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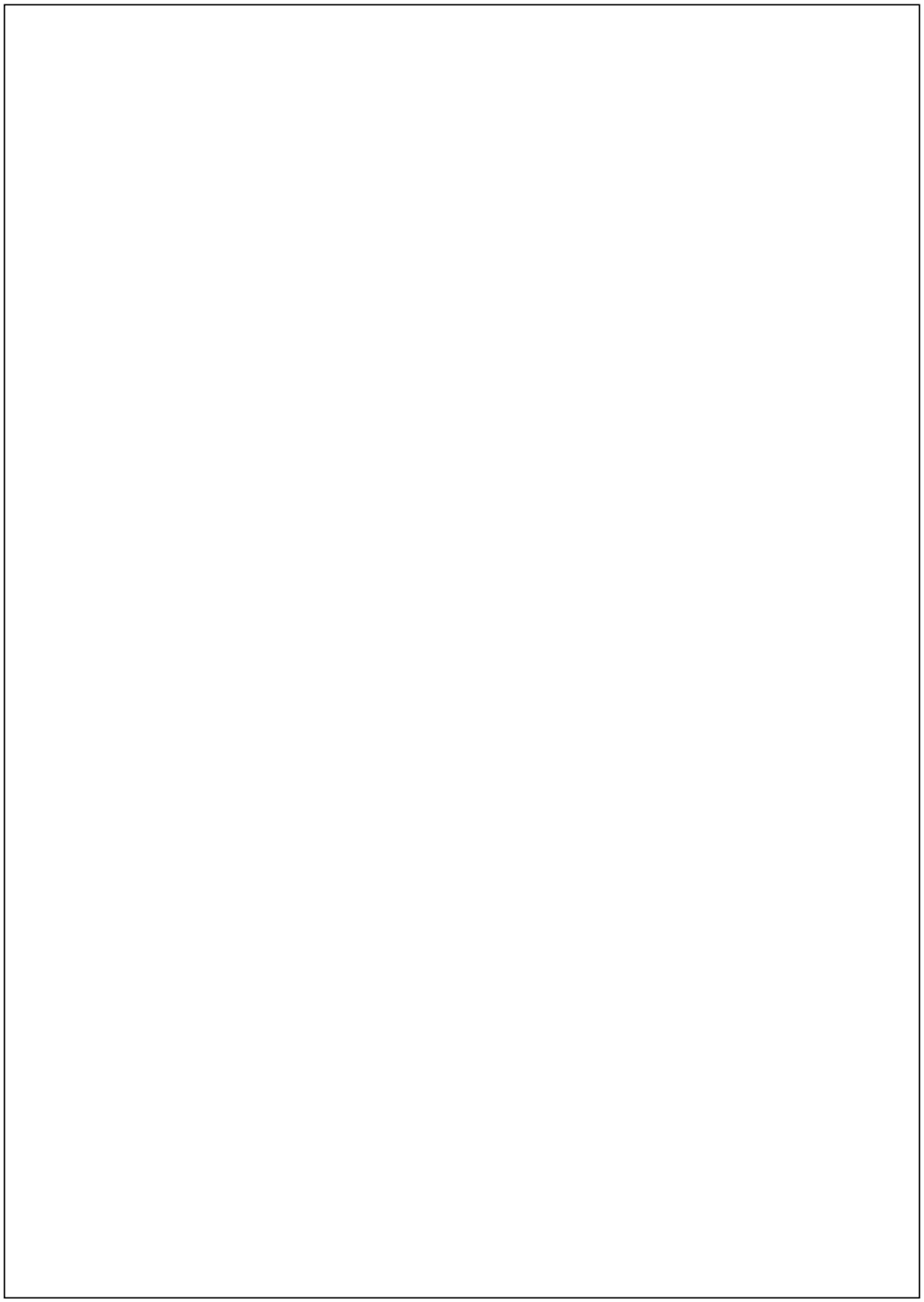

ABSTRACT

Idiopathic Pulmonary Fibrosis (IPF) and Chronic Obstructive Pulmonary Disease (COPD) are progressive respiratory disorders characterized by high mortality rates and pose different clinical manifestations—one by tissue destruction and the other by excessive scarring. However, recent studies are suggestive towards some molecular drivers in common. The present study utilizes an integrative bioinformatics workflow to decode their shared genetic architecture and put forward the common therapeutic targets. The transcriptomic datasets for COPD and IPF were processed to determine differentially expressed genes, thereafter, computational intersection was done using Python and cross-referencing with the Comparative Toxicogenomics Database (CTD) to obtain a set of high confidence common genes. The functional enrichment analysis using Enrichr pointed out the IL-4/IL-13 axis as a major one among the interleukin signalling pathways that had been significantly overrepresented. The human lung co-expression data-based protein-protein interaction network built using NetworkAnalyst showed that TRPV4 was the most centrally located node gene. The incorporation of literature elucidates that TRPV4 acts both as a mechanosensor and an inflammation mediator, thus putting interleukin-driven inflammation in the context of fibrotic remodelling. The outcome of our analysis is that TRPV4 is the commonality of the shared pathology and is thus, a potential common therapeutic target. The results of the present study not only underscore a convergent molecular framework for COPD and IPF but also showcase the potential of integrative bioinformatics in revealing actionable targets for complex, multifactorial diseases.

Keywords – COPD, IPF, IL-4/IL-13 axis, hub genes, TRPV4

CHAPTER 1

INTRODUCTION

1.1 Epidemiology and global prevalence of chronic respiratory diseases

Chronic respiratory disorders contribute substantially to the alleviating global burden and pose a major threat to the public healthcare worldwide. Such respiratory illnesses have been associated with significantly high mortality and morbidity rate. The disease conditions can be primarily categorized into- acute such as influenza or pneumonia, and chronic conditions such as asthma, lung tissue scarring and fibrosis, chronic obstructive pulmonary disease or worse such as lung cancer.[1] Though either of the disease categories pose serious challenge to global healthcare, the case of chronic pulmonary diseases, particularly, Chronic Obstructive Pulmonary Disease abbreviated as COPD and Idiopathic Pulmonary Fibrosis, abbreviated as IPF, deteriorate the quality of life, accounting for the increase in hospitalization rates worldwide[2].

According to the combined GBD (Global Burden of Diseases) data as of 2021, chronic pulmonary ailments affecting primarily the lower region of the respiratory tract attribute to the high morbidity global rate. Amongst chronic pulmonary illnesses, asthma and COPD result for severe loss in productivity among adults, affecting approximately millions across global population. Evidence suggests that COPD alone contributes to 3.2 million morbidity cases globally [1].

The risk factors for pulmonary diseases are multiple, based on the multilayered effect of genetic, clinical and environmental factors. The clinical factors include age, any pre-existing or persisting disease condition, whereas the genetic factors comprise the genotype of an individual. However, primarily in the scenario of respiratory illness, the environmental factors play a vital role. Lifestyle and exposure to environmental toxins, occupational exposure, and poor air quality due to air pollution severely contribute to the disease development. For instance, smoking alone has accounted for almost seventy percent of the COPD cases[1].

1.2 An Overview of Chronic Obstructive Pulmonary Disease (COPD)

Chronic Obstructive Pulmonary Disease (COPD) is attributed to one of the main causes of high mortality and morbidity globally affecting approximately 3 million people annually. The disease is characterized by the limitation of airflow accompanied by inflammation, which is often chronic and is a result of prolonged exposure to harmful particles like cigarette or tobacco particles in smoke[3].

Despite advances in medical sciences and healthcare, COPD has been often neglected owning to the disease complexity and existing heterogeneity across patient population. There has been a gap in research primarily at the molecular level thereby worsening the scenario. Lack of early diagnosis further makes the pulmonary disease irreversible. The major pathological markers associated with COPD include obstructive bronchitis, hypersecretion of mucus and emphysema respectively. The hallmark of this pulmonary disease is the obstruction in airflow and loss of elastic recoil in small airways[4]. Studies indicate that COPD prevails more in the elderly population as compared to younger individuals. Tobacco smoking is one of the major contributors of the disease, accounting

for seventy percent of the cases. The destruction of airflow pathways induced by smoking results in the activation of inflammatory cells such as macrophages and T-cells, resulting in cytokines release. Another factor worsening the disease progression is the release of oxidative reactive species that further destructs the lung tissue. COPD has been categorized into four grades- depending on the disease effects and progression. However, cigarette smoking is not the alone contributor, the disease is a multifaceted effect of multiple factors. Occupational exposure to environmental toxins, leads to the accumulation of harmful air pollutants in the lungs, manganese and cadmium for an instance.

The genetic factors such as absence of the alpha-1 antitrypsin encoding gene also make individuals susceptible to COPD. The protein is absent in the case of autosomal recessive condition for the SERPINA1 gene. Alpha-1 antitrypsin plays a regulatory role in the functioning of proteases released by neutrophils in the inflammatory response[4]. The lack of this antiprotease increases the susceptibility of premature death particularly in the young individuals affected by COPD. Along with the alpha-1 antitrypsin, there are additional genes such as blood group antigens, and alpha-2 macroglobulin, affecting the COPD progression[4].

COPD is categorized as an ageing-related disorder, affecting primarily the elderly population. One of the disease markers, emphysema has been linked to accelerated ageing affecting the lung tissue. Sirtuin 1 gene (SIRT1) is a key regulatory gene affecting the pathophysiology.[4] SIRT1 is pivotal in the regulation of oxidative stress, DNA repair and maintenance of cellular homeostasis. Furthermore, the existing evidence indicates towards the reduced SIRT1 expression in COPD patients, thereby impairing tissue repair and resulting in increased cellular senescence. Another key regulatory gene is SOD2, which regulates the conversion of superoxide dismutase to low toxicity forms, balancing mitochondrial oxidative stress. Ageing thus, has been one of the major clinical factors in COPD development and progression.

The risk of developing a secondary pulmonary infection in the lower respiratory tract, is alleviated in the presence of COPD due to immune dysregulation. This occurs primarily due to weakening of the defense responses due to severe pathological changes induced by COPD such as alveolar macrophage dysfunction and damage to the epithelial barrier.

The current therapies are focused on managing the symptoms, while no therapies aiming at disease progression halting thereby reducing the mortality rate, exist.[2]

1.3 Understanding of Idiopathic Pulmonary Fibrosis (IPF)

Idiopathic Pulmonary Fibrosis (IPF) is characterised by a type of chronic fibrosing pneumonia associated with an unknown cause, defined by the uncontrolled increase in fibroblasts and deposition of the extracellular matrix thus resulting in irreparable lung scarring and most people with this condition live for about 3 to 5 years after they are diagnosed.[5] Dry cough and chronic dyspnoea are the typical symptoms of the disease which is characterized by an irreversible loss in the functioning of lungs,. Though regarded as a rare disease, the cases of IPF patients continues to increase owning to the poor prognosis. Often, there is an overlap between the symptoms of IPF and other respiratory illnesses, resulting in the late diagnosis. IPF prevails more in middle-aged to elderly population, with ageing being a key risk factor.

The risk factors may be multiple, primarily genetic factors, exposure to environmental toxins and smoking. Genetic variations are the main drivers for IPF progression. Several studies point towards the single nucleotide polymorphism associated with the Mucin 5B gene located on the chromosome 11[6]. Another unique cellular process that contributes to the onset of pulmonary fibrosis is disruption of telomere homeostasis. Patients' alveolar epithelial cells have been observed to exhibit telomere shortening.[6]

Oxidative stress induced by exposure to air pollutants and particulate matter drive the disease progression. Air pollutants particularly dust, smoke, and metals such as lead, cadmium, have been associated with the onset of IPF. They impair the cellular homeostasis by releasing free radical species leading to oxidative and nitrosative stress, thereby damaging the lung tissue by fibrosis and inflammation of airways. Air pollution has also been accounted for epigenetic modifications which in turn activate the immune system, thereby causing irreparable damage to alveolar cells. Smoking is one of the most significant risk factors for chronic inflammatory lung conditions. Though, COPD has been the main focus for smoking induced lung tissue damage studies, it has been pointed out that an important link exists between cigarette smoke and the onset of pulmonary fibrosis.

Another risk factor that has often been neglected is the lung microbiota. Bacterial or viral infections affecting the respiratory tract may damage the epithelial barrier by activating immune responses. These immune responses result in the dysregulation of the lung microbiota. As the ratio of lung microbiota becomes disproportionate, the release of pro-fibrotic factors escalates.[6] Thus, the damage to the alveolar epithelial cells increases proportionately with the lung microbiota burden.

Research has also pointed out that the individuals suffering from Gastroesophageal reflux disease (GERD) might develop IPF. The link here is microaspiration.[6] Chronic microaspiration, i.e., the inhalation of gastric contents in the lung airways, increases the risk for IPF significantly. The gastric contents such as proteases, worsen the infiltration and proliferation of fibrosis. This leads to the impairment in tissue repair processes, causing severe damage to the epithelial lining manifested by increased membrane permeability. Long-term or persistent injury attracts fibroblasts and may cause an excessive amount of collagen to be deposited, which thickens the epithelial walls and significantly reduces their flexibility. Current therapeutics aim at anti-fibrotic approaches, targeting at slowing the disease progression[5]. They target the symptoms without curing the pulmonary fibrosis. Potential therapies have been suggested such as the use of monoclonal antibodies or proton-pump inhibitors. Stem cell therapies targeted at the tissue repair and regeneration at the molecular level, utilising the mesenchymal stem cells (MSC) due to their pluripotency are in development.[6]

Thus, it has been implicated that there might exist an overlap between Idiopathic Pulmonary Fibrosis (IPF) and Chronic Obstructive Pulmonary Disease (COPD), which may contribute to the emergence of early diagnostic markers or therapies which may be disease- specific and would be able to target the convergent pathways common to both the chronic pulmonary diseases [2]. The aim of this study is based on this idea, and would be directed towards deciphering the common molecular pathways and identifying the hub genes which are shared between the two diseases, utilising a network-based strategy.

CHAPTER 2

LITERATURE REVIEW

2.1 Understanding the Molecular Basis of COPD

The term "chronic obstructive pulmonary disease" (COPD) refers to a plethora of chronic respiratory conditions that encompasses emphysema and airflow restriction accompanied by inflammation.[7] COPD has been accounted for high morbidity and mortality, affecting approximately 3 million individuals globally. The disease hallmarks include bronchiolitis along with fibrotic condition, small airways obstruction, hypersecretion of mucus and emphysema. As compared to other pulmonary diseases, such as asthma, COPD prevails more in the elderly population, affects predominantly the middle-aged and elder individuals. However, the disease is less explored owing to its complexity and population heterogeneity.

COPD is the multilayered effect of genetic, clinical and environmental factors. However, the highest threat for disease development is posed by tobacco smoking. The estimates reveal that the percentage risk of deaths from smoking-induced COPD is much higher in upper and developed nations than in developing nations. Moreover, genetics play an inherent role over here. It is unlikely that all the smoking individuals develop COPD. But there are chances that such individuals are highly susceptible to develop COPD due to prolonged smoking, at later stages in life. Other environmental factors contributing to the disease are prolonged exposure to fumes, chemical vapours and dust particles especially metal dust, such as lead, cadmium owing to occupation. Air pollution, exposure to second hand smoking, poor ventilation are also significant causes that worsen the disease development and progression.

Genetic factors contribute significantly, such as the absence of alpha-1 antitrypsin gene encoded by SERPINA1, leads to the protease- antiprotease imbalance inducing the epithelial damage. Tumor necrosis factor-alpha (TNF-α), microsomal epoxide hydrolase enzyme, and transforming growth factor (TGF-β) are among the several genes that are linked to an increased risk of getting COPD [8]. Lung microbiota dysregulation, due to bacterial or viral infections in the respiratory tract, also comprise an important risk factor. The lung microbiota burden increases under the effect of any infection, thus, there is an increase in release of inflammatory molecules and proteases due to enhanced activation of the immune cells, leading to fibrosis and destruction of the alveolar cells.

Apart from the environmental and genetic factors discussed above, clinical factors also pose a high risk, making individuals more susceptible to COPD. The major factor here is age. Ageing is mediated by the p-16 and sirtuin protein families, impairing the DNA repair induced by oxidative stress at the cellular level. It has been implicated that the depleted levels of SIRT1 gene in elder individuals, placing them at a higher risk for COPD, causing death in extreme conditions[8]. Gender, though not that much significant, cannot be neglected completely. Men possess higher risk towards COPD as compared to women. However, this ratio does not always hold true, especially while considering the socio-economic status or in the scenario of developed countries. Passive smoking and exposure to smoke particles also influence this ratio.

2.1.1 THE KEY INFLAMMATORY MEDIATORS OF CELLULAR SIGNALING IN COPD

COPD is a very complex condition, and various types of immune cells and fibroblast cells, play a major role in its development. Both the body's natural immune response and the adaptive immune system are involved, helping to drive inflammation by releasing substances like cytokines, proteases, and chemokines.

2.1.1.1 The NF-κB Pathway

There are various pathways- either canonical or non-canonical which result in overexpression of pro-inflammatory factors therefore, driving inflammation and pathogenesis of COPD. NF-κB signaling has been shown to be elevated in COPD patients[8]. A plethora of immunological cells including neutrophils, macrophages, T-lymphocytes, Matrix metalloproteinases (MMPs), proteases, and others, regulated by the NF-κB pathway, are crucial for onset and progression of airway disease. IL-8 released by neutrophils, is a gene regulated by this pathway which leads to the neutrophilia and enhanced neutrophilic infiltration in the small airways, characterizing the small airway disease (SAD).[9]

An increased count in neutrophils is observed in the sputum of COPD patients, indicating to their role in disease progression. By releasing a myriad of tissue destructing and inflammatory mediators, including, Cathepsin-G, proteinase-3, MMP-8 and MMP-9, neutrophil elastase enzyme, neutrophils mediate the alveolar tissue damage and excessive mucus secretion [7]. There is a multi-step process involved in the recruitment and migration of neutrophils, which begins with the adherence to endothelial cells brought on by the overexpression of E-selectin. Trans-endothelial migration is the last stage, which comes after migration to the respiratory system under the influence of chemotactic agents. The survival of neutrophils is prolonged by the release of GM-CSF and G-CSF, growth factors in the respiratory epithelium. Though the precise function and influence of neutrophils in pathophysiology and progression of COPD is unknown, studies indicate towards the release of proteases and oxidative species from the activated neutrophils.

COPD is characterized by the infiltration of immunological cells, both innate and adaptive cells in the airways. All these cells are associated with the release of molecules culminating in hypersecretion of mucus due to wall destruction.

The significant increase in the macrophage count (up to 25-fold) in the mucus and bronchoalveolar lavage fluid of individuals suffering from the disease as compared to the normal smokers suggests a high degree of correlation between macrophages count in the airways and disease severity. This highlights towards the key role played by macrophages in the disease development and pathogenesis. As in the case of neutrophils, the activation of macrophages may be initiated by the tobacco smoke. The macrophages upon activation can produce a variety of cytokines and chemokines, including leukotrienes, IL-8, TNF-α, and CXC chemokines.[7]. Additionally, highly reactive free radicals are released by the activated macrophages. All these inflammatory factors directly or indirectly (through activation of neutrophils or T-lymphocytes) mediate tissue destruction. Apart from these factors, secreted Matrix metalloproteinases (MMP-2, MMP-12 and MMP-9 particularly), cathepsins and neutrophil elastase enzymes by the activated macrophages drive the elastolytic activity. Among all the elastolytic factors, MMP-9 is the most upregulated enzyme in COPD patients.[7] These inflammatory and elastolytic proteins expression are regulated by the NF-κB transcription factor.

Macrophages release various factors leading to the increase in monocytes number, such factors include chemokines like monocyte-selective chemokine (MCP-1), CXCR2, and growth-related oncogene (GRO-α). Another interesting matter of fact is that all monocytes express CCR receptor on their surface, however, the receptors to CXCR2 is expressed by only thirty- percent of monocytes[7]. This points towards the likelihood of such monocytes being transforming into macrophages, leading in turn to the release of more inflammatory proteins. Also, macrophages activate the cytotoxic T-cells or CD8+ T cells by releasing inducible chemokines, for instance, IP-10 and I-TAC.[7] It is known that macrophages undergo phagocytosis for bacterial antigen destruction. Furthermore, a lung microbiota imbalance resulting in elevated bacterial load with respect to the respiratory tract may be caused by compromised phagocytosis.

T-lymphocytes, majorly cytotoxic T-cells (CTLs) and up to some extent, helper T-cells accumulate in the small airways[9]. This infiltration occurs by a process known as lymphocyte homing, similar to neutrophil recruitment and migration as discussed above. T-lymphocyte homing takes place in response to adhesion molecules and chemoattractant molecules secreted by activated macrophages. As compared to healthy people, COPD patients have been found to have higher T-cell counts, particularly CD8+. In the peripheral airways, T-cells display an increased expression of IP-10 and I-TAC. These preferentially express the receptor, CXCR3[7]. Furthermore, bacterial and viral infections mediate the accumulation and colonization of cytotoxic T-lymphocytes in the lower respiratory tract, which becomes responsible for inflammation. Through secretion of granzymes and perforins, additionally TNF-α, the elevated cytotoxic cells significantly drive the inflammatory response and tissue damage. These enzymes lead to cytolysis of alveolar cells. It is still unclear how helper T-cells contribute to the development and course of this disease. However, studies suggest that they may develop memory and initiate the inflammatory response pathway even in the absence of the causative agent, cigarette smoke, for instance. Interferon-γ (IFN-γ), TNF-α, and IL-2 are mainly produced by T-helper cells, especially Th1. [7], activating the macrophages thus promoting the release of proteases and further amplifying the inflammatory response. In some patients, Th17 cells release elevated levels of IL-17 and IL-22 which causes neutrophil recruitment and mucus hypersecretion[9]. All these gene expressions require the presence of the transcription factor, NF-κB, resulting in immune dysregulation.

2.1.1.2 The Matrix Metalloproteinases (MMPs)

Under normal conditions, Matrix Metalloproteinases (MMPs) mediate the tissue repair, wound healing, promote angiogenesis and remodeling of the extracellular matrix. These belong to the family of zinc-dependent proteolytic enzymes, playing an important role in COPD pathophysiology. An imbalance in MMP activity results in emphysema, a characteristic symptom of COPD. Further, emphysema is a result in the proteinase- antiproteinase activity shift in COPD, as the levels of proteinases elevate[8]. Many immunological cells including macrophages, neutrophils, fibroblasts and T-cells release MMPs. Among the key proteinases released by activated macrophages, MMP-8 and MMP-9 are the most essential. An abnormal increase in MMP activity result in impaired tissue repair, damage to extracellular matrix proteins such as collagen and basement membrane components, significantly contributing to airway remodelling.

Additionally, as a result of the MMP activity, Endoplasmic reticulum membrane protein complex (EMC) is degraded and the alveolar cells, structural lung cells, detach and undergo

apoptosis. These EMC fragments further attract the inflammatory cells infiltration into the lung airways, worsening the emphysema in COPD[8].

2.1.2 THE IMPACT OF CELLULAR AGEING ON COPD

Both RNS and ROS are produced under normal physiological conditions as by-products in redox reactions to maintain redox homeostasis. The production of different ROS is achieved by the reduction step of oxygen (O2) to water (H2O) which includes superoxide (O2•-), hydrogen peroxides (H2O2), hydroxyl ions (OH^-) and nitric oxide (NO). The major cellular sites to produce ROS include mitochondria, endoplasmic reticulum, peroxisomes, and NADPH oxidase (NOX). Preliminary studies on ROS suggested it to have detrimental effects on normal cellular functions, but later studies revealed its ability to act as redox signal mediators and defense molecules performing immune functions in the body. The major defense mechanisms used by the immune cells (macrophages and neutrophils) against foreign invasion is the production of ROS/RNS that damages target cell lipids, proteins and DNA.

If we think of ROS and RNS as weapons to fight against microbial invasion, we should also be acquainted with the fact that friendly fire is inevitable just like in any other war. This makes us aware of the deleterious effects of ROS/RNS when present in excess than what is required. Several diseases and disorders, including cancer are linked to the elevated levels of ROS in the body. The disrupted homeostasis caused as a result of sustained oxidative stress is associated with oxidative damage such as increased genetic mutations, altered structures of crucial biomolecules such as lipids, proteins, and nucleic acids. This in turn triggers the antioxidant systems operating in the body to counteract stress conditions and re-establish homeostasis.

An Imperative feature of COPD Is oxidative stress. The Inflammatory cells upregulated by the NF-κB transcription factor, such as neutrophils, macrophages, eosinophils, function by producing reactive oxygen species[9]. Superoxide dismutase, encoded by SOD gene, converts the superoxide ions into hydrogen peroxide (H2O2), which then can be dismuted by catalase enzyme into water. Also, O2•- may combine with H2O2 and NO to form -OH free radical and peroxynitrite respectively. The oxidative damage induced by free radicals produced in the human respiratory system can be counteracted by a myriad of antioxidant processes. These include antioxidants like glutathione (GSH), catalase and superoxide dismutase enzymes. It has been observed that carbon monoxide production is enhanced in COPD patients due to the presence of HO-1, haem oxygenase-1 enzyme, which metabolizes haem, under the effect of oxidative damage in the airways.[7] The effect of ROS on the human airways may be mediated through both ways- directly or indirectly. ROS activates the downstream signal transduction and transcription factors primarily by the NF-κB pathway which in turn leads to the activation of the inflammatory genes, thus amplifying the response.

Oxidative stress also induces the expression of AP-1 transcription factor, and is accompanied by the enhanced release of cytokines such as IL-8, CXC chemokines, MMP-8, MMP-9 and TNF-α.[7] An elevated oxidative stress within the airways also induces the damage in α1-antitrypsin gene, an anti-protease which results in an accelerated breakdown of elastin in the lung parenchymatous tissue. ROS may also directly induce apoptosis of type -1-pneumocytes.[7] Oxidative stress in turn promotes ageing by causing direct DNA damage and telomere shortening. Cellular senescence is induced by an increased oxidative stress by activation of age-related genes such as cyclin-dependent kinase inhibitor (CDKN1A,), HIF1A, MAX dimerization protein 1(MXD1), and Superoxide dismutase-2 (SOD2).[4] All these four

genes have found to be overexpressed in COPD affected individuals. Therefore, there exists a direct link between oxidative stress, ageing and COPD development and progression[8].

2.2 Understanding the Molecular Mechanisms underlying Idiopathic Pulmonary Fibrosis (IPF)

Fibroblast proliferation, excessive extracellular matrix (ECM) deposition, cough, dyspnea, and interstitial fibrosis are the key characteristics associated with Idiopathic Pulmonary Fibrosis, a chronic irreparable respiratory ailment of unknown etiology[10], [11]. This chronic disease results in irreparable lung scarring and most people with this condition live for about 3 to 5 years after they are diagnosed. The intricated pathophysiology associated with this disease requires the complex interaction of a myriad of cells, including the interplay between fibroblasts, cells of the alveolar epithelium, cells of the endothelium, mesenchymal cells (MSC), and most importantly, immunological cells. Studies involving the analysis of multiple IPF samples reflect upon the increased number of airway epithelial cells in comparison to the alveolar epithelial cells. Additionally, the ECM genes were overexpressed and the proportion of activated myofibroblast cells also showed an increase, as compared to the control samples. If we consider the case of the immunomodulatory cells, the dendritic cells as well as the T-cell counts, particularly, T-regulatory cells and alveolar macrophages, increase significantly in IPF patients.[11]

The risk factors are multiple, majorly, environmental and genetic factors. Cellular senescence driven by oxidative stress disrupts the tissue homeostasis, by causing mitochondrial dysfunction, shortening of telomere length and enhanced activation of the transforming growth factor (TGF-β). Disruption in the telomere homeostasis is a rare factor contributing to the development of pulmonary fibrosis.

Air pollutants particularly dust, smoke, and metals such as lead, cadmium, have been associated with the onset of IPF. They impair the cellular homeostasis by releasing free radical species leading to oxidative and nitrosative stress, thereby damaging the lung tissue by fibrosis and inflammation of airways. Air pollution has also been accounted for epigenetic modifications which in turn activate the immune system, thereby causing irreparable damage to alveolar cells. Active or passive smoking is a key risk factor for developing chronic lung ailments. Bacterial or viral infections affecting the respiratory tract may damage the epithelial barrier by activating immune responses. These immune responses result in the dysregulation of the lung microbiota. As the ratio of lung microbiota becomes disproportionate, the release of pro-fibrotic factors escalates.[6] Thus, the damage to the alveolar epithelial cells increases proportionately with the lung microbiota burden. Chronic microaspiration, i.e., the inhalation of gastric contents in the lung airways, increases the risk for IPF significantly. The gastric contents such as proteases, worsen the infiltration and proliferation of fibrosis. This leads to the impairment in tissue repair processes, causing severe damage to the epithelial lining manifested by increased membrane permeability.

Upon exposure of all such risk factors that can potentially cause damage to the lung epithelium, lung homeostasis is disrupted by attenuation of the immune cells, vascular remodelling, activation of pro-fibrotic and pro-inflammatory mediators and signal transduction pathways, for example, TGF-β, Wnt/β catenin, P13K- Akt, resulting in excessive or aberrant thickening of the extracellular matrix (ECM) due to deposition of collagen, fibrinogen. Moreover, there exists an extensive crosstalk between alveolar epithelial cells and mesenchymal or stem cells, further leading to epithelial to mesenchymal transformation.[11]

2.2.1 Role of Alveolar Epithelial Cells (AEC) in IPF Pathophysiology

Pulmonary fibrosis is mainly caused by abnormal alteration in intracellular homeostasis associated with the the alveolar epithelial cells. These cells can be further divided into two categories namely, Type I (AT1) and Type II (AT2) alveolar epithelial cells. Approximately over more than ninety-five percent of the surface area of lung is covered by the cells characterized by a flat morphology. These cells are the AT1 cells. The basement membrane, alveolar wall capillaries and the AT1 cells together, carry out gaseous exchange functions, while also constituting the air-blood barrier. Thus, the efficiency of gas exchange depends upon the transport activities of the AT1 cells. Moreover, the AT1 cells express many receptors on their surface, which induce inflammation, namely the receptors specific for the end products of glycation, known as RAGE and TLR4. However, being terminally differentiated cells, AT1 are not able to further proliferate and differentiate themselves, and are believed to be of relatively less importance in pulmonary fibrosis as compared to the AT2 cells. This is only partially true. The contribution of AT1 cells in the development of fibrosis has been demonstrated further by recent studies. Evidence of the selective expression of a particular receptor RAGE, on the surface of AT1 cells, which controls the inflammatory response which may promote lung fibrosis has also been found. Additionally, recent evidences point out towards the key role of AT1 cells in alveolar regeneration, and could be transdifferentiated into AT2 cells, which could promote alveolar tissue repair[11].

Owning to their unique ability of self-regeneration, proliferation and differentiation, the Type II alveolar epithelial cells (AT2) are also termed as the alveolar stem cells. The important functions of these cells are the surfactant production crucial for lowering of the surface tension, thus preventing the likelihood of alveolar wall collapse. In order to preserve intracellular homeostasis and integrity while encouraging alveologenesis and regeneration, AT2 cells quickly multiply and develop into AT1 cells in response to a variety of stress situations that cause damage to epithelial cells. AXIN2, the Wnt signaling target involved in damage healing and regeneration, is known to be expressed by a fraction of AT2 cells.[11]. Several factors which disrupt the cellular homeostasis, such as, telomere shortening, mitochondrial dysfunction, loss of protein homeostasis, epigenetic changes, alter the AT2 function, causes apoptosis, cytolysis and activates the signaling of fibrotic pathway. Upon initial insult due to various genetic or environmental factors, the alveolar epithelial cells release pro-fibrotic and pro-inflammatory factors. One such pro-fibrotic factor released is TGF-β, which induces fibrogenesis by turning on epithelial to mesenchymal transformation (EMT) and activation of fibroblasts. [11]

Mitochondria provide energy for the cells and also aid in cell growth, proliferation and regulation processes, along with cell apoptosis. DNA damage caused by the free radicals, impair the AT2 cells mitochondrial function, which induces the apoptotic pathway and leads to fibrosis condition. It has been observed that Pink-1, an enzyme responsible for damaged mitochondria phagolysis, is downregulated in individuals affected with IPF, as compared to controls, which in turn accelerates fibrosis by accumulation of damaged mitochondria and apoptosis of AT2 cells. Another mechanism playing a key role in pulmonary fibrosis is the shortening of telomere. Telomere shortening is found to be associated with impaired regeneration capacity of stem cells, AT2 cells. Two key genes which maintain the telomere

integrity and length, Telomere RNA component (TERC) and TERT (Telomere Reverse Transcriptase), are found to be mutated in IPF patients[11]. Additionally, under stress conditions and disrupted AT2 cell function, there is an imbalance of protein homeostasis. All such events lead to endoplasmic reticulum stress, thus activating the TGF-β pathway.[11], [12]

2.2.2 Intercellular Crosstalk in IPF Pathophysiology

Interstitial fibrosis is driven and accelerated by the alveolar epithelial cells, AT1 and AT2 cells in concert with different niche cells including fibroblasts, immunological cells, endothelial cells, and mesenchymal stem cells (MSC). Indeed, the pathophysiology of IPF is based on the intercellular crosstalk between different types of cells. If, we look at the entire process as an example, when there is a stress due to genetic or environmental factors, the epithelium of alveolar cells is damaged. So, in order to repair the compromised fluid transport and gaseous exchange activities, the AT2 cells proliferate and differentiate into the AT1 cells, as discussed above. Cytokines and chemokines, among other pro-fibrotic and inflammatory proteins, are released by the AT1 cells throughout this process. The release of cytokines such as TGF-β is released, which triggers the activation of macrophages and drive the infiltration of neutrophils, causes the aberrant activation of fibroblasts. Formation of more and more aberrant fibroblasts is triggered by the transformation of myofibroblasts, transformation of epithelial to mesenchymal cells (EMT) and endothelial to mesenchymal cell (EndMT) pathways. The growth and normal activity of alveolar epithelial cells (AEC) are further disrupted by dysfunctional fibroblast cells, causing the release of pro-inflammatory proteins, cytokines, into the microenvironment. This promotes immune cell dysfunction which in turn leads to premature cellular apoptosis and senescence, impaired tissue damage repair, and disrupts the networks between epithelial, endothelial and fibroblast cells, thus resulting in persistent tissue damage.[11]

The aberrant function of endothelial cells leads to the marked increase in vascular remodeling, vascular resistance, drives the transformation into fibroblast cells, raises the pulmonary hypertension, thereby, all these actions resulting in fibrinogenesis. A particular subgroup of endothelial cells holds the potential to recruit the immunological cells, promotes extracellular matrix (ECM) deposition, promotes fibroblast proliferation, hence, altogether promoting the pulmonary fibrosis. Unlike all the cells discussed above, mesenchymal stem cells (MSC) prevent aberrant fibroblast cells from proliferating and differentiating. These cells also inhibit the cellular senescence and apoptotic pathways. Subsequently, they are crucial in delaying the process of epithelial to mesenchymal transition (EMT). Hence, they drive the tissue repair process and provide an environment for tissue regeneration.[11]

Table 1. Key cells involved in IPF progression

S.No.	Types of cells	Function	Associated effects in IPF
1.	AT1(Type I alveolar epithelial cell)	Carry out gas exchange and form the air-blood barrier, fluid and ion transport.	Promotes inflammation and impaired gas exchange.
2.	AT2 (Type II alveolar epithelial cell)	Self-regenerating, proliferating stem cells that can differentiate into AT1 cells for tissue damage repair.	Mitochondrial dysfunction, ER stress, activates pro-fibrotic signaling, differentiation into

			fibroblasts, telomere shortening.
3.	Fibroblasts	Responsible for damaged tissue repair.	Mitochondrial dysfunction, telomere shortening, extracellular matrix deposition, vascular remodeling, promotes apoptosis and cellular senescence.
5.	Immunological cells	Release pro-inflammatory cytokines, chemokines, tissue repair.	Immunoregulation, damages the ECM, excessive ECM deposition.
6.	Mesenchymal Stem cells	They possess the ability to heal damage, inhibit apoptosis, proliferate, and give rise to several cell types by the property of differentiation.	Prevent aberrant fibroblast cells from proliferating and differentiating, inhibits apoptotic pathways, and slows down the epithelial to mesenchymal transformation (EMT) process
7.	Endothelial cells	Promote angiogenesis, damaged tissue repair and have immunoregulatory role.	Differentiation into fibroblasts, anti-inflammatory role.

2.2.3 Signal Transduction involved in IPF

As already discussed above, various genetic and environmental factors contribute towards inducing the fibrosis pathway. They act as stimuli, causing chronic inflammation. The excessive accumulation of extracellular matrix (ECM) is the primary cause of fibrosis which can be defined as an abnormal situation marked by tissue hardening and scarring. During the early or initial stages, cells of the immune system are recruited to the site of injury which is triggered by tissue injury. These immune cells along with the alveolar epithelial cells, then become activated and release pro-fibrotic proteins, for instance, TNF-α, PDGF and TGF-β, which become responsible for the cycle of fibrosis[12]. In particular, TGF-β is crucial for the progression of fibrosis by destructing the alveolar epithelial cells (AT1) and inducing apoptosis of AT2 cells. This factor is also linked to myofibroblast activation and the conversion of other cell types (such as endothelium or epithelial cells) into fibroblasts. Research indicates that TGF-β has a significant role in promoting fibroblast cells' non-anchored proliferation, independent of their transformation.

In the cellular signaling involved in IPF, kinases are essential. TGF-β activates the downstream signal transduction pathway by binding selectively to its receptor type II, causing phosphorylation of type I receptor, which is kinase (TβRI). This is followed by the TβRI mediated phosphorylation of SMAD2 and SMAD3 proteins, activating them to bind with SMAD4 protein, forming a complex[12]. This complex upon transported to nucleus then binds to various transcription factors which regulate the expression of the target genes.

TGF-β functions by activating many other pathways also, such as JNK pathway or p38 MAP Kinase pathway. There are several kinases that regulate the fibrosis progression. Sphingosine kinase 1 (SphK1) promotes fibroblast proliferation and migration, while resisting apoptosis.

Another kinase, Pyruvate dehydrogenase kinase 1 (PDK1) regulates the TCA cycle[12], i.e., complete glucose oxidation in the mitochondria, as a result the TGF-β signalling pathway is amplified, resulting in enhanced rate of cell proliferation and myofibroblast differentiation. These kinases are being explored for targeted antifibrotic approach in IPF therapy.[12]

2.3 SIGNALLING BY INTERLEUKINS IN COPD AND IPF

2.3.1 Interleukin signalling involved in COPD progression

The complex nature of this disease necessitates the interplay of multiple cell types, secreting various pro-inflammatory molecules. The most significant inflammatory molecules here are cytokines, a class of low-weight proteins, which are released by activated immune cells including neutrophils and macrophages. One of the biomarkers for COPD is cytokines. Upon the face of any genetic or environmental stress, resulting in initial injury phase of COPD, the recruited immune cells release cytokines that promote inflammation like interleukins. The pathophysiology of COPD is significantly influenced by interleukins (IL-4, IL-6, IL-8, IL-13, and IL-17). Interferon-γ (IFN-γ), IL-2 and TNF-α, produced primarily by T-helper 1 cells [7], which activates macrophages and encourages the release of proteases, hence intensifying the inflammatory response. The T-helper type 2 (Th2) cells release the IL-4 and IL-13 proteins, which control inflammation driven by eosinophils and mucus hypersecretion, which are predominant features of COPD. Additionally, Th17 cells release more IL-17 and IL-22 in some patients, which causes recruitment of neutrophils and mucus hypersecretion. [9].

Another crucial cytokine, which mediates inflammation is Interleukin-6 (IL-6). Evidence suggests that the levels of IL-6 are higher in individuals diagnosed with COPD, in contrast to the normal individuals (controls). Additionally, the levels were found to be varied according to the severity of the disease. This strongly indicates towards the key role of IL-6 in COPD pathogenesis. The inflammatory mediating cytokine, IL-6 has the ability to induce other biomarkers or proteins associated with the acute phase. Furthermore, the elevated levels of IL-6 may exacerbate complications in COPD patients, by contributing towards the development of pulmonary hypertension, thereby increasing the morbidity.[8]

One important cytokine that was first shown to be involved in T-helper 2 (Th2) cell differentiation is IL-4. Additionally, T cells generate it, that activates the proliferation and isotype switching of B-cells, from IgG to IgE[13]. IgE is the antibody associated with allergy and hypersensitivity, expressed on the surfaces of mast cells. IL-4 is also responsible for M2 macrophage differentiation. Thus, significantly contributing towards inflammation and fibrosis in chronic conditions like asthma and COPD. There are two receptors for IL-4. The IL-4 receptor type I has a secondary receptor called γc and an IL-4 receptor α chain. However, IL4 receptor type II is different in the secondary receptor IL-13Rα1, yet it shares the same IL-4 receptor α chain.[14] The interesting matter of fact here is the type I receptor is responsive to only IL-4 protein, on the other hand the type II receptor has is responsive towards IL- 4 as well as IL-13. It is noteworthy that mature T cells have type I receptors, whereas the surfaces of immature T cells express type II receptors. This difference in expression of the secondary receptors, γc and IL-13Rα1, decides the type of receptor the IL-4 binds to initiate signalling.

Upon binding of IL-4 to the Type-I receptor, JAK pathway is activated, and the binding to type II receptor activates both the JAK and TYK2 pathways. Both IL-4 and IL-13 are responsible for STAT6 activation which binds to the docking site on phosphorylated tyrosine, for downstream cellular signalling.[14]. IL-13 binds to the same type II receptor and along with

IL-4, induces, T-helper 2 differentiation, mucus hypersecretion, airway remodeling and IgE driven responses. Furthermore, age and IL-13 expression in COPD patients are correlated, according to recent research.[15]

2.3.2 Interleukins associated with Idiopathic Pulmonary Fibrosis (IPF)

As we know, interleukins are cytokines that mediate inflammation. In the context of IPF, the interleukin family can be subdivided into three categories due to their diverse effects- interleukins that promote pulmonary fibrosis, those which inhibit fibrosis, and interleukins which have dual effect on IPF pathogenesis. Clinical research found that the blood and bronchoalveolar lavage fluid of diseased individuals had greater levels of IL-10, IL-12, IL-1β, IL-2 and IL-17A than controls.[16]

The two isoforms of IL-1 that promote lymphocyte proliferation are IL-1α and IL-1β. IL-1α is produced by both hematopoietic and non-hematopoietic cells and mediates the fibroblasts conversion, whereas IL-1β is produced by mononuclear phagocytes and causes fibrosis by attracting neutrophils and lymphocytes to the site of damage. According to studies, IL-1 may be a useful treatment target for pulmonary fibrosis. IL-6 is produced by monocytes and lymphocytes in the process of wound healing. IL-6 is a promoter of lung fibrosis by switching on the TGF-β pathway. It mediates the acute inflammatory reactions and encourages the growth and development of T and B lymphocytes. Additionally, IL-6 has been found to induce collagen and excess extracellular matrix deposition, thereby, promoting pulmonary fibrosis.

IL-8 is a CXC family chemokine, playing a key role in delayed-type hypersensitivity (DTH), cellular immunity and inflammation, primarily inducing chemotaxis, neutrophil activation and recruitment, while also promoting the phagocytosis of neutrophils by lysosomal enzymes. IL-8 is known to be a mediator of cytotoxicity. It also activates B lymphocytes for production of antibodies. This CXC chemokine is also involved in vascular remodeling, promoting differentiation of myofibroblasts and proliferation of fibroblasts.[10]

The main producers of IL-13 are macrophages, Th2 lymphocytes, and epithelial cells. Because IL-4 as well as IL-13 share amino acid sequences, they operate similarly attributed to their shared homology.[10] The proinflammatory cytokine IL-13 has a well-established involvement in inflammatory bowel disease, asthma, and allergy inflammation. IL-13 promotes mucus formation, smooth muscle cell contraction and extracellular matrix deposition, and fibroblast activation and proliferation. Furthermore, fibronectin and profibrotic cytokines including TGF-β, connective tissue growth factor, PDGF, and collagen type I can be stimulated by IL-13. Inflammatory chemicals including TNF-α, IL-6, and IL-1 are suppressed by IL-10, which stimulates macrophages and can boost the immune system in a range of cell types. IL-10 inhibits the activation, migration, and adhesion of inflammatory cells by downregulating the expression of major histocompatibility antigen II on the surface of monocytes, decreasing its antigen-presenting effect, and downregulating T lymphocyte activity. In addition, IL-10 can attenuate the inflammatory response by lowering inflammatory cytokines such IL-2, INF-γ, TNF-β, and CSF-GM. Additionally, IL-10 suppresses the expression of adhesion molecules and cytokines like TNF-α, IL-1β, and IL-8.

The pathophysiology of PF Is largely dependent on the Th1/Th2 Imbalance, with Th1-type cells represented by IFN-γ, which may aid in the restoration of normal tissue structures, and cytokines released by Th2 cells namely IL-4, which can lead to fibrosis, ECM deposition, and excessive damage repair. Fibrosis may develop when the ratio of Th1/Th2 type cytokines changes in favor of Th2 type cytokines. Th2 cell proliferation and differentiation are inhibited

by IL-12, while Th1 cell multiplication and differentiation are stimulated to produce Th1-type cytokines.[10]

IL-4 is a key cytokine, initially identified as a factor which is responsible for differentiation of T-helper 2 (Th2) cells. The primary contributors of this pleotropic cytokine include activated T lymphocytes, eosinophils, basophils, and macrophages. As discussed in the context of COPD, IL is associated with a dual immunomodulatory effect, i.e., it possesses both pro- and anti-inflammatory actions. While pharmaceutical treatment promotes IL-4-induced autophagy in lung tissue and macrophages in rats administered BLM, IL-4 lowers pulmonary fibrosis by inhibiting collagen deposition, M2 polarization, and macrophage infiltration. However, IL-4 increases in the bronchoalveolar lavage fluid of mice and in the blood of PF patients after BLM poisoning. IL-4's main functions include stimulating dendritic cells to transmit antigens to other immune cells and inducing Th2 responses.[10] Furthermore, the action of IL-24 in synergy with IL-4 stimulates polarization of macrophages toward the M2 which accelerates the growth of pulmonary fibrosis. However, phenotype M2 macrophages can exhibit a pro-wound healing and anti-inflammatory phenotype; when this process is disrupted or impaired, excessive activation of both responses may lead to fibrosis development. Additionally, earlier research has strongly suggested that Th2-type cytokines, particularly those activated by IL-4, play a crucial role in macrophage activation, as well as in the proliferation and differentiation of fibroblasts, and the deposition of extracellular matrix (ECM), all of which are closely associated with the onset of fibrosis and ultimately contribute to the formation of pulmonary fibrosis. Therefore, IL-4 belongs to the category having dual impact on IPF pathogenesis, i.e., both stimulatory and inhibitory. [10]

The dismal and late of prognosis IL-11 results in a number of disorders and is associated with an imperative role in normal growth and development, premature aging, inflammatory process, and fibrosis progression[16]. As a downstream core factor for TGF-β's profibrotic actions, IL-11 has garnered significant research interest. IL-11 protein expression rises with age, contributes to the development of chronic sterile inflammation linked to aging, and is linked to the emergence of aging-related illnesses including IPF. Additionally, TGF-β and other profibrotic factors are positively regulated by IL-11. Nevertheless, little is known about the precise processes via which IL-11 contributes to IPF.[17]

2.4 ROLE OF TRPV4 IN INFLAMMATION AND FIBROSIS

The calcium-permeable, non-selective cation channel known as Transient Receptor Potential Vanilloid (TRPV4) is encoded by 15 exons in mammalian cells. The channel is activated by different kinds of stimuli such as mechanical stress, changes in osmolarity, and inflammatory mediators.[18] The activation of the TRPV4 gene leads to the calcium influx into the cells, which can, in turn, affect the fibroblast activation, immune cell, and epithelial barrier functions.[19]

TRPV4 has been recognized as an important cough mediator, which is a major symptom in COPD, and its levels are high in the airway epithelium and immune cells during lung inflammation.[20] The channel's participation in several signalling pathways, such as ones associated with WNT/β-catenin signalling and mitochondrial iron regulation, reiterates its wide-ranging influence on cellular balance and its likelihood of becoming a therapeutic intervention site.[3]

When the TRPV4 channel is activated in macrophage and airway epithelial cells, cytokines that promote inflammation are released which attracts and recruits neutrophils to the injury

sites, thereby, directly contributor to the persistent inflammatory situation that characterizes COPD.[21] The nonselective ion channel also acts a key mechanosensor which plays an imperative role in determining the connective tissue's stiffness and correlating it to the pro-fibrotic signalling. As a result, fibroblasts become myofibroblasts and collagen is deposited, which are mediated by the calcium-dependent pathways, thus contributing to fibrosis as evident in the case of IPF.[22] It has also been shown that TRPV4 inhibition results in significant reduction in inflammation in COPD progression. Furthermore, inflammatory cytokines released by activated immune cells might make the TRPV4 channels more sensitive and responsive to Calcium ions, resulting in sustained activation of inflammatory cascade. This alternatively results in gradual stiffening of lung extracellular matrix driving the fibroblast activation, and thus, worsening the IPF progression.

2.5 NETWORK BASED BIOINFORMATICS APPROACH FOR THERAPEUTIC TARGET IDENTIFICATION

With high-throughput experimental methods, a massive amount of biological data is produced, making us well-positioned to decipher the molecular basis of the complex diseases. In traditional methods, individual genes or proteins are considered. However, these traditional methods are inadequate in understanding the complicated biological network of multifactorial diseases. It is in this context that the integrated network-based bioinformatics has emerged as a promising systems-level approach to decipher the molecular mechanism underlying diseases and identify potential therapeutic targets. Integrative network-based bioinformatics is the method to combine data sets from different sources, including gene expression profiles, protein interactions, signal transduction pathways, functional annotations and disease related databases, in order to build complex interaction networks representing complex biological interaction between molecules within a cellular system. We can identify the highly interconnected molecules, regulated modules, and important signaling pathways linked to the onset and course of the illness by analysing such an interaction network.[2] The use of network-based methods including enrichment of cellular pathways, gene co-expression analysis, analysis of protein-protein interaction and topological network analysis have enabled identification of significant genes and pathways according to their connectivity and relevance rather than solely on expression change. Using public databases (genomic repositories, disease databases) and bioinformatics tools may allow us to cross validate the disease related molecular signature, thus increasing the reliability and biological significance of the identified targets.[20], [23]

2.6 SIGNIFICANCE OF COMPARATIVE STUDY OF COPD AND IPF

Although the diseases show completely different clinical manifestations—parenchymal destruction in emphysema and excessive scarring in fibrosis—they have molecular similarities that are surprising. The new evidence purports that the pathogenic pathways converge. These common pathways bring to mind the notion of common hub genes which are highly interconnected molecular players in the protein networks that may drive the core pathology in both diseases. The discovery of such common molecular nodes is a critical step for the therapeutic progress. The presently available treatments for COPD and IPF are mainly disease-specific, with little effectiveness and no potential of curing the disease. This accentuates the urgent need to find out the new targets that would deal with the overlapping.[2]

CHAPTER 3

METHODOLOGY

3.1. Dataset Acquisition

The Gene Expression Omnibus (GEO) database, a public source of high throughput genomics data, was used to comprehensively acquire microarray expression datasets from both Idiopathic Pulmonary Fibrosis (IPF) and Chronic Obstructive Pulmonary Disease (COPD). The datasets were selected based on the basis of criterias like human tissue samples, case-control study designs, and availability of raw or normalized expression matrices etc. for each disease. The COPD and IPF datasets collected primarily contained lung tissue of COPD and IPF patients and control healthy subjects.

3.2. Gene Selection

A comprehensive resource integrating chemical-gene-disease interactions, such as the Comparative Toxicogenomics Database (CTD), was utilised for systemic gene retrieval. For each disease under study (COPD and IPF), the first 100 disease-related genes were retrieved with official names or the respective GEO ID. The disease-relatedness of genes was ranked using the pre-compiled gene-disease association score of the CTD (which is an aggregated measure of direct and inferred interactions reported in published literature). To assure biological relevance and limit false positives, only genes with a high confidence association score were taken.

Table 2. List of top 100 retrieved genes involved in COPD

CXCL8	CD8A	LCN1	IL10	NFKB1
TNF	MMP14	TNFSF8	NR3C1	NFKBIA
IL6	EPHX1	HMOX1	IL4	RB1
ICAM1	TNNT2	NPY	IL1B	RELA
MMP9	KLF5	ITGB6	FN1	MAPK1
TGFB1	SERPINA1	ELN	CCL3	IL1RL1
NOS2	VEGFA	SERPINA1	TNFRSF1B	FGF2
NOS3	RAPGEF3	CYP2A6	IL17A	HSPA5
CXCL2	HDAC2	EPHX1	FOS	CD47
SOD3	ELN	MIR218-2	CAT	PPARG
CDKN2A	MTCL1	SCNN1B	CAT	EZR
SFTPD	TRPV4	TGFB1	IFNG	ADM
CXCL1	FAM13A	EDN1	IFNG	
HMOX1	HTR2A	IL13	MPO	
CRP	DSP	CXCL8	CD44	
CDKN1A	AGER	IL1B	IL4	
CYP1A1	TNF	CCL5	ACTA2	
TP53	TNFRSF8	CSF2	SOD2	
HIF1A	MMP9	TNF	TIMP1	
MIF	EEFSEC	IL6	ADRB2	
CYP1A2	MMP2	CSF2	VEGFA	

FOXO3	MMP12	ADRB1	IL2	

Table 3. List of top 100 retrieved genes involved in IPF

STAT3	GRIK3	SFRP1	KIT
ST14	ACTA2	TIMP1	S100A4
TERT	COL1A1	HIST1H2BG	MIR181D
ESR1	S1PR5	CXCL14	TGFBR2
SFTPA2	IL17RD	EDN1	TNFRSF11B
TNF	CDH2	ETS1	LGALS3
SFTPC	PRELP	CX3CL1	ANXA2
TERC	MYO1E	CLDN4	MIR32
PARN	LHFPL6	S100A11	CALCA
RTEL1	CCN2	HPGD	NRG1
WNT5A	DLK1	AQP1	KRT8
DSP	MIR1949	S100A6	ZNF600
GPER1	COL14A1	OVOS2P	ANXA1
ATP11A	IL26	EZR	SMAD2
DPP9	AGTR1	GSK3B	HGF
FAM13A	KAT2B	GPX1	MIR133B
MUC5B	NR2F2	ANXA3	MIR148B
STN1	FGF7	PDGFA	TNFSF11
HIF1A	COL6A3	MUC3A	TRPC2
MMP3	PNP	GATA3	NOX4
PLAU	SOCS1	ANXA2P3	ACE
CCL12	F2RL1	KRT19	ANXA2P2
BIRC5	RAC2	OGG1	MIR342
CDH1	SPP1	PLA2G4A	MMP2
IL12RB2	PODXL	SOX2	AKT1

3.3. Common Genes Identification

The identification of overlapping genetic signatures between COPD and IPF was performed computationally using Python programming language. Specifically, the pandas library—a highly efficient data processing and analysis toolkit—was employed for data manipulation and intersection operations. The top 100 gene lists for COPD and IPF were loaded as separate pandas Series objects, and the common gene set was derived using set intersection methods. The resulting list of shared genes represented the molecular intersection between the two diseases, providing a focused gene set for subsequent network and pathway analyses. All operations were validated by manual cross-referencing to ensure accuracy.

3.4. Construction of Tissue specific-PPI Network

The set of common genes was subjected to an integrative analysis in a web-based comprehensive gene expression analysis and visualization network tool called Network Analyst. A tissue-specific co-expression network was generated by plotting the set of common genes onto precomputed co-expression data from the human lung tissue, representing the etiology of COPD and IPF pathogenesis. Lung tissue was selected based on its role related to the pathophysiology of COPD and IPF, respectively. Using topological

scoring algorithms of Network Analyst, the highest connected nodes (degree centrality) with highest betweenness centrality of the bipartite co-expression network were designated as the hub genes.

3.5. Functional Enrichment Analysis

To determine the biological significance of the discovered hub genes, a functional enrichment evaluation was conducted utilizing well-known bioinformatics methods. Enrichr, a popular gene set enrichment analysis program, was used to conduct the functional enrichment evaluation. Significant biological pathways were identified from the Reactome Pathways 2024 database, which has a collection of curated, peer-reviewed human biological pathways and reactions. Adjusted p-values were used to assess each pathway's statistical significance, with p value less than 0.05 representing significance. The pathways with the highest enrichment were highlighted in regards to inflammation, immune response, and vasculature dysfunction.

3.6. Evaluation of Therapeutic Target

The hub genes from tissue-specific co-expression network were examined as potential therapeutic target using a two- prong approach. Firstly, structural and functional interconnectedness among hub genes in common disease network was identified, where we search for the nodes that act as link between two disease subnetworks (COPD and IPF subnetworks). Second, the hub genes were correlated with aging related pathological progression and in particularly focused on inflammaging. The hub genes, which not only display significant interconnectedness among in disease network but also have established connection with inflammaging or cellular senescence related pathways were chosen as ideal therapeutic targets. The two-prong evaluation helped us identifying the targets that can link two diseases by bridging them in mechanistic way and also can be addressed as targeting the age-related immune dysregulation.

CHAPTER 4

RESULTS AND INTERPRETATION

4.1. Identification of Common Genes

60 common genes to COPD and IPF were identified.

Table 4. List of Common Genes

S.No.	Common Genes	S.No.	Common Genes	S.No.	Common Genes
1.	EDN1	21.	SPP1	41.	TRPV4
2.	CXCL10	22.	CXCR4	42.	CCL4
3.	RAPGEF3	23.	CCN2	43.	ACTA2
4.	IL4	24.	IL13	44.	CASP8
5.	FN1	25.	BCL2	45.	HMOX1
6.	TNFRSF1A	26.	CSF2	46.	TIMP1
7.	STAT3	27.	FAM13A	47.	MAPK14
8.	TGFBR2	28.	IL2	48.	SERPINE1
9.	AKT1	29.	SMAD2	49.	TNF
10.	AQP1	30.	NFE2L2	50.	WNT5A
11.	CCL3	31.	PTGS1	51.	VEGFA
12.	PLAU	32.	NOS2	52.	CCL5
13.	TGFB1	33.	DSP	53.	COL1A1
14.	IL1A	34.	MMP2	54.	RELA
15.	CALCA	35.	GPX1	55.	DLL4
16.	HIF1A	36.	ANXA3	56.	IL12B
17.	CASP9	37.	ANXA1	57.	CTNNB1
18.	CD44	38.	JUN	58.	EZR
19.	MMP9	39.	CYP3A4	59.	RAC2
20.	MAPK8	40.	ICAM1	60.	ATF3

4.2. Reactome Pathway Analysis reveals shared Inflammatory Pathways

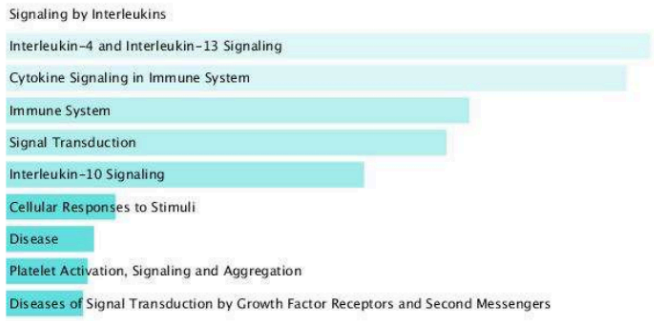

Fig. 1. Reactome Pathways representing shared inflammatory pathways

The functional enrichment analysis based on the 60 common genes retrieved, indicates a set of significantly overrepresented Reactome pathways, sorted by the p-values. The result unambiguously points out that the overlapping genetic signature of COPD and IPF is primarily a specific immune system dysregulation involving interleukin pathways.

The prominence of IL-4 and IL-13 signalling as indicated among the top hits by the lowest p-values is imperative, as they foster alternative (M2) macrophage activation, fibroblast proliferation, and collagen production—all the symptoms of fibrosis in IPF. Their large presence in the overlap of the COPD and IPF profiles seems to indicate a previously overlooked but essential role of this immune axis in the pathogenesis of COPD, perhaps even accounting for the small airway remodelling and fibrotic responses observed in a large number of patients.

4.3. Gene Co-expression Network Analysis Identifies the Hub gene

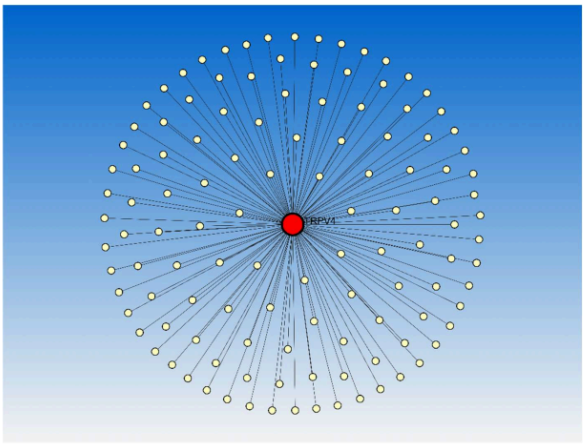

Fig. 2. The tissue specific co-expression network reveals TRPV4 as the central hub gene

One of the most important hub genes, according to topological study, is TRPV4 (Transient Receptor Potential Vanilloid 4), based on its high degree centrality, indicating numerous functional interactions within the shared network. The identification of TRPV4 as a hub gene provides a crosslink between the enriched inflammatory pathways and downstream disease pathology, indicating that certain nodes could connect immune signalling to structural/remodelling functions. TRPV4 is a non-selective, calcium-permeable cation channel, encoded by 15 exons in mammalian cells. The channel is activated by different kinds of stimuli such as mechanical stress, changes in osmolarity, and inflammatory mediators [18].

CHAPTER 5

DISCUSSION

The activation of the TRPV4 gene leads to the calcium influx into the cells, which can, in turn, affect the fibroblast activation, immune cell, and epithelial barrier functions that are relevant to both COPD and IPF [19]. The TRPV4 gene dysregulation has been linked to the chronic inflammatory and fibrotic conditions, thus its position as a potential therapeutic target shared between these complex lung diseases has been underlined. Furthermore, TRPV4 has been recognized as an important cough mediator, which is a major symptom in COPD, and its levels are high in the airway epithelium and immune cells during lung inflammation[20]. The channel's participation in several signalling pathways, such as ones associated with WNT/β-catenin signalling and mitochondrial iron regulation, reiterates its wide-ranging influence on cellular balance and its likelihood of becoming a therapeutic intervention site for both COPD and IPF [2]. The liberation of neutrophilic inflammation and cytokines (IL 8, TNF-α) occurring due to TRPV4 activation in the airway epithelial and macrophage cells is a direct contributor to the persistent inflammatory situation that characterizes COPD [21]. The nonselective ion channel also acts a key mechanosensor which plays an imperative role in determining the connective tissue's stiffness and correlating it to the pro-fibrotic signalling. As a result, fibroblasts become myofibroblasts and collagen is deposited, which are mediated by the calcium-dependent pathways, thus contributing to fibrosis as evident in the case of IPF[22]. It has also been shown that TRPV4 inhibition results in significant reduction in inflammation in COPD progression. This suggests that identifying TRPV4 antagonists may serve as excellent target central to both the disease progression, COPD, and IPF [24].

The TRPV family comprises TRPV1 to TRPV6, however, two of the six ion channels from the family are highly selective to calcium ions, these channels, namely TRPV5 and TRPV6 are temperature insensitive also. In the contrast, TRPV1 to TRPV4 channels are characterized by non-selectiveness and temperature variation sensitivity[25], [26]. Cryo-EM studies have further confirmed the structure of the TRPV4 channel, suggesting that the ion channel possesses a tetrameric structure [27]. Functional genomics and transcriptomics studies using small molecules that can function as agonists or antagonists, have been conducted recently, on knock out mice models. Among the small molecule agonists for TRPV4, 4α-phorbol 12,13-didecanoate, abbreviated as 4αPDD 1 is the most structurally characterized[26]. It is a phorbol ester, which is highly selective for TRPV4 activation. This means, 4αPDD 1 cannot activate other channels of the TRPV family. This small molecule agonist has been administered in separate in vivo studies to determine the functions of TRPV4 primarily in mechanosensation and osmosensation by selective activation of the ion channel. The phorbol esters' structure is crucial in determining how well they bind to the channel. TRPV4 is highly preferential for the phorbol site for binding. [26]

Among another potent agonists for TRPV4, GSK1016790A stands out[26]. It is a piperazine derivative drug, which is responsible for enhancing the intracellular calcium levels. Comprehensive studies conducted in-vivo have pointed out the fact that the binding affinity and thus the current density of TRPV4 for GSK1016790A is higher as compared to 4αPDD 1.

This small molecule drug is developed and currently patented by GlaxoSmithKline, abbreviated as GSK.[26]

In the context of our study, it is of utmost importance to discuss the potential small molecule antagonists for TRPV4, our therapeutic target, and the future scope associated with them.

GlaxoSmithKline has developed antagonists which share structural similarity with the GSK1016790A agonist. One such potential drug under clinical trial studies is GSK2798745, which is selectively blocks the TRPV4 channel. This small molecule antagonist holds immense potential to upscale the field of respiratory as well as cardiac research[27]. GSK2798745 or GSK279 is currently the first antagonist to be studied in vivo human samples. A pyrrolidine diol-based derivative known as antagonist 1 (A1) or GSK3527497 is under pre-clinical studies which can serve as a potential backup for GSK279. Additionally, another orally administrable antagonist, A2 has recently being developed particularly for pain relieve. The A1 and A2 based studies targeted for TRPV4 have been carried out in CHO cell lines. Such studies report that the binding site for A1 and A2 are different on TRPV4[27].

Cryo-EM based studies have revealed that the activation of TRPV family channels is based on the structure of the TRP helix which regulates the channel pore activation by allosteric regulation. Through interactions with the TRP helix, the voltage sensitive domain, also known as the voltage sensor-like domain or VSLD, is crucial to channel activation[25]. It is elucidated that the antagonists, bind to the VSLD domain, leading to the stabilisation of interactions between the VSLD domain and TRP helix which results in closing of the pore[27]. The antagonists A1, A2 as well as GSK279, have been demonstrated by structural studies, to tighten the association between the bundles in VSLD domain and the TRP helix by inducing conformational change. Thus, we can infer that the aforementioned antagonists block the TRPV4 channel indirectly, by binding to the extended pocket of the VSLD domain. This is opposite to the function of agonists which disrupt such interactions, resulting in opening of the pore[27]. Furthermore, the antagonist molecules may result in conformational changes mediated by changing the symmetry.

In addition to such antagonists, many natural products have been developed by the University of Utah which can serve as potential antagonists. For instance, natural compounds isolated from fungi mainly polyketide derivatives have been demonstrated to inhibit the TRPV4 channel activity. However, such natural compounds may be associated with toxicity[26].

However, the current therapeutic approaches targeted to TRPV4 is associated with lower specificity and sensitivity mainly due to the off-target interactions. The future scope of the study should focus on devising highly targeted strategies for delivering the small molecule-based antagonists specifically to the TRPV4 therapeutic target within the lung tissue. Such targeted delivery strategies may include, nanoparticle mediated drug delivery or fibroblast cell specific targeting. Biomarker associated studies could also pave a way in understanding the cellular mechanisms of TRPV4 inhibition by antagonists. Further, it is also necessary to create combination medicines that might incorporate the control of IPF progression and COPD.

CHAPTER 6

CONCLUSION

In this study, we have applied an integrative network-based approach, linking two ageing-related respiratory ailments. The results obtained in our study suggests TRPV4 as a molecular nexus linking interleukin signaling, primarily IL4 /IL13 and mechanical stress. TRP or Transient Receptor Potential channels have been well studied and characterized in several organisms including humans. TRP channels are mainly grouped into several subfamilies, TRPV represents one such family. Moreover, TRPV family channels or the vanilloid receptor subfamily comprises six members. Two of them TRPV5-6 are selective to calcium ions and contribute to calcium homeostasis regulation. The remaining members are nonselective ion channels, and also temperature sensitive[25].

Our study has indicated towards TRPV4 as the hub gene, linking it to the interleukin signalling axis. IL-4 and IL-13 signalling axis emerged as significant contributors to COPD and IPF pathogenesis. These two interleukins exhibit high level of sequence homology and trigger the STAT6 activation which binds to the docking site on phosphorylated tyrosine, for downstream cellular signalling.[14]. IL-13 binds to the same type II receptor and along with IL-4, induces, T-helper 2 differentiation, mucus hypersecretion, airway remodeling and IgE driven responses. Furthermore, inflammatory cytokines might make the TRPV4 channels more sensitive and responsive to Calcium ions, resulting in sustained activation of inflammatory cascade. This alternatively results in gradual stiffening of lung extracellular matrix driving the fibroblast activation. To illustrate, small-molecule inhibitors targeting TRP channels are at present in the preclinical and early-phase clinical trial stages for other ailments, thus indicating a possible route of drug development in COPD and IPF [12]. However, the current therapeutic approaches targeted to TRPV4 is associated with lower specificity and sensitivity mainly due to the off-target interactions[27]. The investigation of the TRPV4 specific isoforms or splice variants expressed in lung tissues that are affected by COPD and IPF could facilitate the targeting of therapies leading to more accurate interventions with lesser off-target effects. Hence, the dual function of TRPV4 in the inflammatory and fibrotic processes suggests it to be an optimal target for the treatment of the common pathophysiology of COPD and IPF.

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