Idiopathic pulmonary fibrosis (IPF): PHMG-P and other disinfectant-associated chemicals as potential causes, the mechanism, and potential treatments

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Pulmonary fibrosis is an irreversible, fatal disease that results in scarring of the lung tissue and decreased function of the lungs. Idiopathic pulmonary fibrosis simply means that the cause is unknown. Patients with IPF typically experience difficulty breathing, with death caused by either respiratory failure or incurrent pneumonia [1]. The disease is characterized by marked collagen deposition and other alterations to the extracellular matrix (ECM), a network of macromolecules that provide structural support to the lungs [1] .These alterations to the ECM remodel and stiffen the lung's airspaces and tissues [1]. It is also characterized by diffuse interstitial inflammation and respiratory dysfunction [2]. Although its cause remains unknown, it is believed that the main steps in the pathogenesis of IPF are initiated by the transforming growth factor β (TGF-β) signaling pathway and involves the migration, proliferation, and activation of lung fibroblasts and their differentiation into myofibroblasts [3]. Fibroblasts are cells that have a high ability to proliferate and to produce ECM and fibrogenic cytokines [3] .Fibrogenic cytokines are multifunctional immunoregulatory proteins that contribute to the inflammatory cell recruitment and activation needed to promote the development of fibrosis [4]. These cytokines can activate myofibroblasts, which are primarily responsible for the synthesis and excessive accumulation of ECM components, collagen and fibronectin, during the repair process that leads to fibrosis [5], [6].

A 2011 outbreak of pulmonary fibrosis in South Korea prompted an onslaught of research as to how IPF may be caused and treated. [7] Specifically, this research has provided evidence that certain chemicals commonly found in household disinfectants can cause IPF through the generation of reactive oxygen species (ROS). ROS have a powerful oxidizing capability that can induce the destruction of cellular and subcellular structures in the lung, including DNA, proteins, lipids, cell membranes, and mitochondria. [8] This damage caused by ROS has been found to promote the activation of the TGF-β signaling pathway and the development of numerous characteristics associated with IPF. [4] This research has been invaluable for the discovery of new potential treatments for patients with IPF.

Potential inducers of idiopathic pulmonary fibrosis

After the 2011 outbreak in South Korea, researchers were able to find a connection between IPF and exposure to chemicals commonly found in household disinfectants, such as those found in humidifiers and pools. They have suspected that these chemicals can cause pulmonary fibrosis by infiltrating the respiratory system as aerosol particles to induce cellular damage. The chemicals polymethylene guanidine phosphate (PHMG-P), didecyldimethylammonium chloride (DDAC), polyhexamethylene biguanide (PHMB), oligo (2-(2-ethoxy) ethoxyethyl guanidinium chloride (PGH), and the mixture of chloromethylisothiazolinone (CMIT) and methylisothiazolinone (MIT) attracted particular interest.

In a study evaluating registered lung disease cases in South Korea, it was revealed that 70 percent of registered patients that suffered from IPF or other forms of household humidifier disinfectant-associated lung injury had used humidifier disinfectants containing the chemicals PHMG, PGH, or a mix of CMIT and MIT prior to their development of the disease [7] It was determined that the aerosol water droplets emitted by the humidifiers may have acted as carriers to deliver these chemicals into the lower part of the respiratory system, causing humidifier disinfectant-associated lung injury. [7] It was also revealed that most of the affected patients in the study had used humidifier disinfectant containing the chemical PHMG. [7]

Another study detailed that even slight exposure to PHMG could cause cell death triggered by the generation of reactive oxygen species (ROS). [8] Injury by ROS is typically followed by a fibrotic repair process involving increases in TGF-β expression, increased fibronectin, collagen synthesis, and a marked increase in the deposition of the ECM, all key characteristics of IPF. [4]

CERTAIN CHEMICALS COMMONLY FOUND IN HOUSEHOLD DISINFECTANTS CAN CAUSE IPF THROUGH THE GENERATION OF REACTIVE OXYGEN SPECIES

One way that ROS promote ECM deposition and IPF is by activating transcription factors like nuclear factor kappa B (NF-κB). [4] NF-κB is a regulator of proinflammatory cytokines that is typically bound to a cytoplasmic inhibitor. [9] One study found that exposure to the biocide (substance that destroys/prevents growth in organisms) and preservative PHMB was able to generate significant ROS levels and activate the NF-κB signaling pathway through the degradation of its inhibitor. [10] This is significant because the activation of proinflammatory cytokines is necessary for the recruitment and activation of myofibroblasts responsible for the increased ECM deposition that is characteristic of IPF patients. PHMB is also a cationic chemical and there is evidence that it can bind to negatively charged mucins, located within the mucous membranes of various organs. This can cause organs located in the re-

spiratory tract to acquire increased susceptibility to PHMB and, in effect, a higher likelihood for the development of IPF. [10] Although the study did not match the exposure conditions of PHMB in humans, it has illuminated another way that individuals may develop IPF. [10]

EXPOSURE TO DDAC CAN RESULT IN THE DEVELOPMENT OF SEVERAL CHARACTERISTICS TYPICALLY ASSOCIATED WITH IPF

In a study investigating the role of DDAC—one of the aerosols—in causing pulmonary fibrosis, mice exposed to DDAC exhibited fibrotic lesions that increased in severity over time. [11] Exposure to the chemical DDAC increased TGF-β signaling and appeared to maintain the differentiation of myofibroblasts. [11] This was complemented by the high expression of genes responsible for the production of collagen in fibrogenic lungs. [11] Overall, the form of pulmonary fibrosis that was induced by DDAC was mild, and so more research must be conducted before it can be concluded that the chemical DDAC is responsible for irreversible, severe pulmonary fibrosis. [11] It is also possible that some of the patients affected with humidifier disinfectant-associated lung injury may have experienced synergistic or additive effects from using multiple humidifier disinfectants, but this can be difficult to determine. [7] However, this study does indicate that exposure to DDAC can result in the development of several characteristics typically associated with IPF.

PHMG-P as a potential causative of IPF

Of the chemicals listed in this literature review, PHMG-P has received the most attention by researchers. PHMG-P is a biocide that exhibits its antibacterial effect by disrupting the cell wall and inner membrane of bacteria, causing cellular leakage. [12] In a similar manner, PHMG-P can infiltrate the lungs in the form of aerosol particles and may cause IPF in individuals through the generation of ROS and the disruption of the ECM's alveolar basement membrane. [4]

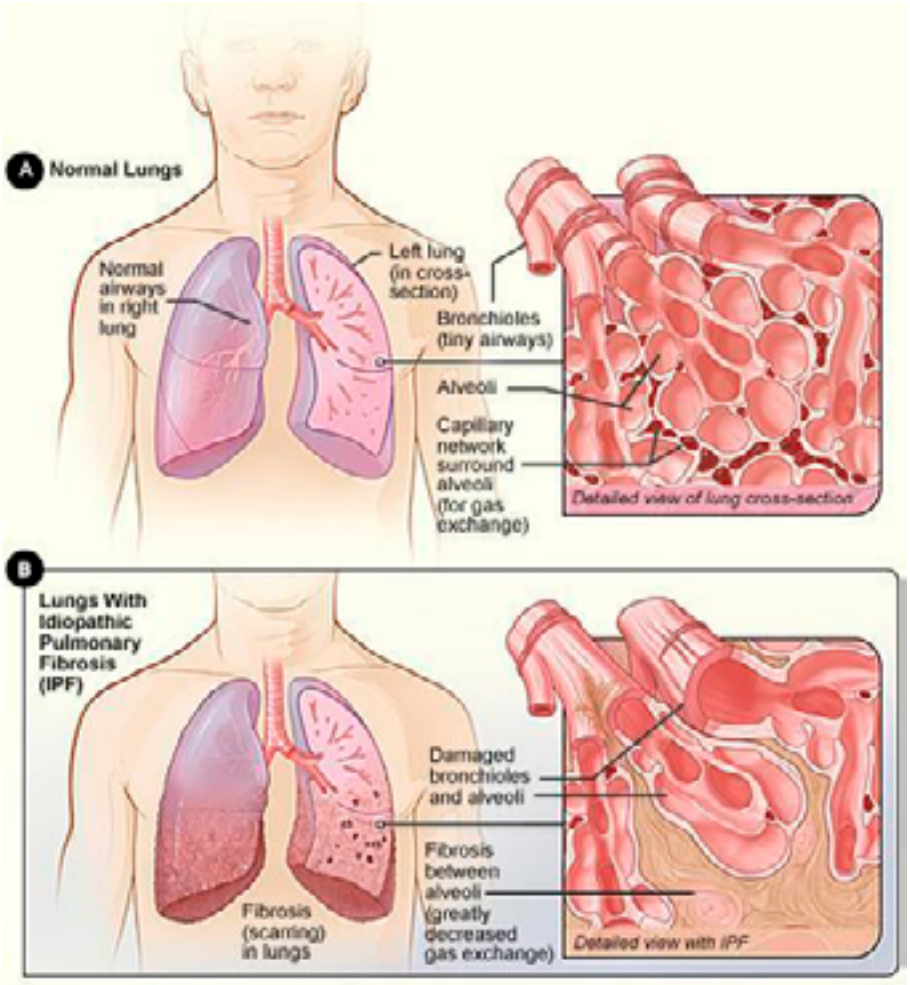
Disruption of the basement membrane occurs through increased expression of matrix metalloproteinases (MMPs), enzymes that degrade various components of connective tissue matrices. [6] Metalloproteinase MMP2, in particular, destroys the basement membrane by solubilizing ECM elastin, fibronectin, and collagen, helping immune cells and fibroblasts migrate to alveolar spaces. [12] This can lead to severe damage of the lung architecture and aberrant ECM deposition typical of IPF. [4]

In a study using an air-liquid interface (ALI) co-culture model to study the pathogenesis of fibrosis, PHMG-P was shown to trigger ROS generation, airway barrier injury, and inflammatory response. [4] Recall that exposure to other chemicals suspected of being potential inducers of IPF had similar effects. Therefore, it can be concluded that PHMG-P infiltrates the lungs in the form of aerosol particles and induces airway barrier injury by ROS. [4] This would result in the release of fibrotic inflammatory cytokines and trigger a wound-healing response that would eventually lead to pulmonary fibrosis. [4]

In an animal study, mice exposed to PHMG-P experienced difficulty breathing and exhibited pathological lesions similar to the pathological features observed in humans affected with IPF. [12] A time course of 10 weeks was even established for PHMG-P-induced pulmonary fibrosis. [12] Throughout this period, it was found that a single instillation of PHMG-P contributed to an increase in proinflammatory cytokine levels and elicited an influx of inflammatory cells into lung tissue. [12] This recruitment of inflammatory cells contributes to the deposition of ECM components in the lungs and, as a result, the development of IPF. The instillation of PHMG-P was also suspected of blocking T cell development and impairing its function in the immune system. [6] This would result in an insufficient resolution of inflammation caused by the increased levels of proinflammatory cytokines and result in stacked fibrotic changes and the progression of IPF. [6]

Another study claimed that PHMG-P could cause pulmonary fibrosis through the activation of the NF-κB signaling path-

Figure 1: A comparison of healthy lungs vs lungs with IPF



way. [9] Recall that the NF-κB signaling pathway is responsible for the production of proinflammatory cytokines associated with the development of IPF. According to the study, mice exposed to PHMG-P generated a large amount of ROS and produced significant levels of the cytokines IL-1β, IL-6, and IL-8 in a dose-dependent manner. [9] These cytokines produced by the NF- κB signaling pathway are known to activate the TGF-β signaling pathway, increase collagen production, and promote wound-healing and tissue remodeling responses. [4] As these responses are characteristic of IPF and the cytokines exhibited in this study are known to be produced through the activation of the NF-κB signaling pathway, there is strong evidence that PHMG-P can induce IPF through the NF-κB signaling pathway.

The Mechanism of IPF

TGF-β's importance in the mechanism

Various studies of IPF have indicated that transforming growth factor β (TGF-β), one of the most significant fibrotic cytokines, plays a key role in the mechanism that induces IPF. TGF-β1 is credited with inducing the differentiation of fibroblasts to myofibroblasts and upregulating the secretion of ECM proteins (like collagen) in IPF. [13]

Specifically, growth factor TGF-β1 binds directly to the TGFβ receptor II (TGFβRII), triggering the recruitment and activation of receptor TGFβRI by TGFβRII. [14] This step leads to the increased production of collagen through the activation of a collection of proteins called the Smad 2/3 complex. [13] The activated Smad 2/3 complex accomplishes this by entering the nucleus to enhance the transcription of profibrotic genes such as those that produce collagen. [13] This idea has been heavily supported by experimental evidence. Exposure to the chemical DDAC was found to increase cellular mRNA levels of TGF-β1 by two-fold. [11] This increase contributed to the activation of the Smad 2/3 complex [11] and induced the differentiation of fibroblasts to myofibroblasts. [15] Overall, this led to the development of pulmonary fibrosis-causing fibrotic lesions in mice. [11]

In another study, TGF-β was found to promote the development of IPF by inhibiting the expression of the microRNA let-7d, driving epithelial-mesenchymal transition (EMT) and increased collagen deposition. [1] Typically, epithelial cells are important to maintaining lung functionality by acting as a barrier against pathogens and other harmful compounds and secreting protective substances. [4] During EMT, however, these cells increase in cellular motility [16] and are transformed into myofibroblasts, resulting in the acceleration of IPF. [4] Additionally, epithelial cells during EMT promote the recruitment of fibroblasts, while simultaneously inhibiting collagen degradation and elevating the levels of the tissue inhibitor of metalloproteinase 1 (TIMP-1). [4] TIMP-1 binds to metalloproteinase MMP2 to promote the growth of fibroblasts and myofibroblasts, accelerating ECM deposition while preventing its degradation. [12] This corroborates the claim that the TGF-β signaling pathway is a crucial component in the mechanism of IPF.

MicroRNA's role in TGF-β regulation and pulmonary fibrosis

THE TGF-B SIGNALING PATHWAY IS A CRUCIAL COMPONENT IN THE MECHANISM OF IPF

MicroRNAs are mRNA sequences that bind to complementary mRNA of proteins to prevent their translation and expression. They are also involved in multiple steps of fibrosis, such as cell proliferation, apoptosis, and differentiation. [16] During the progression of IPF, miRNAs are known to regulate the process in which epithelial cells transition into myofibroblasts (EMT) to promote fibrosis. [16] Since each miRNA is specific to a particular mRNA sequence, miRNAs may function as either promoters or inhibitors of IPF. One study found that the miRNA, miR-433, can act as a promoter of IPF by upregulating receptor TGFβRI and growth factor TGF-β1 to amplify TGF-β signaling. [13] In a separate study, it was confirmed that miR-30c-1-3p may act as a negative regulator of pulmonary fibrosis through targeting the mRNA and preventing the expression of receptor TGFβRII. [15]

In a study headed by the Department of Pathology at the University of Michigan Medical School, it was concluded that the development and pace of progression of IPF may be due to abnormal miRNA generation and processing. [1] It was found that in rapidly progressing IPF biopsies, five miRNAs significantly increased and one decreased when compared to slowly progressive biopsies. [1] This indicates that miRNAs have a significant influence on the mechanism of IPF. Additionally, members of the miR-30c and let-7d family significantly decreased in both forms of IPF when compared with unaffected individuals. [1] As stated previously, certain members of the miR-30c family are believed to be negative regulators of IPF and members of the let-7d family are inhibitors of EMT. All of the stated evidence signifies that miRNAs, in addition to the TGF-β signaling pathway, play important roles in the development of IPF.

NALP3 INFLAMMASOME IS A CENTRAL COMPONENT IN THE IPF MECHANISM

Other factors to consider in the mechanism

The NALP3 inflammasome is another important factor to consider in the mechanism of IPF. The NALP3 inflammasome is an innate immune system receptor suspected of being the main cause of persistent inflammatory response and exacerbation of fibrotic changes. [12] According to a study focused on researching PHMG-P-induced fibrosis in mice, the activation of the NALP3 inflammasome appeared to contribute to fibroblast proliferation and the progression of IPF due to the production of the cytokine IL-1β. [12] IL-1β is known to increase the production of ROS needed to induce lung tissue damage by upregulating the expression of the cytokine chemokine (C-X-C motif) ligand 1 (CXCL1). [6] This upregulation of CXCL1 and resulting tissue damage was exhibited in the study, reinforcing the claim that the NALP3 inflammasome is a central component in the IPF mechanism. [12]

Secretory immunoglobulin A (sIgA), an antibody that has an important role in the immune system, also may have a role in the mechanism of pulmonary fibrosis. In a study supported by the Japan Society for the Promotion of Science, immunoglobulin A, the most abundant human immunoglobulin, was compared with TGF-β in its role in inducing pulmonary fibrosis and inflammation. [3] In this study, sIgA enhanced collagen production and induced responses in cytokines IL-6 and IL-8, and monocyte chemoattractant protein 1 (MCP-1). [3] MCP-1, similar to IL-6 and IL-8, is responsible for stimulating collagen

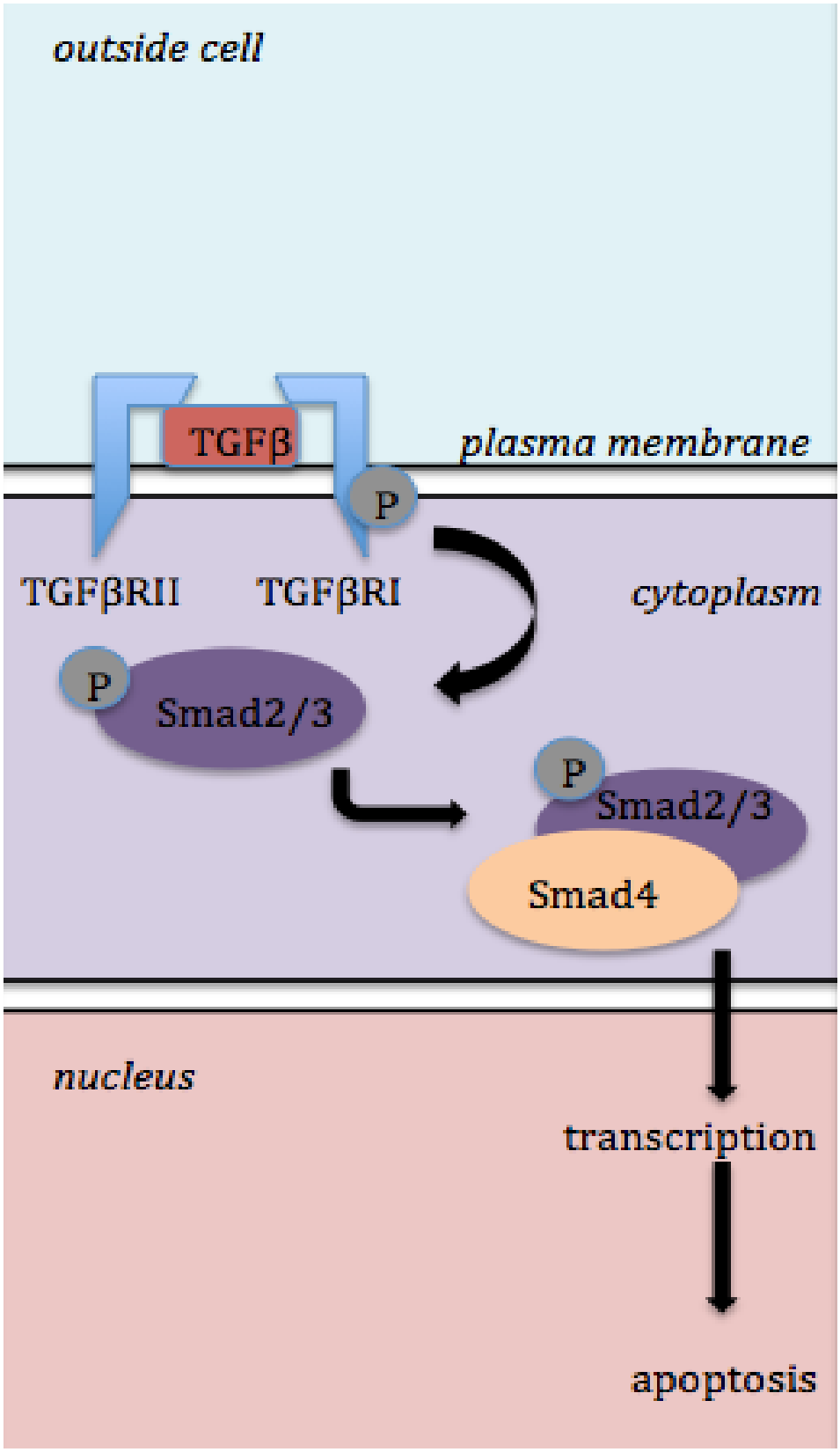


Figure 2: Mechanism of the TGF-β signaling pathway

synthesis and TGF-β production in fibroblasts. [6] It was concluded that under IPF, sIgA may make contact with lung fibroblasts and result in exacerbating airway inflammation and fibrosis through enhancing the production of inflammatory cytokines and ECM collagen. [3]

Potential therapeutic approaches and alternative methods of treatment

According to recent studies, only two drugs, pirfenidone and nintedanib, have been approved by the FDA for IPF treatment, and they have still failed to be significantly effective in treating the disease. [13] However, current studies on therapeutics that inhibit the TGF-β signaling pathway appear promising. Two particular drugs of interest are oridonin and matrine, along with their derivatives.

ORIDONIN ATTENUATED PATHOLOGICAL CHANGES SUCH AS ALVEOLAR SPACE COLLAPSE AND INFILTRATION OF INFLAMMATORY CELLS

Oridonin, a major compound found in the herb Rabdosia rubescens, has been used in traditional Chinese medicine to treat inflammation and cancer for hundreds of years. [2] In a study focused on testing its effectiveness in treating IPF, it was found that exposure to oridonin significantly decreased the levels of three major biomarkers of fibrosis—hydroxyproline (HYP), beta silicomolibdic acid (β-SMA), and collagen, type 1, alpha 1 (COL1A1)—in a dose-dependent manner. [2] Additionally, oridonin attenuated pathological changes such as alveolar space collapse and infiltration of inflammatory cells. [2] Oridonin was able to achieve this through significantly inhibiting the upregulation of collagen production and the activation of Smad 2/3 in lung tissues, an important step in the progression of IPF through the TGF-β signaling pathway. [2] This presents a strong case for the use of oridonin as a treatment for IPF.

Matrine, similar to oridonin, also has roots in traditional Chinese medicine. Matrine has been shown in several studies to exhibit significant antifibrotic effects through the inhibition of the TGF-β pathway. In one study, matrine was shown to have an inhibitory effect against liver fibrosis by reducing the expression of TGF-β1 and instead increasing the expression of hepatocyte growth factor (HGF). [13] Through the inhibition of the TGF-β/Smad pathway, matrine was also shown to exhibit antifibrotic activities on cardiac fibrosis. [13] These antifibrotic effects are not just held by matrine, but their derivatives as well. The matrine derivative MASM was also shown to exhibit potent antifibrotic effects. [13] As the TGF-β signaling pathway is a central component in the mechanism of IPF, matrine and their derivatives present themselves as strong candidates for anti-IPF therapeutics.

Other drug candidates for the treatment of IPF are sesquiterpene lactones. Sesquiterpene lactones are naturally occurring compounds that are known to harbor extensive connections with the TGF-β1 signaling pathway. [5] This makes these compounds and their analogues strong drug candidates for IPF treatment. In one study, two out of 44 semi-synthetic analogues of sesquiterpene lactones were found to highly inhibit the TGF-β1 signaling pathway, ECM production, and the formation of fibroblasts. [5] This inhibition of ECM production and the formation of fibroblasts corroborates the claim that administering sesquiterpene lactones is an effective treatment for IPF.

THESE TREATMENTS COULD BECOME COMMON PRACTICE AND IMPROVE THE QUALITY OF LIFE FOR PATIENTS SUFFERING FROM IPF

As mentioned earlier, studies have shown microRNAs to be negative regulators of IPF. One study suggests that the replenishment of miR-30c may be a promising treatment. [15] Increased levels of miR-30c would promote the negative regulation of the TGF-β signaling pathway, suppressing the differentiation of myofibroblasts and preventing excessive collagen accumulation. In this manner, the replenishment of miR-30c would attenuate IPF symptoms. The inhibition of certain miRNAs, such as miR-34a, has also been shown to be an effective treatment. The inhibition of miR-34a by treatment with the caveolin-1 scaffolding domain peptide (CSP) was found to prevent pulmonary fibrosis by preventing the overgrowth of fibroblasts. [17] Although the manipulation of miRNA expression has been shown to have a large impact on the development of IPF, there is one issue with this method of treatment. A single miRNA can target thousands of mRNAs, making the function miRNAs have in pathophysiological events involved in IPF unclear. [17]

Conclusion

Although there is limited research on the etiology of IPF, this should only serve to motivate researchers to study its causes, mechanism, and potential treatments further. Thus far, the chemicals that have been shown to be potential inducers of IPF are PHMG-P, DDAC, PHMB, PGH, and the mixture of CMIT and MIT. However, out of all the chemicals, only PHMG-P has been heavily researched, and so additional studies are needed to confirm the other chemicals' involvement in inducing IPF. Additionally, this research could be expanded upon through the study of the effects of other household disinfectants on the human body to determine whether they are also factors in inducing IPF. Besides the discovery of potential causatives, studies have also further illuminated details about the mechanism. Specifically attracting interest is the TGF-β signaling pathway in addition to miRNAs and their involvement in the regulation of IPF. Furthermore, the manipulation of TGF-β and miRNA levels with oridonin, matirne, and sesquiterpene lactones has been linked to favorable outcomes in the treatment of IPF. With further research, these treatments could become common practice and improve the quality of life for patients suffering from IPF.

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