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# Recent Findings on Cell-Based Therapies for COVID19-Related Pulmonary Fibrosis

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**Abstract**

COVID-19 has spread worldwide, including the United States, United Kingdom, and Italy, along with its site of origin in China, since 2020. The virus was first found in the Wuhan seafood market at the end of 2019, with a controversial source. The clinical symptoms of COVID-19 include fever, cough, and respiratory tract inflammation, with some severe patients developing an acute and chronic lung injury, such as acute respiratory distress syndrome (ARDS) and pulmonary fibrosis (PF). It has already claimed approximately 300 thousand human lives and the number is still on the rise; the only way to prevent the infection is to be safe till vaccines and reliable treatments develop. In previous studies, the use of mesenchymal stem cells (MSCs) in clinical trials had been proven to be effective in immune modulation and tissue repair promotion; however, their efficacy in treating COVID-19 remains underestimated. Here, we report the findings from past experiences of SARS and MSCs, and how SARS could also induce PF. Such studies may help to understand the rationale for the recent cell-based therapies for COVID-19.

**Keywords**

COVID-19, pulmonary fibrosis

## Introduction

The SARS-CoV-2 virus, originating from bat coronavirus, broke out in Wuhan, a city with a population of 11 million that spent months under strict lockdown since the outbreak. This virus belongs to the family Coronaviridae and genus *Betacoronavirus*, same as the human SARS-CoV, MERS-CoV, and human CoV-HKU, which were found to infect humans. A devastating effect in SARS-CoV-2-infected patients is severe acute respiratory syndrome, the symptoms including fever, cough, fatigue, shortness of breath, and loss of smell. With progress of the disease, acute lung injury might lead to ARDS or PF. While some research groups have reported the ARDS to not be like the typical syndrome[1], histopathological findings have shown the occurrence of interstitial fibrosis in a critical patient with COVID-19[2]. Another feature in patients with COVID-19 is cytokine storm syndrome[3], making a significant difference between the dead and discharged patients[4]. These features are common with those seen in SARS in 2003 and could possibly be due to the similarities between the viruses[5].

## SARS and Pulmonary Fibrosis

Severe acute respiratory syndrome (SARS) is an infectious disease that causes severe respiratory illness and even death. The SARS epidemic originated in southern China in November 2002 and became a global outbreak in 2003. During November 2002–July 2003, a total of 8,098 probable SARS cases were reported to the World Health Organization (WHO) from 29 countries, including 29 cases from the United States; 774 SARS-related deaths (case-fatality rate: 9.6%) were reported (World Health Organization; summary table of SARS cases by country, November 1, 2002–August 7, 2003). The disease was found to be caused by a novel

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coronavirus, which was named SARS-CoV by WHO. Patients with SARS initially presented with fever, cough, chills, malaise, myalgia, headache, shortness of breath, and diarrhea that progressed in severity over the following weeks[6]. Most patients recovered from the infection while approximately one-third developed severe pulmonary complications, leading to ARDS, necessitated intubation, and ventilatory support. Autopsies of patients with fatal SARS showed lung fibrosis at various stages of progression[7]. Many survivors of SARS developed residual PF. In a retrospective study of SARS, 72.7% of patients showed mild or moderate lung function damage after 7-year follow-up[8]. Hui et al. reported that at 1-year follow-up, 27.8% of patients had abnormal CXR findings, and 23.7% of patients had DLCO values < 80% of predicted values, hence indicating PF[9].

Although PF may be seen in other respiratory viral diseases as well, it is more common after SARS-CoV infection. However, the mechanisms of SARS-CoV infection-related PF remain to be fully understood. Activation of transforming growth factor β (TGFβ) pathway and increased degradation of angiotensin-converting enzyme2 (ACE2) or angiotensinogen system-mediated lung fibrosis may play major roles.

## Transforming Growth Factor Beta

In the early phase of SARS infection, elevated serum levels of TGFβ1 have been reported[10]. Activation of TGFβ pathways leads to the production of fibrin, collagen, and secreted proteases (matrix metalloproteinases)[11]. Sime et al. had demonstrated the overexpression of active TGFβ to result in severe interstitial and pleural fibrosis[12]. TGFβ in the lungs is required to promote the differentiation of lung fibroblasts into myofibroblasts, which is necessary for pulmonary tissue repair after lung injury. SARS-CoV infection not only enhances the expression of TGFβ but also facilitates its signaling activity through viral nucleocapsid (N) protein. Overexpression of N protein in lung epithelial and fibroblast cells potentiates TGFβ-induced expression of platelet-activating factor I (PAI-I) and collagen I while attenuating Smad3/Smad4-mediated apoptosis of human peripheral lung epithelial HPL1 cells. Thus, N protein modulates TGFβ signaling and blocks apoptosis of SARS-CoV-infected host cells, besides promoting tissue fibrosis[13].

## Angiotensin-Converting Enzyme 2

Renin-angiotensin system is known to be activated after lung injury to promote tissue repair; when in excess, it may even lead to tissue fibrosis[14]. Angiotensin II (ANG II), converted from angiotensin I via the angiotensin-converting enzyme (ACE), is the major effector peptide in this function. ANG II has been found to be present at high levels in mice treated with bleomycin and in patients with PF, and is known to induce alveolar epithelial cell apoptosis[15]. AngII has profibrotic actions on growth factor expression, extracellular matrix synthesis, migration, and motility of lung fibroblasts mediated through both angiotensin type 1 receptor (AT1) and angiotensin type 2 receptor (AT2). ANGII can stimulate the production of TGFβ in lung tissue mediated by AT1; TGFβ itself can also regulate the level of ANGII. This "autocrine loop" involving ANGII and TGFβ is believed to exist in lung tissues[16]. Application of ACE inhibitors, such as captopril, to inhibit ANGII production has been shown to attenuate experimental PF in animal models induced by bleomycin[17]. In 2000, a novel homolog of ACE, termed angiotensin-converting enzyme2 (ACE2) was identified[18]. ACE-2 could cleave Angiotensin II to a seven-amino acid peptide Ang1–7. Uhal et al. found Ang1–7 to act through its receptor Mas to inhibit bleomycin-induced fibrosis by inhibiting the activation of JNK, which is required for bleomycin and angiotensin II-induced apoptosis[15,19]. Thus, ACE2 can act against lung fibrosis by the negative regulation of local AngII level. ACE2 is a membrane-anchored carboxypeptidase highly expressed in airway epithelial and type I and II alveolar epithelial cells; it was found to be the virus-cell entry receptor during the SARS-CoV outbreak[20]. The spike protein of SARS-CoV binds to ACE2 for entry and infects the target cells in humans[21]. Using a SARS infection model in ACE2 knockout mouse, Kuba et al. were able to show that ACE2 is indeed essential for SARS infection in vivo, and ACE2 expression in lungs is remarkably downregulated in wild-type mice infected with SARS-CoV[22,23]. The reduced expression of ACE2 may result in an increased ANGII level thereby leading to more severe lung fibrosis.

## Cell-Based Therapy in COVID-19 Treatment

In patients with SARS, supportive care is the only proven beneficial treatment, including mechanical ventilation or in-line suction[24]. Antiviral drugs, such as ribavirin, are frequently used in patients, but their efficacy is yet to be proven. Significant toxicity is another issue in ribavirin-treated patients, with chances of approximately 76% hemolysis[25]. Even if steroids are used to prevent the cytokine storm, bone damage can be found in retrospective studies[26]. In this global emergency of the COVID-19 pandemic, physicians have tried every rational treatment, including the drugs against autoimmune and human immunodeficiency virus (like hydroxychloroquine)[27]; however, the studies failed to show expected results. Tissues like bone marrow, adipose, placenta, and cord blood are rich in MSCs. Their characteristics may differ from the source[28], but their common immunomodulating activities have been proven in both experimental research and clinical treatment, as in graft-versus-host disease (GvHD)[29,30]. MSCs are hypoimmunogenic for alloreactive T-cells and have promoted hematological recovery in many preclinical trials[31].

In animal studies, MSCs have shown potential in treating acute lung injury, ARDS, asthma, and fibrosis[32]; their mechanisms of action might include the inhibition of inflammatory cytokines, such as TNFα[33,34]. Thus, based on the

passive defense method, virus-infected cells release interferons (IFNs) and stimulate genes like p21 to restrain cell growth. Stem cells may enhance this intrinsic viral resistance[35]. Khoury et al. had shown systemic MSC administration to potentially reduce lung injury after respiratory tract infections, such as influenza[36]. On the other hand, cell therapy offers an option for direct or indirect effects, like anti-inflammation and improved regeneration through cell-derived microvesicles or exosomes[37,38]. Although many physicians are using the previous regimens with minor modifications for COVID-19 treatment, Chao et al. tested whether MSCs would have any therapeutic potential. In the study, seven patients received MSC infusion, and interestingly, all recovered from the symptoms, including high fever ($38.5 \pm 0.5^{\circ}C$), weakness, shortness of breath, and low oxygen saturation[39]. However, the study was not well-documented in terms of the cell source and manufacturing details.

## Summary

Despite the global pandemic threatening many lives, families, and economies worldwide, safety of any unproven treatment should be established carefully. Recently, many companies have been selling unlicensed cell-based treatments, such as stem cell products or kits for the extraction of exosomes[40]. This irresponsible behavior is not only harmful to the human body but also encroaches on other evidence-based and approved clinical studies. Although the therapeutic effects or benefits are still being evaluated, past experiences obtained from experiments and clinical trials have been contributing to patient and medical care. Based on most of the certified clinical trials, reports have suggested considerable effectiveness in the treatment of COVID-19 and PF. Thus, to improve the confidence of cell-based therapy, safety and rationale would be more important than effectiveness in this hopeless and distressing situation.

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