Alveolar Regeneration in COVID-19 Patients: Network Perspective

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Lung alveolar regeneration to repair the damaged tissue and restoration of normal tissue function could be achieved by transplantation of progenitor or stem cells and exosome-mediated delivery of therapeutic agents, including miRNAs. Not only as a biomarker of COVID-19but also as therapeutic agents, miRNAs have proven to play a crucial role in lung damage and repair. miRNAs can either be regulated locally in the lungsor transported to the damaged site by extracellular vehicles (EVs) secreted by stem cells to induce tissue regeneration by decreasing inflammation and apoptosis, stimulating surfactant production, regulating gene expression of junction proteins to repair microvascular permeability, and reducing fibrosis.

Keywords: COVID-19 ; SARS-CoV-2 ; alveolar regeneration ; alveolar fibrosis

1. Introduction

The novel coronavirus disease 19 (COVID-19), caused by the highly pathogenic SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) virus, has resulted in more than 234 million infections and about 4.8 million global deaths up until October 2021, according to the World Health Organization (WHO) [1]. The SARS-CoV-2, which stands for severe acute respiratory syndrome coronavirus-2, along with the previously existing SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) comprise a group of human coronaviruses that have affected human population adversely in the last two decades. All of them invade by infecting the cells in the upper respiratory tract along with the bronchial epithelial cells as well as pneumocytes, the result of which is a severe lung infection [2]. Every viral infection involves entry and replication of viral nucleic acid. Specifically, SARS-CoV-2 is an RNA virus.

The outcome of critical SARS-CoV-2 infection during the on-going pandemic has been found to be highly destructive and life-threatening for the host organism. It has been established that SARS-CoV-2 primarily affects the respiratory tract and in severe circumstances this virus can induce pneumonia and acute respiratory distress syndrome (ARDS) as well as several extrapulmonary manifestations. ARDS is a severe form of lung injury characterized by noncardiogenic pulmonary edema, bilateral pulmonary infiltrates and profound hypoxemia leading to respiratory failure. In the case of an advanced stage of lung injury or delayed diagnosis, even antibiotic treatment can become insufficient to avoid development of acute lung injury [3]. The population-based incidence of ARDS has been estimated to range from 10 to 86 instances per 100,000, but it is also believed that the condition is underdiagnosed especially in low-income countries [4]. Additionally, ARDS is a life-threatening illness with a high mortality rate [5]; there have been very modest improvements in recent decades, and increased mortality in the elderly [6]. Patients with ARDS frequently show diffuse alveolar damage (DAD) [7]. The acute phase of ARDS results from DAD and endothelial injury, while late phase is associated with proliferation of alveolar epithelial type2 (AT2) cells and fibroblasts, followed by chronic inflammation and extensive pulmonary fibrosis of the alveoli leading to a loss of normal lung architecture [4]. Of note, with the promotion of fibrosis, reestablishment of normal lung architecture is extremely challenging in human.

ARDS has also been established as one of the major hallmarks of severe COVID-19 infections. In fact, around 40% of COVID-19 patients developing pneumonia also developed ARDS and around 70% of deaths in COVID-19 infected critically ill patients occurred as a result of ARDS [7]. However, the disease when it occurs as part of COVID-19 behaves differently and there is insufficient data to explain explicitly how pathophysiology of COVID-19 ARDS is different from that of the typical ARDS [8]. A clinical study compared the histopathological and molecular features of lungs obtained from autopsies of patients who died from a COVID-19 infection to those who died from ARDS after an influenza A infection. Although similar morphological patterns were observed in both types of lungs, unique vascular features such as severe endothelial cell injury, extensive vascular thrombosis and a higher degree of vascular angiogenesis were particularly noticed in the COVID-19 infected lungs [9]. Thus, there is a pressing need for finding an effective treatment for COVID-19 ARDS, which is also a current challenge for doctors and researchers.

Even though the on-going SARS-CoV-2 pandemic has unprecedentedly and severely impacted our health-care system and economic growth, a conclusive cure for it still does not exist. Though the majority of COVID-19 cases are mild, around 5–8% of patients develop life-threatening critical illnesses. The vaccination drives for COVID-19 are in progress in most countries, and seemingly, it may require a considerable amount of time before the vaccine reaches all over the globe. Additionally, growing numbers of incidents have been reported worldwide, where patients who survived the illness of COVID-19 and tested negative clinically, still continue to struggle through the symptoms of the disease and post virus-clearance complications, including significant lung damage and cardiac arrhythmias [10][11]. Several autopsy studies of patients who died of COVID-19 have reported extensive alveolar damage to the lungs, while long-term lung impairment has been reported in the survivors of COVID-19 disease [11]. Thus, there is a critical need for a deepened understanding of the biomolecular mechanisms that govern these virus–host interactions so that effective preventive, as well as treatment strategies, can be developed for post-COVID-19 lung infections. Notably, in order to restore normal functioning of lungs post-COVID-19 infection, a highly regulated inflammatory response is usually triggered in the host organism. Alveolar regeneration and fibrotic repair are two main processes by which a host organism can heal the damaged tissue after injury. Both processes are tightly regulated and involve crosstalk between different cell types. Hence, understanding of key proteins and cellular pathways underlying regenerative cells can be helpful to identify the drug targets for pathological intervention. In this review, we summarize our current understanding of the regeneration and fibrotic repair processes, especially in the context to the SARS-CoV-2 infection. Furthermore, we emphasize the network biology potential and demonstrate its applicability in order to understand the mechanisms of regeneration switching.

2. Alveolar Regeneration

The lung, despite its general quiescent state, has a remarkable regenerative capacity, in which resident progenitor cell populations proliferate and differentiate into a variety of cell types in response to injury [12]. Recent evidence suggests that the lung contains several spatially and temporally restricted progenitor cells capable of producing all types of pulmonary cells [12]. Among these, alveolar type I cells (AT1) and alveolar type II cells (AT2) constitute two major cell populations found in the alveolar epithelium [13]. During lung injury, AT2 cells behave as adult tissue stem cells and play a critical role in tissue regeneration by differentiating into AT1 cells [14][15][16][17][18]. Notably, despite being the most abundant cells in the alveolar space, AT2 cells only cover ~5% of the total surface due to their unique cuboidal morphology [19]. The remaining ~95% of the surface is covered by large squamous attenuated AT1 cells. Bronchial and alveolar epithelial cells are key targets of viral pathogens. SARS-CoV-2 can infect both AT1 and AT2 cells ex vivo [20]. Long-term infections can result in uncontrolled inflammation and lung damage. Together with ciliated airway cells, AT2 cells are primary targets for SARS-CoV-2 infection [21][22]. A detailed examination of lungs from patients who died from SARS-CoV-2 infection demonstrated that AT2 cells were the most extensively proliferating cell population in severely damaged lungs. This suggests that AT2 cells might be implicated in the alveolar regeneration following SARS-CoV-2 infection [23]. In other words, the alveolar regeneration capability may be directly modulated by the SARS-CoV-2 infection. However, limited information exists so far on the molecular mechanisms involved in repairing and rebuilding an operational respiratory system after lung injury due to SARS-CoV-2.

To date, rodent models have been the most effective for providing direct experimental evidence for in vivo alveolar regeneration. However, despite the conservancy in lung development among mammals, the structure, cellular composition, molecular mechanisms, and reactions of mouse and human lungs are vastly different [24][25]. In rodents, complete alveolar regeneration, and restoration of lung functions after injury takes only a few weeks [26][27]; whereas in humans, it requires a very long time scale. There is a study which showed that a 33-year-old woman who had undergone pneumonectomy had complete alveolar regeneration over a period of 15-years [28]. Intriguingly, several recent studies have also confirmed the enrichment and implication of various types of progenitor cells in the regeneration of damaged respiratory epithelium following a SARS-CoV-2 infection. For instance, Chen et al. (2020) showed evidence of alveolar regeneration in 54-year-old and 58-year-old COVID-19 patients [26]. In these patients, AT2 cells could differentiate into AT1 like cells, although the regeneration process began on the 38th day after the commencement of the first symptoms [26]. In another study, tracheas and lungs from five COVID-19 deceased patients were examined. Interestingly, an extensive population of proliferating Krt5 + basal cells was found to be enriched in the trachea and larger airways. In addition, a population of extensively proliferating AT2 cells was observed in the intrapulmonary airways and alveoli of these patients. This suggests that distinct populations of proliferating progenitor cells become enriched at the area of lung damage in order to regenerate the damaged trachea and alveoli following SARS-CoV-2 infection [23]. Zhao et al. (2020) also identified a possible mechanism of lung repair following severe SARS-CoV-2 infection [29]. Their single-cell RNA-sequencing analysis confirmed a significant increase of lung progenitor Tm4sf1+ and Krt5 + cells in critical COVID-19 patients, both of which could act together synergistically to restore epithelial barriers and regenerate alveolar cells [29]. One other investigation assessed the magnitude of lung regeneration after recovery from SARS-CoV-2 infection [30]. It

was found, through an analysis of serum cellular markers in COVID-19 infected patients, that AT1 cellular damage was no longer discernible during the recovery period after virus clearance. Conversely, AT2 cells, as well as lung structures, were still found to be damaged two weeks after clearance of SARS -CoV-2 [30].

3. Alveolar Fibrosis

The regenerative process is often capable of restoring the function and structure of the lung after damage. In the case of prolonged and pervasive damage, the lung is healed by an accumulation of fibers at the site of injury. Although this fibrotic repair mechanism produces scar tissue, it gradually results in epithelial and endothelial damage causing a significant loss of lung function and an increase in morbidity [31]. Pulmonary fibrosis is a common result of most chronic inflammatory lung disorders, and it can have an impact on lung function, ultimately leading to its failure and death [32]. Fibrosis is scarring of the affected tissue due to the accumulation of extracellular matrix (ECM) components such as collagen and fibronectin but in abnormal excessive amounts. The transformation of normal repair to fibrosis also depends on the severity and duration of the damage as long-lasting damages tend to develop fibrosis as compared to small-scale injuries [33][34].

It has been established that interactions between different types of cells is very crucial for the onset of fibrosis. In pulmonary fibrosis, mesenchymal cells and fibroblasts are recognized as key cell types which partake in heavy ECM deposition, leading to fibrosis and decreased lung function [35]. These cells undergo a lot of changes after an event of tissue damage. Activation of glycolysis in fibroblasts after lung damage initiates a cascade of enzymatic activations which elevate cell proliferation, collagen synthesis and production of secondary metabolites thereby promoting fibrosis. Increased glutaminolysis and fatty acid oxidation play an important role in fibroblast activation. Inflammatory monocytes and tissue-resident macrophages are also critical regulators of tissue fibrosis, helping to initiate, maintain, and resolve tissue damage. Macrophages are also involved in the recruitment of fibroblasts which leads to fibrosis. In addition, the severity of fibrosis depends on factors such as age, genetics, and environmental factors [36]. Recent studies have revealed the significance of epithelial cells in fibrogenesis. Epithelial cells under special circumstances undergo transformation and gain fibroblast-like properties by a process known as epithelial-mesenchymal transition. These cells can migrate to the site of inflammation and contribute to fibrogenesis. Thus, all these cell types interact and contribute to tissue repair but any disparity in their normal activity or function can lead to fibrosis [37]. In other words, fibrosis is an outcome of any shortcoming in the cellular crosstalk during the tissue repair process. Notably, chemokines play a central role in the onset of fibrosis especially the transforming growth factor (TGF-β1), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). TGF-β1 especially plays a central role in pulmonary fibrosis by promoting the production of ECM as well as by regulating its own expression. It is further regulated by different cell types which serve dominant roles in fibrogenesis and include fibroblasts, macrophages, and epithelial cells [38].

Viral infections play a key role in fibrogenesis and increase the risk of fibrosis [39]. These infections cause severe inflammation and can pair with genetic factors, as well as old age, eventually leading to fibrosis. Pulmonary fibrosis is also central to the SARS-CoV-2 infection, as indicated by the available radiographic, autopsy or other emerging clinical evidence [40][41][42]. In COVID-19 patients, alveolar damage and changes in the fibroblast niche are two prime causes of pulmonary fibrosis [43]. The damage to the alveolar epithelium can stimulate the injured cells to release molecules such as damage-associated molecular patterns (DAMPs) that are recognized by macrophages which further trigger downstream inflammatory responses including activation of toll-like receptors (TLRs) and inflammasomes as well as the release of cytokines such as IL-1 and TNF [41][44][45]. The damage further leads to the stimulation of endothelial cells and endothelial leukocyte adhesion molecules (ELAMs) that recruit leukocytes to the site of damage. Following alveolar damage, fibroblast growth factor (FGF) along with TGF-β1 and chemokines are also stimulated, leading to the recruitment of fibroblasts to the site of injury. These fibroblasts then proliferate and differentiate into myofibroblasts, further activating the inflammatory responses, including epithelial or endothelial to mesenchymal transition. The accumulation of fibroblasts and myofibroblasts can thus lead to over-active secretion of extracellular matrix components. Altogether, dysregulation in any of these response elements as well as aberrant accumulation of various chemokines, pro-fibrotic growth factors, and myofibroblasts can disrupt a well-organized healing response eventually leading to pulmonary fibrosis. There are an increasing number of reports of pulmonary fibrosis in COVID-19 patients as confirmed by radiological scans. For instance, in two different studies conducted by Zhou et al. and Pan et al. , chest CT scans showed fibrotic changes especially during the advanced phase of the disease [46][47]. Furthermore, autopsy reports of patients who died due to COVID-ARDS or post-COVID pneumonia confirmed extensive alveolar damage as well as lung fibrosis [48][49][50]. Yu et al. (2020) compared the CT findings and clinical features of COVID-19 discharged patients who did or didn’t develop pulmonary fibrosis and reported that fibrosis is the more likely outcome in patients who are old age and have serious clinical conditions including an increase in inflammatory indicators CRP and IL-6 [51]. Thus, SARS-CoV-2 induced lung fibrosis may altogether modify lung biomechanics resulting in irreversible tissue damage [52]. However, a direct relationship

between viral infection and pulmonary fibrosis and molecular mechanisms involved in this process are still not apparent [53]. At the RNA level, the downregulation of miR203 might be a key factor involved during pulmonary fibrosis [54]. Another key factor is the transcriptional factor SNAI1 which plays a direct role in epithelial to mesenchymal transition (EMT) and fibroblast activation. SNAI1 is regulated by TGFβ which, as discussed above, plays a key role in fibrosis and causes EMT, which is crucial to fibrosis [55]. Genome wide studies are further required in order to identify specific genes associated with post-COVID-19 lung fibrosis.

4. Fibrotic Repair Is Dominant over Regeneration

The level of inflammation in the lungs following tissue injury is a crucial factor to determine whether regeneration or fibrotic repair will take place [56]. Both repair processes are often sequential and interrelated [57]. In the case of limited damage, regeneration usually occurs first to restore the integrity and function of the tissue. If this process fails because of severe damage, fibrotic repair initiates. Again, if the fibrotic repair fails or if there is excessive fibrotic repair in response to high levels of injury or inflammation, it may lead to chronic lung disease or collapse. This suggests that regeneration is a better healing process while repair is only good when it is moderate. Unfortunately, in the case of virus induced severe infection, fibrotic repair is the dominant process over regeneration [31][53].

Due to lack of post-injury lung samples, suitable animal models, and regeneration-specific molecular markers, alveolar regeneration in humans is still not well characterized. In this pursuit, single-cell RNA sequencing (scRNA-seq) technology has revealed the cellular architecture of the lung and the intermediate state of AT2 to AT1 transition known as Krt8+ cells [17]. AT2 and AT1 cells are classes of distal airway stem cells and Krt8+ cells are the transitional stem cells between AT2 and AT1 as mentioned before. Krt8+ cells appear in both small and big injury and normally peak at 10 days after the injury. They also have the ability to transition back to AT2 cells. These cells are distinguished by their high expression of keratin 8 and distinct gene sets having some similarities with AT1 cells. In the case of injury, these progenitor cells are expressed in the damaged tissue in high numbers. Apart from AT2 cells, activated club cells also play a role in the differentiation to Krt8+ cells by transcriptional regulation in these two stem cells but the detailed mechanism behind this process is still not clear [14][17][58]. After injury, all these cell types, including AT2 cells, Krt8+ cells, and activated club cells, undergo proliferation leading to fibrosis. Krt8+ cells were also found to have an affinity for macrophages, fibroblasts, and endothelial cells. In addition, they also secrete many factors involved in fibrogenesis. Accumulation of Krt8+ cells and not transitioning to AT1 cells can actively lead to fibrosis confirming that Krt8+ cells are profibrogenic. Krt8+ cells were found to be interacting with the cellular niche to coordinate the regeneration process. However, Krt8+ cells do not express similar ligands and receptors to its endpoint AT1 cells and thus are considered a different cell lineage. However, they play an essential role in processes such as inflammation, angiogenesis, and fibrogenesis [15][16][17].

Given the increasing number of incidences of post-COVID lung fibrosis, it is paramount to understand the underlying molecular mechanisms and develop effective targeted strategies for its treatment. A potential approach in this regard is to design therapeutic approaches that can promote regeneration over repair mechanisms. For instance, use of corticosteroids such as dexamethasone indirectly affects this by delaying the repair rate and reducing inflammation [59]. The successful use of dexamethasone in reducing mortality in severe COVID patients has been demonstrated in many trials already [53][60]. Another approach would be to design anti-fibrotic therapeutic strategies in order to slow down the rate of fibrotic progression [61][62]. The two anti-fibrotic drugs already available commercially and currently being tested in COVID-19 patients are pirfenidone and nintedanib [52]. It has also been suggested to use anti-inflammatory drugs such as steroids in combination with the anti-fibrotic drugs as a better treatment strategy [63]. A study performed in mice demonstrated that the blocking of platelet-derived growth factor receptor β (PDGFR-β), where the growth factor PDGF has been shown to be involved in the pathogenesis of fibrosis, reduces the effects of pulmonary fibrosis [64]. It would be further intriguing to perform similar studies using animal models in order to investigate post-COVID lung fibrosis. Moreover, inflammatory cytokines such as IL6, which play an important role in the development of lung fibrosis, might also act as potential therapeutic targets for its treatment. We hypothesize that understanding the cellular network of AT2 cells could be an important step to elucidate critical factors involved in state transition.

References

1. WHO. Coronavirus Disease 2019 (COVID-19) Weekly Operational Update on COVID-19—5 October 2021. Available online: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20211005_weekly_epi_update_60.pdf?sfvrsn=7b566b06_3&download=true (accessed on 12 October 2021).
2. V'Kovski, P.; Kratzel, A.; Steiner, S.; Stalder, H.; Thiel, V. Coronavirus biology and replication: Implications for SARS-CoV-2. Nat. Rev. Microbiol. 2021, 19, 155–170.

3. Berger, S.; Goekeri, C.; Gupta, S.K.; Vera, J.; Dietert, K.; Behrendt, U.; Lienau, J.; Wienhold, S.M.; Gruber, A.D.; Suttorp, N.; et al. Delay in antibiotic therapy results in fatal disease outcome in murine pneumococcal pneumonia. Crit. Care 2018, 22, 287.
4. Thompson, B.T.; Chambers, R.C.; Liu, K.D. Acute Respiratory Distress Syndrome. N. Engl. J. Med. 2017, 377, 562–572.
5. Bellani, G.; Laffey, J.G.; Pham, T.; Fan, E.; Brochard, L.; Esteban, A.; Gattinoni, L.; van Haren, F.; Larsson, A.; McAuley, D.F.; et al. Epidemiology, Patterns of Care, and Mortality for Patients with Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries. JAMA 2016, 315, 788–800.
6. Phua, J.; Badia, J.R.; Adhikari, N.K.; Friedrich, J.O.; Fowler, R.A.; Singh, J.M.; Scales, D.C.; Stather, D.R.; Li, A.; Jones, A.; et al. Has mortality from acute respiratory distress syndrome decreased over time? A systematic review. Am. J. Respir. Crit. Care Med. 2009, 179, 220–227.
7. Cardinal-Fernandez, P.; Lorente, J.A.; Ballen-Barragan, A.; Matute-Bello, G. Acute Respiratory Distress Syndrome and Diffuse Alveolar Damage. New Insights on a Complex Relationship. Ann. Am. Thorac. Soc. 2017, 14, 844–850.
8. Gibson, P.G.; Qin, L.; Puah, S.H. COVID-19 acute respiratory distress syndrome (ARDS): Clinical features and differences from typical pre-COVID-19 ARDS. Med. J. Aust. 2020, 213, 54–56, e1.
9. Ackermann, M.; Verleden, S.E.; Kuehnel, M.; Haverich, A.; Welte, T.; Laenger, F.; Vanstapel, A.; Werlein, C.; Stark, H.; Tzankov, A.; et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N. Engl. J. Med. 2020, 383, 120–128.
10. Ng, M.Y.; Ferreira, V.M.; Leung, S.T.; Yin Lee, J.C.; Ho-Tung Fong, A.; To Liu, R.W.; Man Chan, J.W.; Wu, A.K.L.; Lung, K.C.; Crean, A.M.; et al. Patients Recovered From COVID-19 Show Ongoing Subclinical Myocarditis as Revealed by Cardiac Magnetic Resonance Imaging. JACC Cardiovasc. Imaging 2020, 13, 2476–2478.
11. Vasarmidi, E.; Tsitoura, E.; Spandidos, D.A.; Tzanakis, N.; Antoniou, K.M. Pulmonary fibrosis in the aftermath of the COVID-19 era (Review). Exp. Ther. Med. 2020, 20, 2557–2560.
12. Kotton, D.N.; Morrisey, E.E. Lung regeneration: Mechanisms, applications and emerging stem cell populations. Nat. Med. 2014, 20, 822–832.
13. Chen, Q.; Liu, Y. Heterogeneous groups of alveolar type II cells in lung homeostasis and repair. Am. J. Physiol. Cell Physiol. 2020, 319, C991–C996.
14. Jiang, P.; Gil de Rubio, R.; Hrycaj, S.M.; Gurczynski, S.J.; Riemondy, K.A.; Moore, B.B.; Omary, M.B.; Ridge, K.M.; Zemans, R.L. Ineffectual Type 2-to-Type 1 Alveolar Epithelial Cell Differentiation in Idiopathic Pulmonary Fibrosis: Persistence of the KRT8(hi) Transitional State. Am. J. Respir. Crit. Care Med. 2020, 201, 1443–1447.
15. Kobayashi, Y.; Tata, A.; Konkimalla, A.; Katsura, H.; Lee, R.F.; Ou, J.; Banovich, N.E.; Kropski, J.A.; Tata, P.R. Persistence of a regeneration-associated, transitional alveolar epithelial cell state in pulmonary fibrosis. Nat. Cell Biol. 2020, 22, 934–946.
16. Riemondy, K.A.; Jansing, N.L.; Jiang, P.; Redente, E.F.; Gillen, A.E.; Fu, R.; Miller, A.J.; Spence, J.R.; Gerber, A.N.; Hesselberth, J.R.; et al. Single cell RNA sequencing identifies TGFbeta as a key regenerative cue following LPS-induced lung injury. JCI Insight 2019, 5, e123637.
17. Strunz, M.; Simon, L.M.; Ansari, M.; Kathiriya, J.J.; Angelidis, I.; Mayr, C.H.; Tsidiridis, G.; Lange, M.; Mattner, L.F.; Yee, M.; et al. Alveolar regeneration through a Krt8+ transitional stem cell state that persists in human lung fibrosis. Nat. Commun. 2020, 11, 3559.
18. Wu, H.; Yu, Y.; Huang, H.; Hu, Y.; Fu, S.; Wang, Z.; Shi, M.; Zhao, X.; Yuan, J.; Li, J.; et al. Progressive pulmonary fibrosis is caused by elevated mechanical tension on alveolar stem cells. Cell 2021, 184, 845–846.
19. Stone, K.C.; Mercer, R.R.; Gehr, P.; Stockstill, B.; Crapo, J.D. Allometric relationships of cell numbers and size in the mammalian lung. Am. J. Respir. Cell Mol. Biol. 1992, 6, 235–243.
20. Chu, H.; Chan, J.F.; Wang, Y.; Yuen, T.T.; Chai, Y.; Hou, Y.; Shuai, H.; Yang, D.; Hu, B.; Huang, X.; et al. Comparative Replication and Immune Activation Profiles of SARS-CoV-2 and SARS-CoV in Human Lungs: An Ex Vivo Study with Implications for the Pathogenesis of COVID-19. Clin. Infect. Dis. 2020, 71, 1400–1409.
21. Carcaterra, M.; Caruso, C. Alveolar epithelial cell type II as main target of SARS-CoV-2 virus and COVID-19 development via NF-Kb pathway deregulation: A physio-pathological theory. Med. Hypotheses 2021, 146, 110412.
22. Hou, Y.J.; Okuda, K.; Edwards, C.E.; Martinez, D.R.; Asakura, T.; Dinnon 3rd, K.H.; Kato, T.; Lee, R.E.; Yount, B.L.; Mascenik, T.M.; et al. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell 2020, 182, 429–446.e14.

23. Fang, Y.; Liu, H.; Huang, H.; Li, H.; Saqi, A.; Qiang, L.; Que, J. Distinct stem/progenitor cells proliferate to regenerate the trachea, intrapulmonary airways and alveoli in COVID-19 patients. Cell Res. 2020, 30, 705–707.
24. Nikolic, M.Z.; Sun, D.; Rawlins, E.L. Human lung development: Recent progress and new challenges. Development 2018, 145, dev163485.
25. Pan, H.; Deutsch, G.H.; Wert, S.E.; On behalf of the Ontology Subcommittee & NHLBI Molecular Atlas of Lung Development Program Consortium. Comprehensive anatomic ontologies for lung development: A comparison of alveolar formation and maturation within mouse and human lung. J. Biomed. Semant. 2019, 10, 18.
26. Chen, J.; Wu, H.; Yu, Y.; Tang, N. Pulmonary alveolar regeneration in adult COVID-19 patients. Cell Res. 2020, 30, 708–710.
27. Hogan, B.L.; Barkauskas, C.E.; Chapman, H.A.; Epstein, J.A.; Jain, R.; Hsia, C.C.; Niklason, L.; Calle, E.; Le, A.; Randell, S.H.; et al. Repair and regeneration of the respiratory system: Complexity, plasticity, and mechanisms of lung stem cell function. Cell Stem Cell 2014, 15, 123–138.
28. Butler, J.P.; Loring, S.H.; Patz, S.; Tsuda, A.; Yablonskiy, D.A.; Mentzer, S.J. Evidence for adult lung growth in humans. N. Engl. J. Med. 2012, 367, 244–247.
29. Zhao, Z.; Zhao, Y.; Zhou, Y.; Wang, X.; Zhang, T.; Zuo, W. Single-cell analysis identified lung progenitor cells in COVID-19 patients. Cell Prolif. 2020, 53, e12931.
30. Shao, H.; Qin, Z.; Geng, B.; Wu, J.; Zhang, L.; Zhang, Q.; Wu, Q.; Li, L.; Chen, H. Impaired lung regeneration after SARS-CoV-2 infection. Cell Prolif. 2020, 53, e12927.
31. Lucas, A.; Yasa, J.; Lucas, M. Regeneration and repair in the healing lung. Clin. Transl. Immunol. 2020, 9, e1152.
32. Salvati, L.; Palterer, B.; Parronchi, P. Spectrum of Fibrotic Lung Diseases. N. Engl. J. Med. 2020, 383, 2485.
33. Keane, T.J.; Horejs, C.M.; Stevens, M.M. Scarring vs. functional healing: Matrix-based strategies to regulate tissue repair. Adv. Drug Deliv. Rev. 2018, 129, 407–419.
34. Xue, M.; Jackson, C.J. Extracellular Matrix Reorganization During Wound Healing and Its Impact on Abnormal Scarring. Adv. Wound Care 2015, 4, 119–136.
35. Herrera, J.; Henke, C.A.; Bitterman, P.B. Extracellular matrix as a driver of progressive fibrosis. J. Clin. Investig. 2018, 128, 45–53.
36. Henderson, N.C.; Rieder, F.; Wynn, T.A. Fibrosis: From mechanisms to medicines. Nature 2020, 587, 555–566.
37. Lekkerkerker, A.N.; Aarbiou, J.; van Es, T.; Janssen, R.A. Cellular players in lung fibrosis. Curr. Pharm. Des. 2012, 18, 4093–4102.
38. Kinoshita, T.; Goto, T. Molecular Mechanisms of Pulmonary Fibrogenesis and Its Progression to Lung Cancer: A Review. Int. J. Mol. Sci. 2019, 20, 1461.
39. Sheng, G.; Chen, P.; Wei, Y.; Yue, H.; Chu, J.; Zhao, J.; Wang, Y.; Zhang, W.; Zhang, H.L. Viral Infection Increases the Risk of Idiopathic Pulmonary Fibrosis: A Meta-Analysis. Chest 2020, 157, 1175–1187.
40. Zhang, C.; Wu, Z.; Li, J.W.; Tan, K.; Yang, W.; Zhao, H.; Wang, G.Q. Discharge may not be the end of treatment: Pay attention to pulmonary fibrosis caused by severe COVID-19. J. Med. Virol. 2021, 93, 1378–1386.
41. Ojo, A.S.; Balogun, S.A.; Williams, O.T.; Ojo, O.S. Pulmonary Fibrosis in COVID-19 Survivors: Predictive Factors and Risk Reduction Strategies. Pulm. Med. 2020, 2020, 6175964.
42. Gentile, F.; Aimo, A.; Forfori, F.; Catapano, G.; Clemente, A.; Cademartiri, F.; Emdin, M.; Giannoni, A. COVID-19 and risk of pulmonary fibrosis: The importance of planning ahead. Eur. J. Prev. Cardiol. 2020, 27, 1442–1446.
43. Yang, J.; Pan, X.; Wang, L.; Yu, G. Alveolar cells under mechanical stressed niche: Critical contributors to pulmonary fibrosis. Mol. Med. 2020, 26, 95.
44. Roh, J.S.; Sohn, D.H. Damage-Associated Molecular Patterns in Inflammatory Diseases. Immune Netw. 2018, 18, e27.
45. Wilson, M.S.; Wynn, T.A. Pulmonary fibrosis: Pathogenesis, etiology and regulation. Mucosal Immunol. 2009, 2, 103–121.
46. Zhou, S.; Wang, Y.; Zhu, T.; Xia, L. CT Features of Coronavirus Disease 2019 (COVID-19) Pneumonia in 62 Patients in Wuhan, China. AJR Am. J. Roentgenol. 2020, 214, 1287–1294.
47. Pan, Y.; Guan, H.; Zhou, S.; Wang, Y.; Li, Q.; Zhu, T.; Hu, Q.; Xia, L. Initial CT findings and temporal changes in patients with the novel coronavirus pneumonia (2019-nCoV): A study of 63 patients in Wuhan, China. Eur. Radiol. 2020, 30, 3306–3309.

48. Spagnolo, P.; Balestro, E.; Aliberti, S.; Cocconcelli, E.; Biondini, D.; Casa, G.D.; Sverzellati, N.; Maher, T.M. Pulmonary fibrosis secondary to COVID-19: A call to arms? Lancet Respir. Med. 2020, 8, 750–752.
49. Tian, S.; Xiong, Y.; Liu, H.; Niu, L.; Guo, J.; Liao, M.; Xiao, S.Y. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. Mod. Pathol. 2020, 33, 1007–1014.
50. Wang, Y.; Dong, C.; Hu, Y.; Li, C.; Ren, Q.; Zhang, X.; Shi, H.; Zhou, M. Temporal Changes of CT Findings in 90 Patients with COVID-19 Pneumonia: A Longitudinal Study. Radiology 2020, 296, E55–E64.
51. Yu, M.; Liu, Y.; Xu, D.; Zhang, R.; Lan, L.; Xu, H. Prediction of the Development of Pulmonary Fibrosis Using Serial Thin-Section CT and Clinical Features in Patients Discharged after Treatment for COVID-19 Pneumonia. Korean J. Radiol. 2020, 21, 746–755.
52. Kiener, M.; Roldan, N.; Machahua, C.; Sengupta, A.; Geiser, T.; Guenat, O.T.; Funke-Chambour, M.; Hobi, N.; Kruithof-de Julio, M. Human-Based Advanced in vitro Approaches to Investigate Lung Fibrosis and Pulmonary Effects of COVID-19. Front. Med. 2021, 8, 644678.
53. John, A.E.; Joseph, C.; Jenkins, G.; Tatler, A.L. COVID-19 and pulmonary fibrosis: A potential role for lung epithelial cells and fibroblasts. Immunol. Rev. 2021, 302, 228–240.
54. Rajasekaran, S.; Rajaguru, P.; Sudhakar Gandhi, P.S. MicroRNAs as potential targets for progressive pulmonary fibrosis. Front. Pharm. 2015, 6, 254.
55. Baulida, J.; Diaz, V.M.; Herreros, A.G. Snail1: A Transcriptional Factor Controlled at Multiple Levels. J. Clin. Med. 2019, 8, 757.
56. Strieter, R.M. What differentiates normal lung repair and fibrosis? Inflammation, resolution of repair, and fibrosis. Proc. Am. Thorac. Soc. 2008, 5, 305–310.
57. Jiang, D.; Rinkevich, Y. Scars or Regeneration? Dermal Fibroblasts as Drivers of Diverse Skin Wound Responses. Int. J. Mol. Sci. 2020, 21, 617.
58. Aspal, M.; Zemans, R.L. Mechanisms of ATII-to-ATI Cell Differentiation during Lung Regeneration. Int. J. Mol. Sci. 2020, 21, 3188.
59. Aubin Vega, M.; Chupin, C.; Pascariu, M.; Prive, A.; Dagenais, A.; Berthiaume, Y.; Brochiero, E. Dexamethasone fails to improve bleomycin-induced acute lung injury in mice. Physiol. Rep. 2019, 7, e14253.
60. Tomazini, B.M.; Maia, I.S.; Cavalcanti, A.B.; Berwanger, O.; Rosa, R.G.; Veiga, V.C.; Avezum, A.; Lopes, R.D.; Bueno, F.R.; Silva, M.; et al. Effect of Dexamethasone on Days Alive and Ventilator-Free in Patients with Moderate or Severe Acute Respiratory Distress Syndrome and COVID-19: The CoDEX Randomized Clinical Trial. JAMA 2020, 324, 1307–1316.
61. George, P.M.; Wells, A.U.; Jenkins, R.G. Pulmonary fibrosis and COVID-19: The potential role for antifibrotic therapy. Lancet Respir. Med. 2020, 8, 807–815.
62. Zhang, H.; Cui, Y.; Zhou, Z.; Ding, Y.; Nie, H. Alveolar Type 2 Epithelial Cells as Potential Therapeutics for Acute Lung Injury/Acute Respiratory Distress Syndrome. Curr. Pharm. Des. 2019, 25, 4877–4882.
63. Udwadia, Z.F.; Koul, P.A.; Richeldi, L. Post-COVID lung fibrosis: The tsunami that will follow the earthquake. Lung India 2021, 38 (Suppl. 1), S41–S47.
64. Kishi, M.; Aono, Y.; Sato, S.; Koyama, K.; Azuma, M.; Abe, S.; Kawano, H.; Kishi, J.; Toyoda, Y.; Okazaki, H.; et al. Blockade of platelet-derived growth factor receptor-beta, not receptor-alpha ameliorates bleomycin-induced pulmonary fibrosis in mice. PLoS ONE 2018, 13, e0209786.