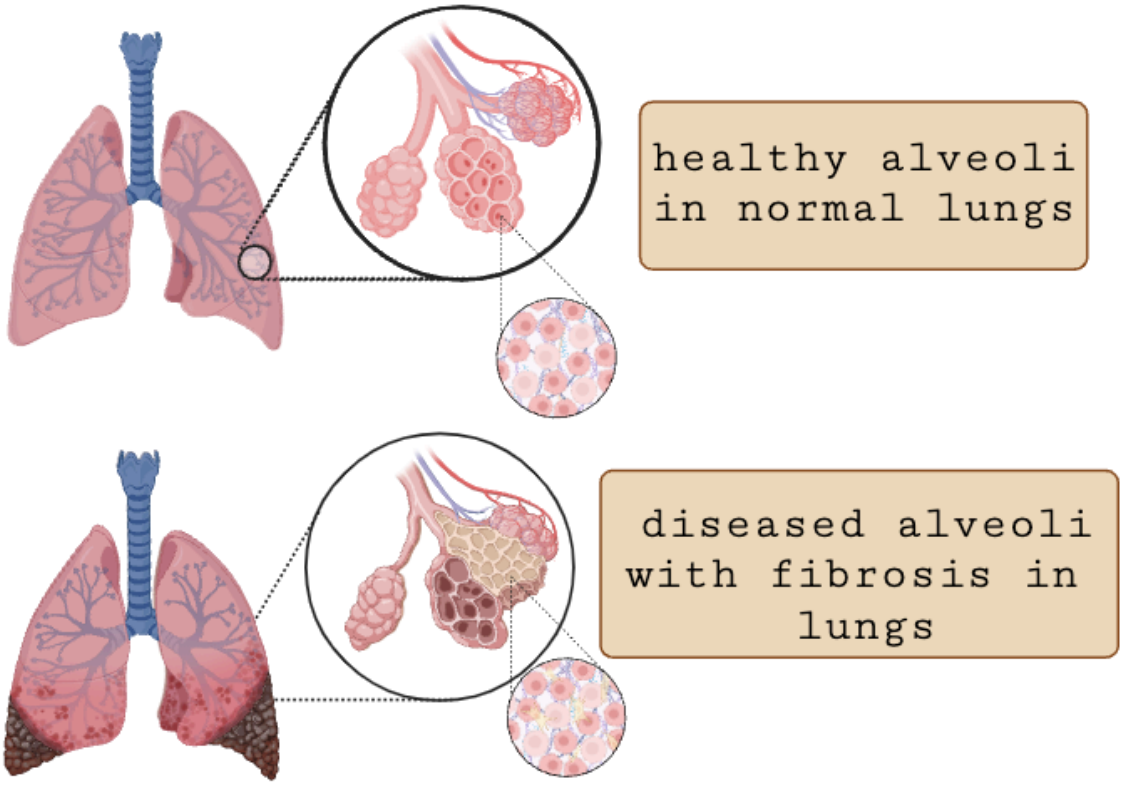

Figure 3: Detailed representation of structural differences between normal and fibrotic lungs.

Treatment

The only two FDA-approved treatments for ILD are nintedanib or pirfenidone which are antifibrotic agents. These drugs mainly target slowing down the progress of fibrosis and/or blocking the immune system to reduce inflammation in the lung tissues. Although these drugs can help in slowing the disease from getting worse, it doesn't actually help in repairing any of the already lost function in the lungs and scarring. Some non-pharmaceutical approaches taken to helping the patient breathe easier are automatic oxygen ventilation and quitting smoking as these can help relieve some of the symptoms they face. Many novel therapies and drugs are being researched now such as TGF-β inhibitors and other stem-cell approaches as well. One particular treatment of interest is metformin due to its notable role in being in AMPK activator, mainly suppress fibroblast differentiation into myofibroblasts and also potentially reverse fibrosis existing already in the lungs (which is something that has not been done with other drugs before).

HYPOTHESIS

Pulmonary fibrosis is associated with the upregulation of the AMPK signaling pathway. The AMPK enzyme helps play a role in regulating energy in the cell and sending stress signals. So, could Metformin, being a key and well known activator of the AMPK pathway, help cure pulmonary fibrosis by reversing lung scarring and serve as a potential treatment option?

METHODOLOGY

For this report, the study GSE81293 was used. This specific dataset contains data from open lung biopsies containing 5 control patients and 15 Systemic Sclerosis-Associated Interstitial Lung Disease (SSc-ILD) patients. This dataset was chosen as it allowed for the comparison of the differential expressed genes between the control group and the diseased patients.

Identification of Differentially Expressed Genes (DEGs) with GEO2R

GEO2R is an interactive online tool within NCBI used to compare and analyze two datasets collected under the same experimental conditions. It employs GEO query and limma to perform differential expression analysis. In this study, GEO2R was used to investigate differentially expressed miRNAs (DEM) between GSE81293 and control samples. DEMs are micro RNAs that exhibit statistically significant changes in expression levels in response to different experimental conditions. These such miRNAs were analyzed further. DEMs were considered statistically significant when $|\log FC| \geq 2$ and the P value < 0.05, serving as the cutoff criteria. The probe IDs were converted to gene symbols. Any single probe corresponding to multiple genes was removed.

Using this data set, different miRNAs were analyzed to determine which were upregulated and which were downregulated using GEO2R. Four of the main upregulated miRNAs were selected, and using the miRDB database, the genes they regulated were identified. Since each miRNA controlled thousands of genes, the genes that were shared by three of the miRNAs were taken and analyzed. There were only two genes that overlapped across all four miRNAs, but using the Human Protein Atlas, these genes, the CLOCK and GMFB genes were not highly expressed in the lungs and were prevalent in nearly all cell types, so hence they were excluded as a possible lead. Using the DAVID analyzer, functional annotation clusters were examined, and their associated functions were evaluated. From this analysis, histone kinase controlling activities had appeared a lot, so they were studied more. This led to the identification of the PRKAA2 gene and it was traced to the AMPK pathway. Reactome was then used to analyze the signaling pathway, which was helpful in understanding possible treatment mechanisms. Using Enrichr with the shortlisted genes common across at least three miRNAs, phosphorylation appeared much as a key process. Connecting all these findings, metformin, an AMPK activator, became a major focus of the project. Since there are no clinical trials including metformin as a treatment for pulmonary fibrosis,

mainly since metformin as an antibiotic drug is still in its preclinical stages, most of the research comes from animal studies and theoretical approaches. Metformin is a promising candidate for fibrosis reduction but clinical trials and patients data is needed to validate this.

RESULTS

Differential Gene Expression Analysis

Using GEO2R to analyze SSc-ILD lung biopsy samples identified, four main upregulated miRNAs (hsa-mir-141, hsa-mir-205, hsa-mir-31, hsa-mir-182) were shortlisted for further analysis. Figure 4 below depicts the differential expression of miRNAs between healthy controls and patients with SSc-ILD. The x-axis represents the log2 (fold change) in expression, and the y-axis shows the -log10 (p-value). The red dots indicate the significantly upregulated miRNAs. The blue dots indicate significantly downregulated miRNAs, and the gray dots represent non-significant changes (Padj < 0.05). These miRNAs regulate thousands of genes each so, to shortlist genes for analysis, only two genes were found acting on all four miRNAs (CLOCK and GMFB). These genes were excluded since they did not have a high expression in the lungs and relative presence in all other cell types so including them would not help to further analyze one pathway.

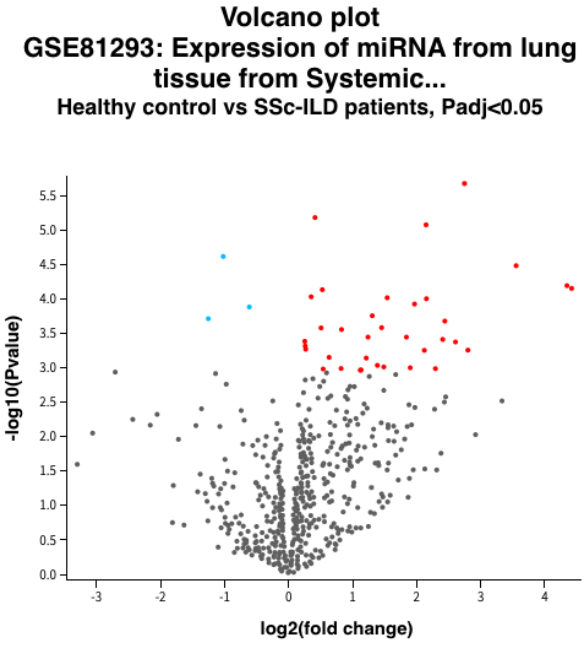

Figure 4: Volcano Plot of differentially expressed miRNAs between SSc-ILD patients and healthy control patients.

Annotation Cluster 3	Enrichment Score: 1.05	G		Count	P_Value	Benjamini
GOTERM_BP_DIRECT	chromatin remodeling	RT		6	1.1E-2	4.5E-1
GOTERM_MF_DIRECT	protein serine kinase activity	RT		4	3.5E-2	4.8E-1
GOTERM_MF_DIRECT	protein serine/threonine kinase activity	RT		4	3.9E-2	4.8E-1
GOTERM_MF_DIRECT	histone H3S10 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H2AXS139 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H3S28 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H4S1 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H2AS1 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H2BS14 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H3T3 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H2AS121 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	Rho-dependent protein serine/threonine kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H2BS36 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H3S57 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H2AT120 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	3-phosphoinositide-dependent protein kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	eukaryotic translation initiation factor 2alpha kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	ribosomal protein S6 kinase activity	RT		3	8.7E-2	4.8E-1
GOTERM_MF_DIRECT	histone H3T45 kinase activity	RT		3	8.8E-2	4.8E-1
GOTERM_MF_DIRECT	DNA-dependent protein kinase activity	RT		3	8.8E-2	4.8E-1
GOTERM_MF_DIRECT	histone H3T11 kinase activity	RT		3	8.8E-2	4.8E-1
GOTERM_MF_DIRECT	histone H3T6 kinase activity	RT		3	8.8E-2	4.8E-1
GOTERM_MF_DIRECT	AMP-activated protein kinase activity	RT		3	8.9E-2	4.8E-1
UP_KW_MOLECULAR_FUNCTION	Kinase	RT		5	1.1E-1	6.5E-1
GOTERM_MF_DIRECT	ATP binding	RT		6	1.9E-1	6.7E-1
UP_KW_LIGAND	ATP-binding	RT		6	4.5E-1	1.0E0
UP_KW_LIGAND	Nucleotide-binding	RT		7	5.2E-1	1.0E0

Figure 5: Functional Annotation Cluster from DAVID displaying high number of genes associated with histone kinase activity.

Meta-Analysis of Target Genes

Using the miRDB, target genes were identified and comparing genes from all miRNAs helped narrow down 43 genes regulated by at least three mi-RNAs. In Figure 5 above, the functional annotation chart using DAVID showed a strong enrichment of genes related to kinase activity with a large number specifically tied to histone kinase functions. Histone kinases are important because they modify chromatin structure and influence whether certain genes are active or silenced. The presence of so many histone associated kinases in this cluster could mean that changes in chromatin remodeling and gene regulation could be important factors of disease process. Using functional annotation clustering tools from DAVID, phosphorylation and histone protein kinase activity were involved in the function of a lot of different genes. Analyzing this further in DAVID led to the finding of the PRKAA2 gene which codes for a subunit of the AMPK enzyme. The AMPK enzyme is key for regulating the cell's metabolism and is crucial for homeostatic processes in the cell.

Pathway Analysis (DAVID and Enrichr)

Using Enrichr and DAVID, the AMPK pathway was associated with the genes the shortlisted mi-RNAs controlled significantly. Reactome analysis also showed this as phosphorylation had connected with the

AMPK pathway a lot. As seen above in ***Figure 5***, many of the genes are associated with histone kinase activity. The activity of these histone proteins mainly relate to when a specific enzyme transfers a phosphate group to a histone protein which is also known as phosphorylation (Mihaylova & Shaw, 2011).

DISCUSSION

Since the AMPK signalling pathway is related in ways to homeostasis of the cell, its connection with fibroids activity was further investigated and multiple other studies had validated this relationship. Hence, looking into possible AMPK activity was the next step. Metformin as a significant activator for the AMPK signalling pathway was seen a lot and further investigated to see its potential impacts in lung fibrosis. The results affirm that metformin can help inhibit the profibrotic signalling in PF as the upregulation of the AMPK pathway has been shown to reprogram fibroblast into a less fibrogenic phenotype. This reprogramming of the fibroblast leads to less fibroblast differentiation and ECM deposition of the cell. Due to this novel function, metformin can reduce fibrosis but also reverse the existing scarred lung tissues present. Seen in figure 6 is the process of metformin being involved in fibrosis reversal. Metformin acts as an AMP-activated protein kinase (AMPK) activator causing inhibition of the transforming growth factor-β1 (TGF-β1) signaling and inhibits phosphorylation of Smad3. These combined effects reduce the differentiation of fibroblasts into myofibroblasts which overall helps prevent fibrosis.

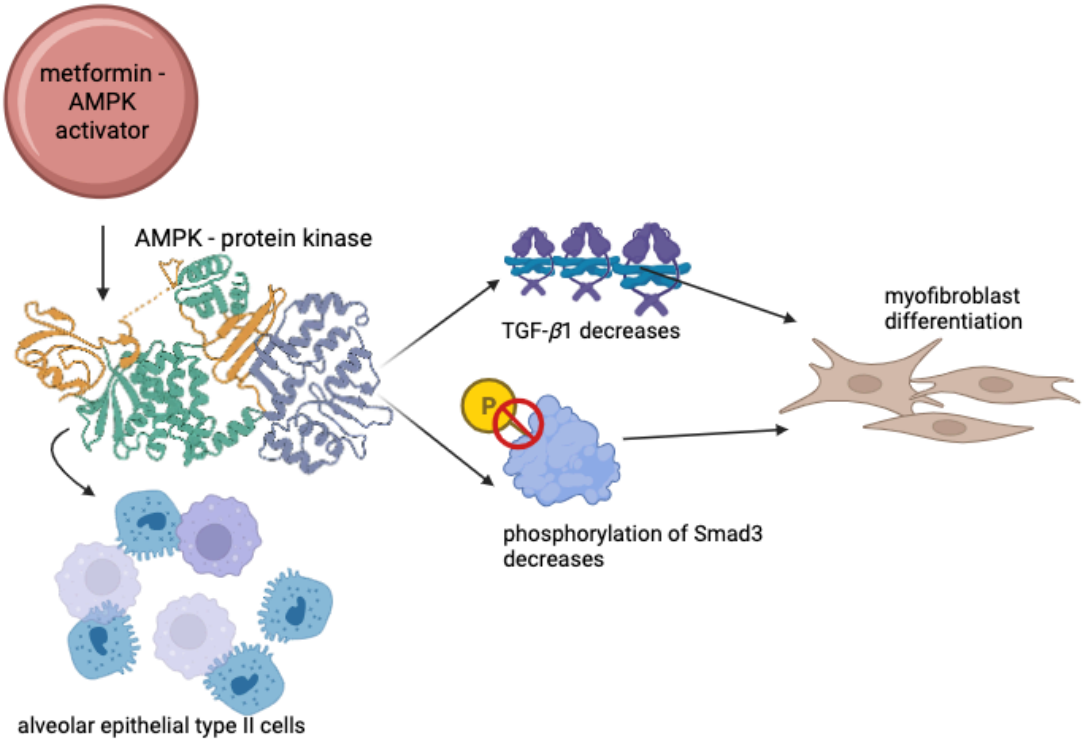

Figure 6: Detailed image of AMPK activation done by Metformin suppressing profibrotic signaling in alveolar epithelial cells.

CONCLUSION

Metformin is a strong candidate as a treatment option for PF considering its AMPK activating functions. In pre-clinical models, metformin has shown to change the fibroblast into a non-fibrogenic phenotype and help reduce myofibroblast differentiation. A total of 19 studies involving 368 animals had shown results where the drug had increased the concentration of the AMPK enzyme in lung tissues therefore has a protective effect on lung tissues in PF animal models and reducing animal death (Wu et al., 2022). This will help reduce the ECM deposition overall decreasing fibrosis. These results provide the potential for using metformin widely for multiple subtypes of ILD, considering that multiple of them include fibrosis as a factor. Further work should be done to validate these findings with larger patient data sets and human clinical studies to investigate the efficacy of metformin in human PF. Other studies focusing on connecting AMPK activation to fibroblast repro-program in the human lung tissues especially will be helpful to further validate the hypothesis. Overall, this study identifies AMPK signalling as a key regulating pathway in PF and describes Metformi as a possible treatment for fibrosis reversal in PF.

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