Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

International Immunopharmacology 116 (2023) 109418

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Lung fibrosis: Post-COVID-19 complications and evidences

Rishabh Hirawat [a], Namrata Jain [a], Mohd Aslam Saifi [a], Mahesh Rachamalla [b], Chandraiah Godugu [a,*]

[a] Department of Biological Sciences, NIPER Hyderabad, Balanagar, Hyderabad, India
[b] Department of Biology, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada

ARTICLE INFO

Keywords:
COVID-19
Pulmonary fibrosis
Epithelial-mesenchymal transition
TGF-β
WNT
YAP/TAZ

ABSTRACT

Background: COVID 19, a lethal viral outbreak that devastated lives and the economy across the globe witnessed non-compensable respiratory illnesses in patients. As been evaluated in reports, patients receiving long-term treatment are more prone to acquire Pulmonary Fibrosis (PF). Repetitive damage and repair of alveolar tissues increase oxidative stress, inflammation and elevated production of fibrotic proteins ultimately disrupting normal lung physiology skewing the balance towards the fibrotic milieu.

Aim: In the present work, we have discussed several important pathways which are involved in post-COVID PF. Further, we have also highlighted the rationale for the use of antifibrotic agents for post-COVID PF to decrease the burden and improve pulmonary functions in COVID-19 patients.

Conclusion: Based on the available literature and recent incidences, it is crucial to monitor COVID-19 patients over a period of time to rule out the possibility of residual effects. There is a need for concrete evidence to deeply understand the mechanisms responsible for PF in COVID-19 patients.

1. Introduction

In December 2019, a series of reports consisting of atypical pneumonia-like aetiology had surfaced from Wuhan, a province in China, of which the cause was later confirmed to be originated from a novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Within the three months, the infection had spread in all the major countries, at a breakneck speed. The disease caused by SARS-CoV-2, known as Coronavirus Disease 2019, abbreviated as COVID-19 had escalated across the globe creating a major health crisis. This deadly virus belongs to a genus β-coronavirus [1], which had previously caused (SARS-CoV) and Middle East Respiratory Syndrome Corona Virus (MERS-CoV) infections. In 2003, SARS-CoV infected thousands of people and caused nearly 800 deaths whereas MERS-CoV killed around 700 lives in 2012 [2,3]. Due to low transmissibility, the previous viral attacks did not invade much into the population count, but SARS-COV-2 has put a halt to millions of breaths. As of 4th May 2022, the worldwide confirmed active cases numbered approximately 516 million and estimated death toll to about 6.25 million. Looking at the alarming situation, WHO declared COVID-19 as a pandemic on 11th March 2020 (see Figs. 1–4).

The SARS-CoV-2 virus has a higher binding affinity towards Angiotensin Converting Enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), present in the lungs [4] due to certain protein residents (discussed ahead) in the viral structure. Apart from the previously described receptors the virus also binds to other receptors like Cathepsin L1, CD147 and GRP78 [5] and activates pro-inflammatory factors such as cytokines (IL-6, IL-2, IL-1 and TNFα), chemokines (IL-8 and oxanthin), Cell adhesion proteins (ICAM, V-CAM-I and ε-selectin) and inducible enzymes (NOS and COS II) thus, resulting into severe inflammation [6]. Once the virus invades into alveolar epithelial cells, it replicates and stimulates the production of various cytokines that starts agitating inflammation [7] accompanied with tissue injury, further provoking myofibroblast cells which not only initiates collagen deposition [8] but also stimulates mediators like TGF-β [9]. TGF-β belongs to the cytokine family which is known to play a substantial role in the activation of fibroblasts and enhancing fibrosis, giving a signature mark of chronic vascular detriment, indicating Pulmonary Fibrosis (PF) [10].

During emergent cases, efforts were much concreted in controlling the infectious process to prevent further lung impairment [11,12] although patients recovered after a long-term treatment from SARS-CoV-2 infection unveiled pathological findings of PF. To observe the

* Corresponding author at: Department of Biological Sciences (Regulatory Toxicology), National Institute of Pharmaceutical Education and Research (NIPER), Balanagar, Hyderabad, Telangana 500037, India.
E-mail address: chandra.niperhyd@gov.in (C. Godugu).

https://doi.org/10.1016/j.intimp.2022.109418
Received 24 August 2022; Received in revised form 13 October 2022; Accepted 31 October 2022
Available online 4 November 2022

progression of PF, CT scan images resulted to be a remarkable marker to identify the severity of the disease. Based on the percentage of infection in an experiment conducted by Yazan and associates where they developed FPN DenseNet201 encoder model which was proven to be more sensitive in determining the severity of PF [13]. CT scan images could also be used to differentiate between ILD and PF relying on the data of ground glass opacities [14]. PF usually arises after acute respiratory distress syndrome (ARDS). ARDS is divided into two phases: an immediate exudative inflammatory phase and a proliferative phase marked by alveolar epithelial cell hyperplasia [12] Along the line of understanding the unknown post-COVID-19 consequences, short follow-ups have demonstrated fibrotic changes in the lungs at various durations considering the severity spectrum [15,16]. There is a need to unveil the incomplete mechanistic pathway of PF interlinking post COVID observations in patients lungs. Our review majorly focuses on the important pathways that can form a base in an attempt to identify the crucial targets and minimise the risk of post COVID PF for severely affected patients undergoing long term treatment.

2. Structure & pathogenesis

The coronavirus pertains to the order of nidovirales belonging to the family; coronaviridae, which are further categorized into 4 different genera namely: α, β, γ and δ coronaviruses [17]. Amongst the aforementioned viruses, α and β coronaviruses are more pathogenic and infect mammals, whereas γ and δ coronavirus afflict avians more than mammals [18]. The SARS-CoV-2 structure consists of a clustered envelope, positive-sense, single-strand RNA comprising of 30,000 bases along with a 5′cap structure and a 3′poly A tail [19]. The genomic sequence of SARS-CoV-2 is 89 % similar to that of Bat-CoV which possibly connotes bat origin [20].

The major structural proteins assembled in the viruses which are held accountable to penetrate host cells and initiate replications are Surface spike (S) protein, Membrane (M) protein, Envelope (E) protein, Nucleocapsid (N) protein. Surface spike (S) protein: The SARS-CoV-2 entry is mediated by S-protein [21] which consists of 2 subunits specifically S1 and S2 subunits. The S1 subunit consists of the *N*-terminal domain and receptor binding domain, both the domains help in binding with the hACE2 receptor. While S2 subunit is made up of seven components notably, Fusion peptide (FP), Heptad Repeat 1 (HR1), Central Helix (CH), Connector Domain (CD), Heptad Repeat 2 (HR2), Transmembrane Domain (TMD) and Cytoplasmic tail (CT) as its main function is to initiate fusion between host-viral cell membrane [22]. The S protein undergoes 2 step cleavage events, the initial splitting of S1 and S2 followed by splitting of S2 into FP and S2′ which is common in both SARS-CoV-1 and SARS-CoV-2, however SARS-CoV-2 additionally contains furin cleavage. In particular, the furin cleavage initiates cell–cell union, which facilitates efficient viral entrance and propagation from one cell to another in human lung cells [23]. Membrane (M) Protein: It consists of three major domains; *N*-Terminal ectodomain, three transmembrane helices and C-Terminal domain, it plays a major role in the congregation of new virions by interacting with S, E & N proteins which are necessary for initiating membrane budding [24]. Envelope (E) protein: The E protein has the smallest transmembrane and includes 3 domains, an *N*-terminal hydrophilic ectodomain, a domain of hydrophobic transmembrane lastly a long hydrophilic C-terminal endodomain. This protein is responsible for causing loss of membrane potential and actuates host inflammasomes [25]. Nucleocapsid (N) protein: The N protein acts as a package of viral proteins that converts viral RNA into helical ribonucleocapsid proteins (RNP) which allows alteration with other structural proteins during virion assembly further encompassing genome encapsidation [26]. There are two domains assembled into the N protein, an *N*-terminal RNA binding domain and a C terminal domain that helps in the formation of dimers, as reported by Qiaozhen et al. N protein also possesses self-assemble properties and the N2b domain is relatively similar to beta coronavirus structure [27]. The S, M and E protein invades the endoplasmic reticulum whereas the N protein gets attached to the genomic RNA (+) strand to produce nucleoprotein complex [26].

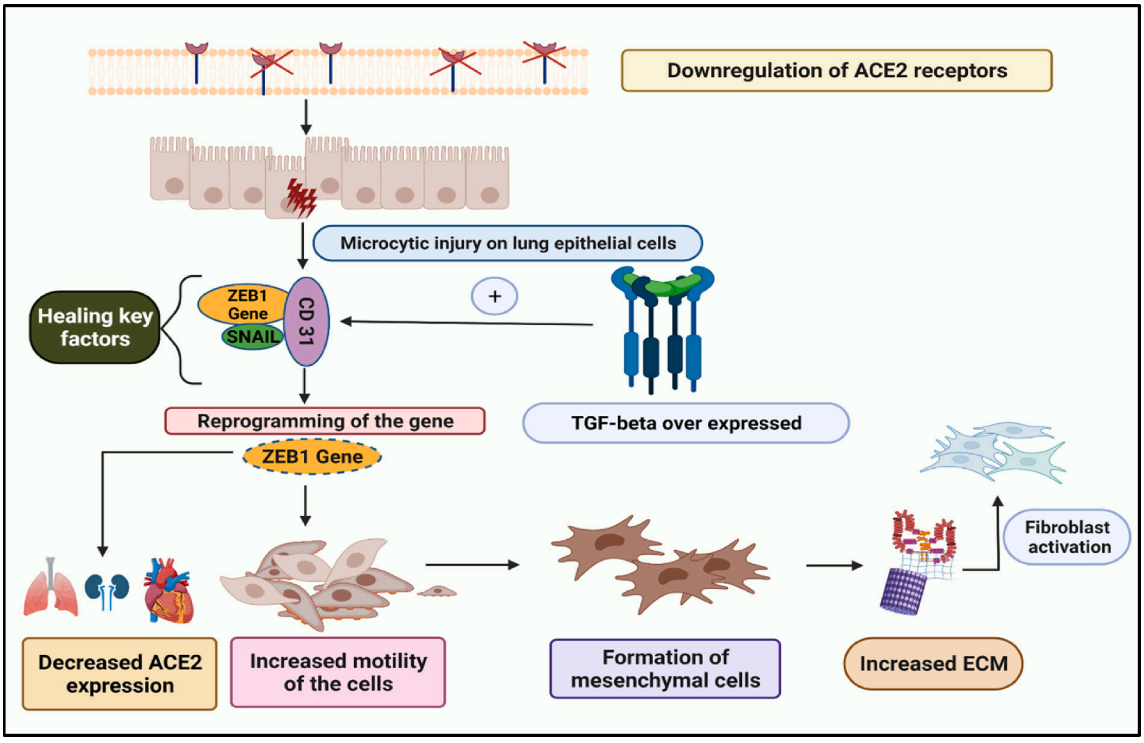

Fig. 1. An overview of the EMT pathway. The alveolar injury results in decreased expression of ACE2 receptors, resulting microcytic injuries in epithelial cells. Epithelial injury can increase the expression of TGF-β and force reprogramming of the ZEB-1 gene to alter characteristic function of healing factors. Genetic reprogramming along with altered functions of the growth factors increase motility of epithelial cells and helps in their transition into mesenchymal subtype that ultimately increases the risk of pulmonary fibrosis.

Additionally, the virus also possesses 16 non-structural proteins (nsp 1–16) [19] and 5–8 accessory proteins along with 14 open reading frames (ORF) that encompass 2/3rd part of the genome which is more prone to mutation. Repetitive mutations in the SARS-CoV-2 genome resulted in numerous variants which have altered its pathogenic ability, in due triggering new symptoms like conjunctivitis, blurred vision, itchiness, tingling in peripheral limbs, etc. leaving the physicians perplexed. A list of important mutations in SARS-CoV-2 is shown in Table 1.

3. Pathogenesis

The SARS-CoV-2 virus primarily binds to type-2 pneumocyte cells in the alveoli of the lungs which further invades into cell surfaces of specific receptors like hACE2 and CD90L [43]. The two subunits of S proteins S1 and S2 have a significant and specific role; on the binding of the virus, the Receptor Binding Domain (RBD) present in the S1 subunit fosters down-regulation of ACE2 receptors, and the S2 subunit assimilates binding with ACE2 receptors and illustrates the fusion between host-viral cell membrane [44]. The ACE2 receptors play a key role in the regulation of the Renin-Angiotensin System (RAS) by maintaining a balance between homeostasis and fluidic ions. The Angiotensin Converting Enzyme (ACE) processes and converts Angiotensin I (AT1) to Angiotensin II (AT2) [45,46], further, the increased production of AT2 activates myriads of signalling pathways. Moreover, AT2 helps in releasing aldosterone and induces vasoconstriction due to imbalance between the sodium ions and fluid, causing excess fluid retention as well as exacerbating pulmonary vascular permeability inducing profibrotic effects. AT II is also responsible to activate the collagen 1 gene through MAPK/ERK pathway and promoting TGF-β, being essential factors for provoking fibrotic responses. Apart from binding to ACE2 receptors, a transmembrane serine protease 2 (TMPRSS2) provides direct entry for the S protein of SARS-CoV-2 [47] and permits penetration into the targeted cells [48]. Upon invading the host's cell, the virus enters into the Endoplasmic Reticulum (ER), and amplifies ER modification through promoting synthesis of viral RNA via orf1a and orf1ab proteins. These viral proteins are transported to the cell surfaces and get released through exocytosis. This transport is greatly involved in the replication and proliferation of the virus. Certain host kinases such as Casein Kinase II (CK2), Cyclic Dependent Kinase (CDK) and Protein Kinase C (PKC) phosphorylate the host receptors and play a pivotal role during replication of the SARS-CoV-2 virus [49]. The aforementioned host kinases regulate the cell cycle and are also responsible for detaining cell function that abides cell death. Once the cell cycle is impaired, the viral proteins incorporated into the structural N protein of the SARS-CoV-2 are elevated and become liable for impeding host translation.

During the infection, the virus gets replicated employing mitosis and facilitates migration assisting deeper invasion into the respiratory cells through a process known as cytoskeletal rearrangement. This rearrangement induces internal as well as external mechanical forces, recognized by integrins and is further transduced through activating the mechano-sensitive pathways. The integrins act as a linker between the cytoskeleton and extracellular matrix (ECM) which provides a biochemical mechanism, commencing vascular contraction and compelling rigidity to the matrix present inside the alveolar lung epithelial cells, leading to fibroblast activation, macrophage phagocytosis and transmuting the endothelial barrier function [50]. Integrins such as $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ not only induces profibrotic pathway but also directly activate TGF-β. The rise of TGF-β levels results in the actuation of fibroblasts, cellular contraction, stiffening of matrix and aggravated collagen deposition, these conformational changes instigate the host's fibrinogenic mechanism ultimately causing the lung injury [46,51]. Considering the antigen-presenting cells (APC) in the SARS-CoV-2 virus also stimulate the release of pro-inflammatory cytokines [IL-6, IL-1β, IL-

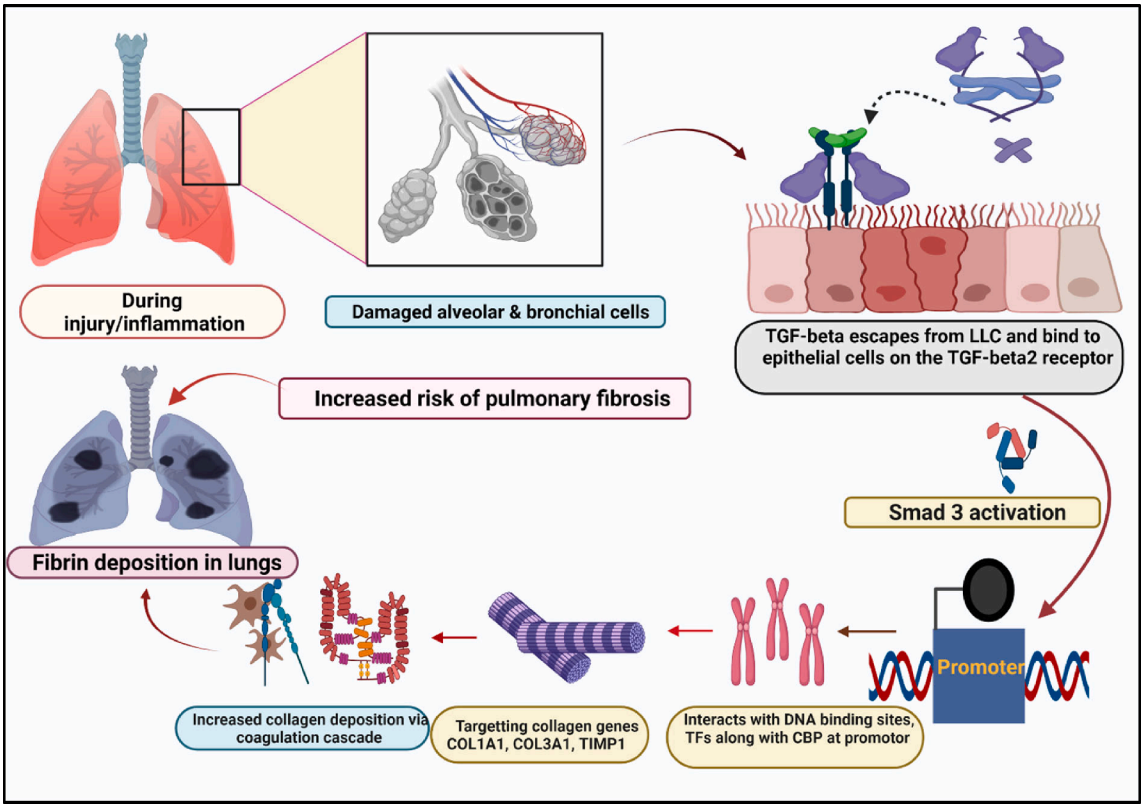

Fig. 2. TGF-β signalling pathway activation. The damaged alveolar epithelial cells stimulate TGF-β signalling, during this process the latent TGF-β escapes the LLC complex and binds with the TGF-β1 receptor which activates SMAD along the way interferes with the collagen genes like COL1A1, COL1A3, TIMP1 and increases the collagen deposition leading to varied ECM depositions.

12, IL-18, IL-33, TNF-α, TGF-β] and chemokines like IL-8, CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, etc. instigating cytokine storm, henceforth mutilates the lining of epithelial cells and reaches into the blood circulation causing multi-organ injuries [43,52,53]. A retrospective cohort study analysed the chemokine levels of 72 patients infected from SARS-CoV-2. The report illustrated levels of different inflammatory cytokines including IL-6, IL-8, IL-10, TNF-α, etc. during the stay period right after hospitalisation till the unprecedented death. It was observed that IL-8 levels always remained high in severely affected COVID-19 patients stating it as one of the prognostic marker for untimely death [54]. Similar conclusions were drawn by Francesco and associates inclusive of 14 days. Observations were compared between IL and 6, IL-8, IL-10 and other pro-inflammatory factors, out of which levels of IL-8 constantly surged during the hospital stay in severely ill patients. Thus, emphasising on the fact that IL-8 can be considered as one of the prognostic markers based on the evaluations [55]. Along the line of understanding the unknown post-COVID-19 consequences, short follow-ups have demonstrated fibrotic changes in the lungs at various durations considering the severity spectrum [15,16]. The direct viral mechanism involved in induction of lung fibrosis in recovered COVID-19 patients is still unknow (see Table 2).

4. COVID-19 and PF

PF (PF) is a sequela of the tissue repair responses which prevail following multiple tissue injuries during chronic inflammatory conditions. PF usually arises after acute respiratory distress syndrome (ARDS). ARDS is a severe, acute form of microvascular injuries present in lungs which can be divided into two phases: an immediate exudative inflammatory phase and a proliferative phase marked by alveolar epithelial cell hyperplasia [12]. It has been observed that patients infected from SARS-CoV-2 virus are at higher risk for developing ARDS as reported in one of the cohort studies where 40 % patients acquired ARDS after hospitalisation marking it as one of the early death predictors. The characteristics of PF involve re-epithelialization, fibroblast activation and increased collagen deposition. PF can be evoked by SARS-CoV-2 virus in two forms. 1) Direct lung injury caused by continuous viral latching of Spike proteins on the ACE2 receptors. The injuries caused during this process activates repair mechanism; however, due to excessive viral invasions there is abnormal healing. The irregular and incessant restoration of wound leads to development of PF. 2) During viral infection, immune mediated inflammatory responses are initiated because of continuous aggregation of macrophages, eosinophils, neutrophils cells at the site of injury and propagate profibrotic and pro-inflammatory cytokines/factors [68]. Particularly in PF, ECM deposition is hugely increased and infuriates normal lung parenchyma further contributing to the expansion of interstitial matrix that weakens the capillaries, leading to ventilatory failure [60–62]. Whenever the lung tissue is injured, collagen and fibronectin help in the formation of fibrotic tissue being an important and normal step of the tissue restoration process. Initially, these injuries activate fibroblasts, helping induce vascular contractions along with the secretion of inflammatory mediators that aid in the healing of impaired tissue [69,70]. Repetitive and extended repairing of lung tissues induce many inflammatory mediators and disturbs the normal architecture of the lungs. In general, alveolar epithelial injury induced from exogenous (infections, toxin, radiation) or endogenous stimuli (inflammation, oxidative stress and aberrant immune response) trigger the release of profibrotic cytokines like TGF-β, TNF-α, Connective Tissue Growth Factor (CTGF), Insulin-like Growth Factor-1 (IGF-1) and Platelet Derived Growth Factor (PDGF) which collectively initiate PF. The increased circulating levels of profibrotic cytokines/growth factors stimulate and enhance the proliferation of lung fibroblasts [69,71–73]. Activated lung fibroblasts also known as myofibroblasts are liable for the production of excess amount of ECM. These myofibroblasts alter ECM balance between matrix, metalloproteinases and their natural inhibitors (tissue inhibitor of MMPs) to promote PF [72,74].

Usually, myofibroblasts are not circulated in tissue in normal conditions [75]. As a study done by Hinz, during the restoration of scars present on lung tissue, myofibroblasts emerge for a brief period and are

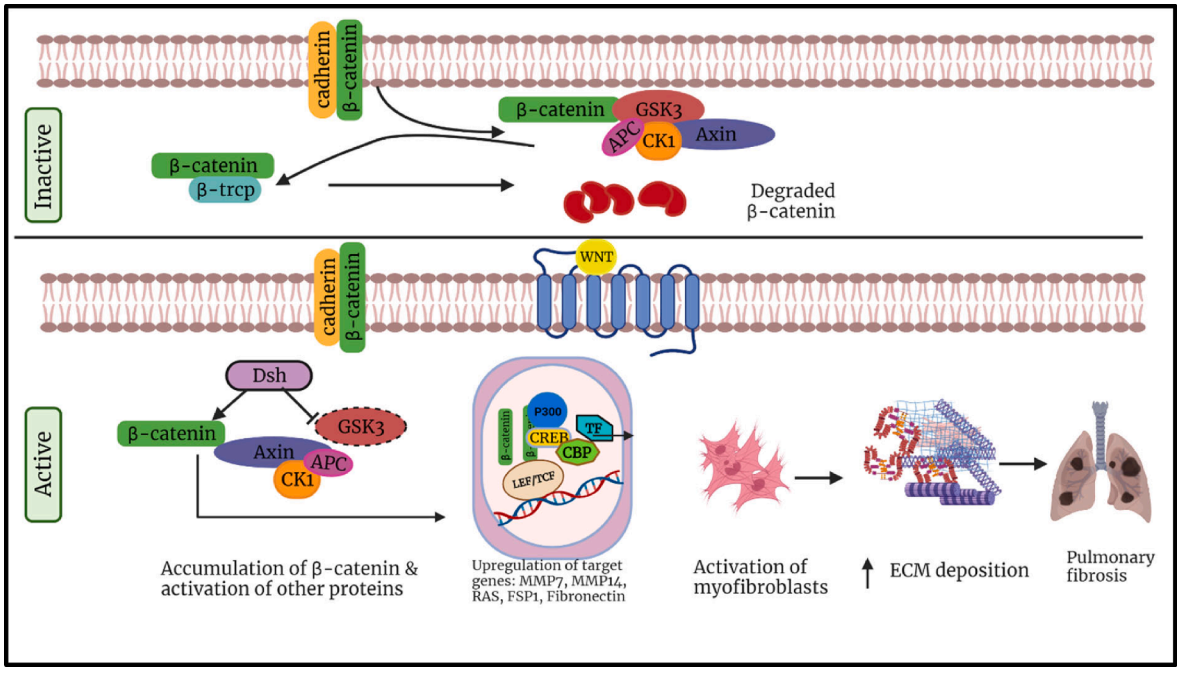

Fig. 3. A simplified diagrammatic representation of WNT signalling pathway: During inactive state β-catenin and Cadherin form a conjugate that combine with other factors like Casein kinase 1 CK1, Glycogen synthase kinase 3 GSK3, APC, Axin, which are degraded with the help of a β-trcp protein. Apparently, in the active phase the WNT signalling helps β-catenin to transduce from the membrane while it forms the complex at the same time stimulation of Dishevelled protein results in inhibition of GSK3 and frees the β-catenin, this unbundled β-catenin accumulates in the cytoplasm and target genes like MMP7, MMP14, RAS, FSP1 and alters their mechanism leading to myofibroblast activation causing excess ECM deposition.

released in a controlled manner for maintaining the normal tissue framework utilizing intense collagen formation on the scars [76]. Various progenitors that activate myofibroblasts are smooth muscle cells in the arterial wall, chondrocytes present in cartilage, osteoblasts in bones and pericytes in vascularized tissues [77]. Apart from these pro-activators, cells of the epithelium and/or endothelium also generate myofibroblasts upon activation via a process known as an epithelial mesenchymal transition (EMT) [78]. An overview of EMT pathway is shown in Fig. 1. Abhijeet P. D and associates exemplified cases and suggested a detailed study considering cytokine TGF-β1 as a critical key factor inducing cellular and molecular changes that interfere with EMT signalling. According to the data obtained it was observed that SARS-CoV-2 induced viral infection could deplete the epithelial and endothelial cellular activity and restrain the easy permeability of spike proteins of SARS-CoV-2. The enhanced permeation via cellular changes could make respiratory cells more prone to edema and increase the risk of PF [79].

5. Molecular mechanism of PF

To understand the molecular mechanism of PF various studies were put forth, it was noted that *trans*-differentiation of epithelial cells into mesenchymal is a major contributor for progression of PF. EMT itself is an intricate mechanism that has both protective and detrimental effects in normal and pathological lung conditions, respectively. It has been demonstrated that EMT plays a necessary role in embryogenesis, gastrulation, and during the formation of the neural crest, heart, and other organs [80]. EMT is responsible for the healing of abrasive tissue in the lung that utilizes several transcription factors like Snail, Slug, Twist, β-catenin, Zinc-finger E box-binding (ZEB), Zeb-1 Zeb-2 and Basic helix-loop-helix that collectively help in regulating the transcriptional, translational and post-translational sequences [81,82]. Initially, epithelial cells lose their apical-basal polarity and change their cytoskeleton structure by redefining their cell shape, encroaching a reprogrammed gene of ZEB1 which directly suppress ACE2 expression and consequently elevate vimentin levels, inferring increased mesenchymal shifts; as a result, cells become more motile and advance its invasion [83–86]. EMT is flexible and varies according to the cell type, tissue context and signalling pathways that are responsible to activate them. The normal infrastructure of lung has organised matrix fibres and uniform basal lamina of alveolar epithelial tissue. ECM consists balanced proportions of proteins such as elastin, laminins, collagens while in PF conditions, the orientation of the matrix fibres in lungs get disrupted along with the upregulation of collagens. There is over expression of TGF-β, glycosaminoglycans (GAGs) and decreased production of laminins [87]. Imbalanced proteins expressed during the continuous repair of alveolar tissues hamper the normal ECM flora making it denser followed by differentiation of fibroblasts [88] which initiate EMT allowing epithelial cells to undergo apoptosis and gain senescence resistance [89]. Likely, endothelial cells transit themselves into mesenchymal cells known as Endothelial Mesenchymal Transition (EndMT) also stimulate myofibroblasts genes [90]. When TGF-β activates EMT and EndMT via SMAD pathway it unveils the expression of Vascular Endothelin (VE) – Cadherin, CD 31 and claudins. However, SMAD4′s dominant-negative form blocks TGF-β induced EndMT, decreases Snail expression and VE-Cadherin as well as CD 31 which in a way increases the Fibroblast – Specific Protein 1 (FSP1) and alpha-smooth muscle actin (α-SMA) expression [91]. The downregulation of E-cadherin in EMT destabilizes the adherent junctions in the epithelial cells and alters the gene expression that impairs the barrier function of epithelial cells [92]. The E-cadherin downregulation is balanced by upregulation of Neural (N) cadherin which is expressed in mesenchymal cells results in a cadherin switch, which changes cell adhesion. The affinity towards *N*-cadherin acquires via homotypic *N*-cadherin interactions and causes epithelial

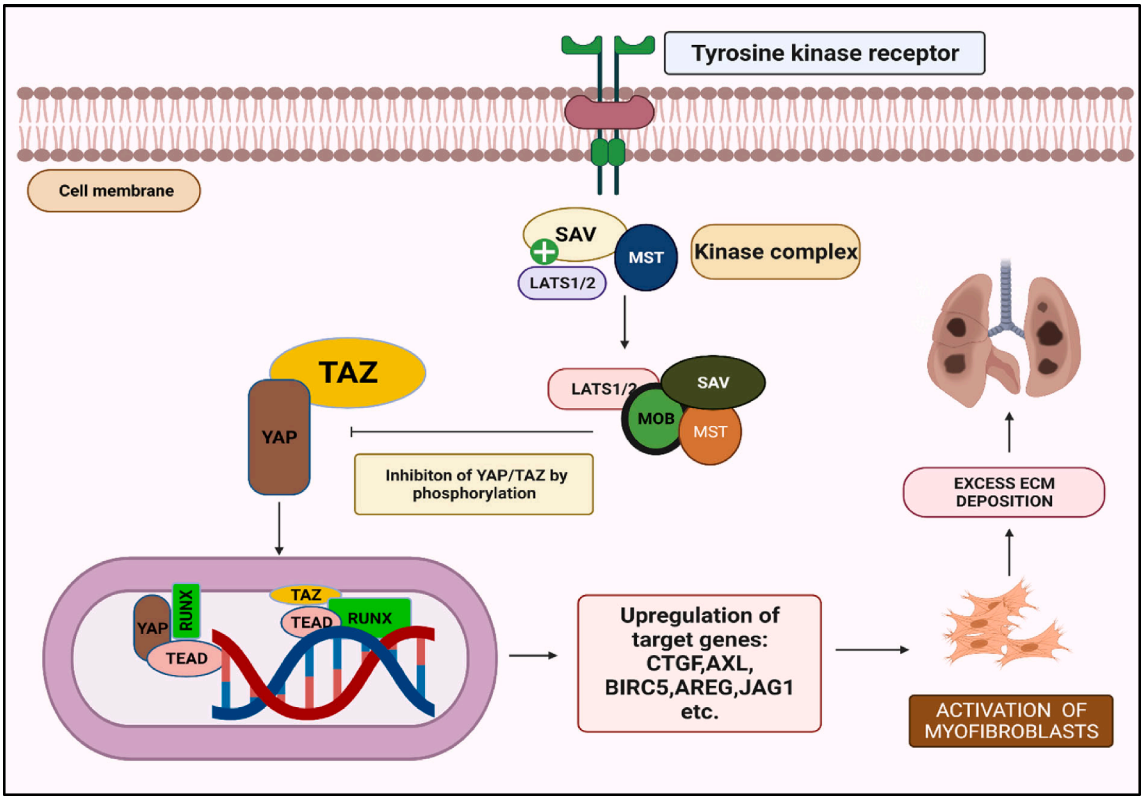

Fig. 4. YAP/TAZ Signalling pathway. In the active state, a kinase complex is formed between LATS1/2 and MST1/2 via stimulation of MOB, which inhibits the YAP/TAZ and further initiates nucleus transduction where YAP and TAZ bind with RUNX, TEAD whilst upregulating targeted genes CTGF, AXL, BIRC5, AREG, JAG1, that activates myofibroblasts ultimately exceeds ECM deposition.

Table 1
Few important mutations reported in SARS-CoV-2.

Sr No.	Variant	Mutation reported	Effects	References
1	B.1.17	17 mutations in spike protein (Δ69-70, Δ144 deletion N501, P681H, T7161, S982A)	Enhances the affinity towards ACE2 receptor	[19,26–32]
2.	B.1.351	9 mutations in spike protein (L18F, D80A, N501Y, D215G, R2461, K471N, D614G A701V, E484)	Decreased monoclonal antibody therapy	[33–36]
3.	B.1.1.28.1	P1 Lineage, 10 mutations in spike protein (LI8F, 501Y, D138Y)	Decreased convalescent & post vaccination sera	[36–38,40]
4.	B.1.617.2	Delta variant harbours 10 mutations, was 1st detected in India, December 2020.	Increased transmissibility, decreased monoclonal antibody therapy	[38,41]
4.	B.1.427 &B.1.429	Specific mutation (427: L452R, D614G & 429: W152C, S131, L452R, D614G)	Increased transmissibility related to wild type	[39,42]
5.	B.1.525 & B.1.526	Harbors spike mutations (A67V, Δ69/70, Δ144, E484K, Q677H, & 526: T951, D253G, D614G)	Increased transmissibility related to wild type circulating strains	[38]
6.	B.1.1.28.2	Spike mutations (L18F, T20N, P265, F157L, E484K, D614G, S929I, V1176F)	Potential reduction of monoclonal antibody and vaccine sera	[38]
7.	B.1.1.28.3	Key spike mutations (141–143 deletion, E484K, N501Y, P681H)	Reduction in convalescent therapy	[38]
8.	B.1.617.1	Variant harbour key mutations (G142 Deletion, D66H, Y144V, D215G, V483A, D614G, H655Y, G669S, Q949R, N1187D)	Decreased plasma convalescent therapy and monoclonal antibody	[38]

cells to lose their adherence whilst connecting more towards mesenchymal cells [93]. Summarizing EMT, it signifies dynamic interconversion of epithelial cells to quasi mesenchymal cells being reversible as these mesenchymal cells can be converted back to epithelial cells through a mechanism known as mesenchymal epithelial transition (MET). It plays a central role in repeated wound healing and exhibiting features like invasiveness, increased motile function of quasi cells, shedding of epithelial cells, unbalanced ECM, leading to fibroblast activation that elevates the collagen deposition, aggravated fibronectin, causing deeper abrasions and finally subjecting it into an aggressive form of PF.

6. Pathways that may interlink COVID-19 and PF

In most cases, COVID-19 death is a result of lung failure due to severe acute respiratory distress syndrome. This syndrome is particularly attributed as an uncontrolled inflammatory response by a "cytokine storm", ultimately leading to edema and fibrosis in the lungs at later stages. Hypothesizing the below pathways may allow us to understand

Table 2
Cases of pulmonary dysfunctionalities observed in patients post COVID-19.

S no.	Title	Studies conducted	Complications / findings	References
1.	Risk factors associated with ARDS & death in patients with coronavirus disease 2019 pneumonia in Wuhan, China.	40 % COVID infected patients developed ARDS of which 20 % cases were severe	Diffused alveolar damage (DAD) characterized by an initial acute inflammatory exudative phase with hyaline membrane, followed by the fibrotic phase	[56]
2.	Risk factors associated with disease severity and length of hospital stay in COVID-19 patients.	61 patients with a disease duration of more than 3 weeks developed fibrosis	Duration of disease plays an important role in signifying lung fibrosis.	[57]
3.	Impaired pulmonary function in discharged patients with COVID-19:- more work ahead.	In a cohort study, 25.45 % of patients manifested lung abnormalities post COVID	Impaired diffusion capacity Decreased alveolar volume Decreased coefficient of the lung for carbon monoxide (kCO)	[53]
4.	Pulmonary post-mortem finding series of COVID-19 cases from Northern Italy: a two-centre descriptive study.	38 patient's lung tissue samples were analysed who died from COVID-19 and reported 33 cases in which platelet fibrin thrombi were found, an ideal marker for Interstitial lung disease (ILD)	DAD Capillary congestion Interstitial and intra-alveolar edema Type-2 pneumocyte hyperplasia Squamous metaplasia Atypia and Coagulopathy	[59]
5.	Rapid Onset honeycombing fibrosis in spontaneously breathing patient with COVID-19	Individual case report	High ACE2 expression, indicative with IPF in the intimal, medial and advential layers of pulmonary arteries. Expression of mesenchymal proteins like S100A4 and vimentin and EndMT	[60]
6.	Fatal Pulmonary fibrosis, a post-COVID-19 autopsy case	Individual case report of Caucasian Female aged 80	CT scan revealed bilateral development of widespread fibrosis DAD Fibrosis honeycomb-like remodelling Bronchial metaplasia	[61]
7.	Abnormal pulmonary function in COVID-19 patient at the time of hospital discharge.	In a cohort study of 110 patients of which 47.2 % cases reported features of Lung fibrosis	Decreased diffusion capacity in the alveolar septal fibrous proliferation Capillary damage	[62]
8.	Clinical characteristics of 138 hospitalised patients with	A cohort study of 138 patients revealed that most patients with ARDS or severe	CT Scans revealed bilateral ground-glass opacities with or without consolidation	[63]

(*continued on next page*)

Table 2 (*continued*)

S no.	Title	Studies conducted	Complications / findings	References
	2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China.	pneumonia were susceptible to Pulmonary fibrosis		
9.	Residual ground glass opacities 3 months after COVID-19 pneumonia correlate to alteration of respiratory function. The post COVID M3 study	A retrospective study amongst 145 patients reported 79.3 % bilateral pneumonia along with extensive intra alveolar fibrin deposition	Extensive injury of alveolar epithelial cells and endothelial cells Secondary fibroproliferation Pulmonary hypertension	[10]
10.	Post COVID-19 Pneumonia Pulmonary Fibrosis	Individual case report of a male patient aged 48	Chest imaging reported fibrotic changes in the form of traction bronchiectasis, architectural distortions and septal thickening	[64]
11.	Evolution of COVID-19 Pulmonary Fibrosis – Like Residual Changes Over Time-Longitudinal Chest CT up to 9 months after Disease Onset: a Single-Centre Case Series	Follow ups of COVID-19 infected patients were conducted after a long-term treatment. Out of 192 patients, 22 patients CT chest images were observed with some deformities in lungs.	CT images signified persistent signs of lung interstitial damages along with DAD.	[65]
12.	CT Lung Abnormalities after COVID-19 at 3 Months and 1 Year after Hospital Discharge	73 patients CT scan images post COVID follow up lead to identify some histopathological changes.	The CT scan images consisted of persistent abnormalities in lungs.	[66]
13	Temporal Patterns of COVID-19-Associated Pulmonary Pathology: An Autopsy Study	Autopsy images of pulmonary tissues of 27 patients who were severely infected with COVID-19 represented anomalies in the reports.	The morphological oddity after histopathological studies included deteriorated hyaline membrane and DAD.	[67]

the connections between pro-inflammatory factors and other signalling mechanisms giving an undue rise of deaths even after complete treatment.

6.1. *TGF-β signalling pathway*

According to a few pieces of evidence reported during COVID-19 infection, the infection cases were associated with a sharp rise in inflammatory and immune response, resulting in activation of various mediators particularly, TGF-β, right after declined lung function [93]. In most of the patients, the severe infection had worsened the lung condition, probably considering ARDS which is the main stakeholder of inducing edema and fibrosis in the lungs at later stages [93]. It is stated that the uncontrolled release of TGF-β retrogrades the homeostasis fluid in the lungs and causes improper coagulation cascade along with irregular fibrinolytic pathway [94]. SARS-CoV-2 causes apoptosis of bronchial cells, alveolar cells, pneumocytes and *T*-lymphocytes which further subjugate to death of neutrophils, to clear the debris, macrophages wander and invade into the lungs where they engulf and digest the dead cells and subsequently releases more amount of TGF-β [95]. Usually, in normal circumstances, TGF-β forms a Large Latent Complex (LLC) which is conjoined with Latency Associated Peptides (LAP's) and Latent TGF-β Binding Proteins (LTBP's) [96,97]. Subordinate to this any injury or inflammation in lung tissues compel TGF-β to escape the LLC and forces it to bind with type 2 receptor (TGF-β-2) present on the membrane of epithelial cells. Furthermore, the continuous activation of TGF-β-2 results in the down-streaming of signal molecules namely, Suppressor of Mothers against Decapentaplegic homologues (SMAD 2) and (SMAD 3) [98]. SMADs are a cluster of intracellular proteins that help in transmitting the nuclear signals from TGF-β towards the surface of cell [97]. [99]During the SARS-CoV outbreak, it was noted that the Nucleoprotein (NP) present inside the virus increased the levels of TGF-β1 in mucosal tissue [100]. Moreover, SARS-CoV-2 NP is 90 % homologous to SARS-CoV which indicates direct stimulation of TGF-β mediating the gene expressions via SMAD3 activation. The SMAD 2/3 and 4 interact with DNA sequence-specific binding sites primarily, transcription factor (TF) and CBP at the promoter, which brings out stimulation of fibrosis-related gene (COL1A1, COL3A1, TIMP1 etc.) and excess deposition of ECM [101]. Thus, an uncontrolled amount of TGF-β released via activated cytokines and pro-inflammatory factors results in edema and fibrosis which block the airway, finally causing irreversible lung failure and death. The activation and critical role of TGF-β signalling in pathophysiology of COVID-19 is shown in the Fig. 2.

6.2. *WNT-signalling pathway*

WNT plays a crucial part in controlling cell proliferation and migration during inflammation and PF [102]. This pathway is distorted in various pathological conditions such as CNS disorders, disturbed psychological behaviour, improper metabolism, etc. [103] In presence of TGF-β, the WNT pathway is upregulated and as reported by COVID-19 survivors, some clinical findings exhibited increased WNT expression [104,105]. WNT is encoded by a large family of secreted protein factors in humans [106,107]. WNT signal transduction can be classified as 1. Canonical pathway or (WNT/ β-Catenin pathway), 2. WNT/PCP (Planar Cell Polarity pathway), 3. WNT/Ca^{+2} pathway [108]. Amongst these pathways, the canonical pathway influences majorly during inflammation and helps in the activation of the fibroblasts [108–110]. In the present scenario, there is some evidence which points out WNT canonical pathway being somehow linked to cytokine storm syndrome which is the considerable factor responsible for inducing ARDS in severe COVID-19 infected patients [106,111]. To explore more, researchers analysed reports of COVID-19 patients who were severely infected, observed higher WNT levels than that of normal, specifically WNT5a in blood samples, which if over stimulated induces ARDS and makes one prone to PF in short span [106]. A study forecasted on WNT and TGF-β showed synchronized positive feedback to each other [112,113]. WNT ligands play a very crucial role in repairing injured tissues, by several mechanisms, including signal control, embryogenesis, cell proliferation, apoptosis, organogenesis, migration and sustains polarity [102]. To maintain normal tissue function in the lungs one of the foremost activities is to knock off the accumulated β-catenin by destructive complexed proteins, like Casein kinase 1 (CK1), Glycogen synthase kinase (GSK3), Axis inhibitors (Axin) and Adenomatous polyposis colon (APC), under the phosphorylation process. It also interacts with β-Transduction repeat containing protein (β-trcp), which regulates the protein mechanism resulting in ubiquitination of β-Catenin and simultaneous proteasome decay. The transduction takes place via Seven transmembrane receptors of the frizzled family and the co-receptor of Lipoprotein Receptor Related Protein 5/6 (LRP 5/6). During inflammation in lung tissues, the WNT pathway is upregulated at higher levels and it further stimulates Dishevelled (Dsh) protein plays a major role in inhibiting GSK3 protein, hindering the destructive process, which releases unresolved β-Catenin

to rack up in the cytoplasm. Consequently, these free β-Catenin bind to other ancillary factors viz. T Cytokines/Lymphokines TCF/LEF, Transcription Factors, p300, CREB and CBP and regulate targeted genes like MMP7, MMP14, RAS, FSP1 that directly or indirectly aid in the progression of PF by increasing the levels of ECM along with hyperactivation of myofibroblasts [114–116]. A simplified diagrammtic presentation of WNT signalling is demonstrated in Fig. 3.

6.3. YAP/TAZ signalling pathway

Yes-Associated Proteins (YAP)/Transcriptional co-activator with PDZ-binding motif (TAZ) combinedly is transcriptional co-activator that acts as a key regulator in maintaining organ size and as a mechanical property it also activates fibroblasts, through down-streaming hippo pathway [117]. The hippo pathway is a type of inhibitory pathway, its main function is to regulate cell proliferation, stem cell differentiation and apoptosis. In humans, the hippo pathway constitutes of complexed protein structures of MST1/2 with Salvador (SAV) that imparts direct stimulation of Mps One Binder (MOB) and Long-Acting Thyroid Stimulator (LATS1/2) [118]. Further, the MOB activation propels MST1/2 and LATS1/2 to form a kinase complex (MST1/2-LATS1/2) that initiates phosphorylation of the YAP/TAZ and degrades the complex proteosome considering the fact that it is a normal process [119]. In a situation where the addition of serine residues consisting of Ser127, S381 in YAP and TAZ, respectively [106,109], interferes with the normal phosphorylation process and inactivates it. Further assisting, YAP/TAZ to get transferred into the nucleus from the cytoplasmic origin and forcing it to interact with transcription factors like Runt Related Transcription factor (RUNX) and TEA domain (TEAD) family that regulates the targeted genes and causes up-regulation of (Connective tissue growth factor) CTGF, AXL, Baculoviral inhibitor of apoptosis repeat-containing 5 (BIRC5) genes and ultimately increases ECM deposition and progresses PF [118,120]. An in-vitro study was conducted by Gustavo G. and associates, wherein the lung samples of normal control and COVID-19 patients were collected to observe the level of YAP. The study revealed significant increase in the level of YAP in COVID-19 lung samples when compared to normal [118,119,121,133–140]. Fig. 4 shows activation of myofibroblasts in response to YAP/TAZ signalling.

To summarize, the above-discussed pathways give an explanation towards the significance of fibrosis in the long term treated and severely infected COVID-19 patients, where repeated repairing in lung tissue results in uncontrollable inflammatory response of TGF-β [122] and other cytokines [97,123,124] causing an increased series of inflammatory pathways, myofibroblasts activation [8,125–129], dysregulated collagen deposition [70,130] and pro-fibrotic factors stimulation [126]. Moreover, these inducers damage the barrier function of the cells and continuously delineate epithelial cells present in alveoli [131,132] giving rise to impaired pulmonary function resulting in untimely death in COVID-19 patients. According to the studies performed, a clear indication is imparted where the rise of TGF-β results in fibrosis and also activates other mechanistic pathways including WNT/β-catenin and YAP/TAZ, which unwillingly triggers fibroblasts and disturbs the normal homeostasis in lungs. These pathways are yet elusive and stipulated, hence it becomes important to unveil the incomplete pathways to understand the liable risks preceding post COVID.

7. Can post COVID-19 PF resolve with time?

In a wake of the existing quo, the rise of new cases is being witnessed in some countries, the strains of the mutated virus didn't spare embarking the new symptoms along with unknown multiple complications even after being successfully treated [141]. One of the major threats still wandering is PF; an unavoidable consequence emerging just after ARDS in patients undergoing long COVID-19 treatment, the repetitive detriment of capillaries causes plasma protein to leak, which implies an inevitable risk of ARDS [142]. A proportion of acute ARDS survivors are likely to develop a fibroproliferative response, which comprises fibroblast accumulation, collagen deposition, and perhaps other pulmonary ECM components [143], the severity of which has been linked to the duration of the disease [144]. Furthermore, despite the fact that invasive mechanical ventilation has profoundly altered ARDS care, ventilator-associated lung damage is thought to be a significant contributor to PF in ventilated ARDS patients [145]. In addition to Diffuse alveolar damage (DAD) and hyaline membranes, literature analysis of reported histopathological findings of COVID-19 lungs postmortem highlighted the frequent occurrence of PF, whereas aberrant pulmonary architecture and functioning have been described in many recovered COVID-19 patients [62,146,147], it has been revealed that SARS-CoV-2 infection causes the production of many pro-fibrotic factors, including TGF- β [148]. Interstitial lung diseases (ILD) patients, on the other hand, had a higher risk of developing ARDS and severe COVID-19 [149–152], and COVID-19 related ILD acute exacerbations had a worse prognosis than non-COVID-19 ILD acute exacerbations, [59] suggesting PF as an inevitable risk and a possible COVID-19 complication. Taking into account one of the studies evidenced 41 % of SARS-CoV-2 infected patients developed ARDS. Foreseeing the cytokine storm syndrome, the patient's condition worsens because of uncontrolled release of pro-inflammatory factors resulting in repetitive inflammation making patients susceptible for hyperactive reactions, untimely combating of immune cells circulated from blood to the site of infection along with destructive effects [2,141,142]. However, the mechanism of PF, occurring post-COVID-19 is yet to be precisely resolved. Initially, during the onset of infection, neutrophils, macrophages and helper *T*-cells attack the SARS-CoV-2 invaders but due to the absence of specific antibodies in humans these saviour cells encounter apoptosis, and results in cell death [153]. Although complete treatment does not guarantee whether the patient survives or gets succumbed to death, if the patient has some prior pulmonary disease, then infection makes the patient's case worrisome and later at the end-stage it increases the fatality risk thriving patient to a condition where it becomes impossible to survive without an Extracorporeal membrane oxygenation (ECMO) [154]. Most of the evidence set forth, manifests a worrisome condition PF, due to prolonged COVID-19 treatment in severe and comorbid cases. Even after accurate treatment is attested it is hard to escape from post COVID illnesses faced by the infected population, not only does it subsidize the daily lifestyle but also has rampant effects etched onto the livelihood. Considering the duration and spectra of post COVID management does not either promise or repay a healthy life once infected with SARS-CoV-2. This arises an uncertainty whether fatal PF is itself resolved with time or does it need a permanent solution?

8. Future perspective

In a plight to impose, the review article mainly focuses on pathways that may interlink PF with post-COVID complications. However, the concept of persistent PF in COVID-19 is debatable issue due to availability of contrasting reports. Although, there are definite evidence of the residual fibrosis in the COVID-19 survivors; the developed fibrosis seems to be self-resolutory in nature. The PF developed in the COVID-19 patients resolved over time without any need of antifibrotic regimen (Pirfenidone and Nintedanib). Some reports also highlight the use of anti-inflammatory drugs like ibuprofen, naproxen, could be used to improve the pulmonary condition in severely affected COVID-19 patients. According to a case study conducted by Castro et al., the use of ibuprofen and naproxen at an early stage would help patients avoid the risk of mechanical ventilation during their hospital stay and would slow down the inflammatory responses [155]. Interestingly, Hong and colleagues discovered the potential use of celecoxib, a COX-II inhibitor that improved pulmonary ground opacities and prevented disease progression in severely affected COVID-19 patients [156]. These findings may suggest the possible role of anti-inflammatory drugs to impede PF progression. In the absence of any long-term data in COVID-19, it is quite

early to reach a consensus on whether PF persists or resolves automatically gradually. However, in the light of previous viral attacks, it seems more plausible to state that the PF could become one of the major complications associated with COVID-19. Due to widespread and high number of affected individuals by COVID-19, we cannot neglect the development of PF in a minor fraction of the population. In addition, the residual effects of viral attacks generally persist over a considerable time of period. As the development of PF is a slow progressive phenomenon; there are chances that PF in COVID-19 survivors can appears after months or even years of the infection. Due to these issues, the PF progression in COVID-19 patients demands our attention. However, the development of PF in COVID-19 survivors seems to be an indirect effect of proinflammatory mechanisms and current treatment of COVID-19 patients with anti-inflammatory agents might have an inhibitory effect on the development of PF making antifibrotic therapy redundant. Our limited knowledge about the basic pathological mechanisms behind the development of PF in COVID-19 is still limited which hinders our decision to add antifibrotic therapy to the ongoing regimen or not. Further, as the COVID-19 infection affects multiple signalling pathways implicated in PF; focusing on a single pathway may not be a good approach for the management of COVID-induced PF. Furthermore, the presence of comorbid conditions adds to the complexity to an already complex pathological phenomenon. However, with increasing horizon of our knowledge in this respect will ultimately help to achieving better management of PF observed in COVID-19 survivors. Nevertheless, the emergence of PF in COVID-19 patients represent itself as one of the greatest concerns associated with COVID-19 infection. There is an urgent need to focus on this neglected aspect of the deadly viral infection for better management of COVID-19 associated complications.

9. Conclusion

PF in COVID-19 patients is one of the most fatal, post COVID complications. COVID-19 induced damage to the lungs could result into a progressive PF leading to multiple organ failure. Moreover, rapid mutations and newly hijacked pathways also make it difficult to delineate the underlying mechanisms responsible for PF. However, in the light of recent incidences, it is clear that PF in COVID-19 survivors resolved over time, at least, in a considerable number of patients. On the other hand, there are reports to demonstrate persistence of PF in other subjects which complicates the picture. Nevertheless, PF in COVID-19 remains a challenge given the lack of suitable antifibrotic agents. At present, we are far from reaching the consensus on developed fibrosis in COVID-19 infection. A deeper understanding of the novel mechanisms used by virus to cause/sustain PF will likely result in better management of PF and other associated complications to serve mankind.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

The authors are thankful to ministry of chemicals and fertilizers, Govt. of India.

References

[1] A.E. John, C. Joseph, G. Jenkins, A.L. Tatler, COVID-19 and pulmonary fibrosis: A potential role for lung epithelial cells and fibroblasts, Immunol. Rev. 302 (2021) 228–240.
[2] A.M. Al-Osail, M.J. Al-Wazzah, The history and epidemiology of Middle East respiratory syndrome corona virus, Multidiscip. Respir. Med. 12 (2017).
[3] D.S.C. Hui, A. Zumla, Severe Acute Respiratory Syndrome: Historical, Epidemiologic, and Clinical Features, Infectious Disease Clinics 33 (2019) 869–889.
[4] M. Hoffmann, et al., SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor, Cell 181 (2020) 271–280.e8.
[5] J.A. Aguiar, et al., Gene expression and in situ protein profiling of candidate SARS-CoV-2 receptors in human airway epithelial cells and lung tissue, Eur. Respiratory Soc. 56 (2020).
[6] J.L. Ríos, Effects of triterpenes on the immune system, J. Ethnopharmacol. 128 (2010) 1–14.
[7] C. Zhang, Z. Wu, J.W. Li, H. Zhao, G.Q. Wang, Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality, Int. J. Antimicrob. Agents 55 (2020), 105954.
[8] T.A. Wynn, Cellular and molecular mechanisms of fibrosis, J. Pathol. 214 (2008) 199–210.
[9] P, K. M., Sivashanmugam, K., Kandasamy, M., Subbiah, R. & Ravikumar, V. Repurposing of histone deacetylase inhibitors: A promising strategy to combat pulmonary fibrosis promoted by TGF-β signalling in COVID-19 survivors. Life Sci 266, 118883 (2021).
[10] J. Frija-Masson, et al., Residual ground glass opacities three months after Covid-19 pneumonia correlate to alteration of respiratory function: The post Covid M3 study, Respir. Med. 184 (2021), 106435.
[11] P. Pandey, S. Agarwal, Rajkumar, Lung pathology in COVID-19: A systematic review, Int. J. Appl. Basic Med. Res. 10 (2020) 226.
[12] Pellegrino, R., Sterk, P., … J. S.-E. R. & 1998, undefined. Assessing the effect of deep inhalation on airway calibre: a novel approach to lung function in bronchial asthma and COPD. Eur Respiratory Soc 12, 1219–1227 (1998).
[13] Y. Qiblawey, et al., Detection and Severity Classification of COVID-19 in CT Images Using Deep Learning, Diagnostics 11, 893 (2021).
[14] M. Orlandi, et al., The role of chest CT in deciphering interstitial lung involvement: systemic sclerosis versus COVID-19, Rheumatology (Oxford) 61 (2022) 1600–1609.
[15] X. Li, et al., CT imaging changes of corona virus disease 2019(COVID-19): A multi-center study in Southwest China, J. Transl. Med. 18 (2020).
[16] Z. Ye, Y. Zhang, Y. Wang, Z. Huang, B. Song, Chest CT manifestations of new coronavirus disease 2019 (COVID-19): a pictorial review, Eur. Radiol. 30 (2020) 4381–4389.
[17] A. Wu, et al., Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China, Cell Host Microbe 27 (2020) 325–328.
[18] A.R. Fehr, S.C. Perlman, An Overview of Their Replication and Pathogenesis, Coronaviruses: Methods and Protocols 1282 (2015) 1–23.
[19] F. Wu, et al., A new coronavirus associated with human respiratory disease in China, Nature 2020 579:7798 579, 265–269 (2020).
[20] M. Ciotti, et al., COVID-19 Outbreak: An Overview, Chemotherapy 64 (2019) 215–223.
[21] W. Li, et al., Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus, Nature 2003 426:6965 426, 450–454 (2003).
[22] M.Y. Wang, et al., SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development, Front. Cell. Infect. Microbiol. 10 (2020) 724.
[23] X. Xia, Domains and Functions of Spike Protein in SARS-Cov-2 in the Context of Vaccine Design, Viruses 13 (2021) 109 .
[24] M. Ujike, F. Taguchi, M. Johnson, S.-L. Liu, Incorporation of Spike and Membrane Glycoproteins into Coronavirus Virions, Viruses 7 (2015) 1700–1725.
[25] V.S. Mandala, et al., Structure and drug binding of the SARS-CoV-2 envelope protein transmembrane domain in lipid bilayers, Nat. Struct. Mol. Biol. 2020 27: 12 27, 1202–1208 (2020).
[26] M. Sarkar, S. Saha, Structural insight into the role of novel SARSCoV-2 E protein: A potential target for vaccine development and other therapeutic strategies, PLoS ONE 15 (2020).
[27] Q. Ye, A.M. v West, S. Silletti, K.D. Corbett, C.D. Kevin Corbett, Architecture and self-assembly of the SARS-CoV-2 nucleocapsid protein, Wiley Online Library 29, 1890–1901 (2020).
[28] E. Volz, et al., Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England, Nature 593 (2021) 266–269.
[29] D.J. Grint, et al., Case fatality risk of the SARS-CoV-2 variant of concern B.1.1.7 in England, 16 November to 5 February, Euro. Surveill. 26 (2021).
[30] N.G. Davies, et al., Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7, Nature 593 (2021) 270–274.
[31] R. Challen, et al., Risk of mortality in patients infected with SARS-CoV-2 variant of concern 202012/1: matched cohort study, BMJ 372 (2021).
[32] N.G. Davies, et al., Association of tiered restrictions and a second lockdown with COVID-19 deaths and hospital admissions in England: a modelling study, Lancet Infect. Dis. 21 (2021) 482–492.
[33] R.P. Walensky, H.T. Walke, A.S. Fauci, SARS-CoV-2 Variants of Concern in the United States-Challenges and Opportunities, JAMA 325 (2021) 1037–1038.
[34] N.G. Davies, et al., Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England, Science 372 (2021).

[35] K. Wu, et al., mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. *bioRxiv* (2021) doi:10.1101/2021.01.25.427948.
[36] H. Tegally, et al., Detection of a SARS-CoV-2 variant of concern in South Africa, Nature 2021 592:7854 592, 438–443 (2021).
[37] C.K. Wibmer, et al., SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma, bioRxiv (2021), https://doi.org/10.1101/2021.01.18.427166.
[38] M. Mwenda, et al., Detection of B.1.351 SARS-CoV-2 Variant Strain - Zambia, December 2020, MMWR Morb Mortal Wkly Rep 70 (2021) 280–282.
[39] P. Wang, et al., Increased Resistance of SARS-CoV-2 Variant P.1 to Antibody Neutralization. bioRxiv (2021) doi:10.1101/2021.03.01.433466.
[40] N.R. Faria, et al., Genomics and epidemiology of a novel SARS-CoV-2 lineage in Manaus, Brazil. *medRxiv* (2021), https://doi.org/10.1101/2021.02.26.21252554.
[41] M. Cascella, M. Rajnik, A. Cuomo, S.C. Dulebohn, R. di Napoli, Features, Evaluation, and Treatment of Coronavirus (COVID-19). StatPearls (2022).
[42] W. Zhang, et al., Emergence of a Novel SARS-CoV-2 Variant in Southern California, JAMA 325 (2021) 1324–1326.
[43] X. Li, M. Geng, Y. Peng, L. Meng, S. Lu, Molecular immune pathogenesis and diagnosis of COVID-19, J. Pharm. Anal. 10 (2020) 102–108.
[44] F. Li, W. Li, M. Farzan, S.C. Harrison, Structural biology: Structure of SARS coronavirus spike receptor-binding domain complexed with receptor, Science 1979 (309) (2005) 1864–1868.
[45] Y. Imai, et al., Angiotensin-converting enzyme 2 protects from severe acute lung failure, Nature 2005 436:7047 436, 112–116 (2005).
[46] K. Kuba, et al., A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury, Nat. Med. 2005 11:8 **11**, 875–879 (2005).
[47] F. Li, et al. Distinct mechanisms for TMPRSS2 expression explain organ-specific inhibition of SARS-CoV-2 infection by enzalutamide, Nat. Commun. 2021 12:1 12, 1–14 (2021).
[48] N.A. Bhowmick, et al., COVID-19 and androgen-targeted therapy for prostate cancer patients, Endocr. Relat. Cancer 27 (2020) R281–R292.
[49] M. Bouhaddou, et al., The Global Phosphorylation Landscape of SARS-CoV-2 Infection, Cell 182 (2020) 685–712.e19.
[50] G. Burgstaller, et al., The instructive extracellular matrix of the lung: basic composition and alterations in chronic lung disease, Eur. Respir. J. 50 (2017) 1601805.
[51] B. Hinz, B. Suki, Does breathing amplify fibrosis? Am. J. Respir. Crit. Care Med. 194 (2016) 9–11.
[52] M.J. Cameron, J.F. Bermejo-Martin, A. Danesh, M.P. Muller, D.J. Kelvin, Human immunopathogenesis of severe acute respiratory syndrome (SARS), Virus Res. 133 (2008) 13–19.
[53] R. Chen, Y. Gao, M. Chen, W. Jian, C. Lei, J. Zheng, S. Li, Impaired pulmonary function in discharged patients with COVID-19: more work ahead, Eur. Respir. J. 56 (1) (2020) 239–241.
[54] H. Li, et al., The prognostic value of IL-8 for the death of severe or critical patients with COVID-19, Medicine 100 (2021) e23656.
[55] F. Salton, et al., Cytokine Profiles as Potential Prognostic and Therapeutic Markers in SARS-CoV-2-Induced ARDS, J. Clin. Med. 11 (2022) 2951.
[56] C. Wu, et al., Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China, JAMA Intern. Med. 180 (2020) 934–943.
[57] X. Liu, et al., Risk factors associated with disease severity and length of hospital stay in COVID-19 patients, J. Infect. 81 (2020) e95–e97.
[59] L. Carsana, et al., Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study, Lancet Infect. Dis. 20 (2020) 1135–1140.
[60] M. Combet, A. Pavot, L. Savale, M. Humbert, X. Monnet, Rapid onset honeycombing fibrosis in spontaneously breathing patient with COVID-19, Eur. Respir. J. 56 (2020).
[61] H.F. Schwensen, et al., Fatal pulmonary fibrosis: a post-COVID-19 autopsy case, J. Clin. Pathol. 74 (2021) 400–402.
[62] X. Mo, et al., Abnormal pulmonary function in COVID-19 patients at time of hospital discharge, Eur. Respir. J. 55 (2020).
[63] D. Wang, et al., Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China, JAMA 323 (2020) 1061–1069.
[64] S. Tale, et al., Post-COVID-19 pneumonia pulmonary fibrosis, QJM: An Int. J. Med. 113 (2020) 837–838.
[65] A. Poerio, et al., Evolution of COVID-19 Pulmonary Fibrosis-Like Residual Changes Over Time — Longitudinal Chest CT up to 9 Months After Disease Onset: a Single-Center Case Series, SN Compr. Clin. Med. 4 (2022).
[66] B. Vijayakumar, et al., CT Lung Abnormalities after COVID-19 at 3 Months and 1 Year after Hospital Discharge. https://doi.org/10.1148/radiol.2021211746 (2021) doi:10.1148/RADIOL.2021211746.
[67] G.S. Stoyanov, et al., Temporal Patterns of COVID-19-Associated Pulmonary Pathology: An Autopsy Study, Cureus 13 (2021).
[68] W.J. Huang, X.X. Tang, Virus infection induced pulmonary fibrosis, J. Transl. Med 19 (2021) 496.
[69] S. Fredj, J. Bescond, C. Louault, D. Potreau, Interactions between cardiac cells enhance cardiomyocyte hypertrophy and increase fibroblast proliferation, J. Cell. Physiol. 202 (2005) 891–899.
[70] J. Prakash, P.K. Bhattacharya, S. Priye, N. Kumar, Post-COVID-19 Pulmonary Fibrosis: A Lifesaving Challenge, Indian J. Crit. Care Med. 25 (2021) 104–105.
[71] J.A. Lucas, et al., Inhibition of transforming growth factor-β signaling induces left ventricular dilation and dysfunction in the pressure-overloaded heart, Am. J. Physiol. Heart Circ. Physiol. 298 (2010) 424–432.
[72] J.C. Horowitz, V.J. Thannickal, Mechanisms for the resolution of organ fibrosis, Physiology 34 (2019) 43–55.
[73] B.C. Berk, K. Fujiwara, S. Lehoux, ECM remodeling in hypertensive heart disease, J. Clin. Invest. 117 (2007) 568–575.
[74] T. Tsuruda, L.C. Costello-Boerrigter, J.C. Burnett, Matrix metalloproteinases: Pathways of induction by bioactive molecules, Heart Fail. Rev. 9 (2004) 53–61.
[75] B. Hinz, Myofibroblasts, Exp. Eye Res. 142 (2016) 56–70.
[76] B. Hinz, Mechanical Aspects of Lung Fibrosis. (2012), https://doi.org/10.1513/pats.201202-017AW 9, 137-147.
[77] J.J. Tomasek, G. Gabbiani, B. Hinz, C. Chaponnier, R.A. Brown, Myofibroblasts and mechano-regulation of connective tissue remodeling, Nat. Rev. Mol. Cell Biol. 2002 3:5 3, 349–363 (2002).
[78] B. Hinz, The myofibroblast in connective tissue repair and regeneration, Regenerative Medicine and Biomaterials for the Repair of Connective Tissues 39–80 (2010), https://doi.org/10.1533/9781845697792.39.
[79] A.P. Deshmukh, et al., Identification of EMT signaling cross-talk and gene regulatory networks by single-cell RNA sequencing, Proc. Natl. Acad. Sci. 118, (2021).
[80] C.A. Stewart, et al., SARS-CoV-2 infection induces EMT-like molecular changes, including ZEB1-mediated repression of the viral receptor ACE2, in lung cancer models, bioRxiv 31, (2020).
[81] S.v. Vasaikar, et al., EMTome: a resource for pan-cancer analysis of epithelial-mesenchymal transition genes and signatures, Br. J. Cancer 2020 124:1 124, 259–269 (2020).
[82] J. Roche, The Epithelial-to-Mesenchymal Transition in Cancer, Cancers 10 (2018) 52.
[83] J.P. Thiery, J.P. Sleeman, Complex networks orchestrate epithelial–mesenchymal transitions, Nat. Rev. Mol. Cell Biol. 2006 7:2 7, 131–142 (2006).
[84] J.P. Thiery, H. Acloque, R.Y.J. Huang, M.A. Nieto, Epithelial-Mesenchymal Transitions in Development and Disease, Cell 139 (2009) 871–890.
[85] R. Huang, P. Guilford, science, J. T.-J. of cell & 2012, undefined. Early events in cell adhesion and polarity during epithelial-mesenchymal transition. journals.biologists.com (2012) doi:10.1242/jcs.099697.
[86] W. Jia, A. Deshmukh, S.A. Mani, M.K. Jolly, H. Levine, A possible role for epigenetic feedback regulation in the dynamics of the epithelial–mesenchymal transition (EMT), Phys. Biol. 16 (2019), 066004.
[87] P. Confalonieri, et al., Regeneration or Repair? The Role of Alveolar Epithelial Cells in the Pathogenesis of Idiopathic Pulmonary Fibrosis (IPF), Cells 11 (2022).
[88] A. Pardo, S. Cabrera, M. Maldonado, M. Selman, Role of matrix metalloproteinases in the pathogenesis of idiopathic pulmonary fibrosis, Respir. Res. 17 (2016).
[89] V.A. Ptasinski, J. Stegmayr, M.G. Belvisi, D.E. Wagner, L.A. Murray, Targeting Alveolar Repair in Idiopathic Pulmonary Fibrosis, Am. J. Respir. Cell Mol. Biol. 65 (2021) 347.
[90] L.A. van Meeteren, P. ten Dijke, Regulation of endothelial cell plasticity by TGF-β, Cell Tissue Res. 347 (2012) 177–186.
[91] D. Medici, S. Potenta, R. Kalluri, Transforming growth factor-β2 promotes Snail-mediated endothelial–mesenchymal transition through convergence of Smad-dependent and Smad-independent signalling, Biochem. J. 437 (2011) 515–520.
[92] E. Theveneau, R. Mayor, Cadherins in collective cell migration of mesenchymal cells, Curr. Opin. Cell Biol. 24 (2012) 677–684.
[93] M.C. Montalvo Villalba, et al., Interferon gamma, TGF-β1 and RANTES expression in upper airway samples from SARS-CoV-2 infected patients, Clin. Immunol. 220 (2020), 108576.
[94] S.S. Batah, A.T. Fabro, Pulmonary pathology of ARDS in COVID-19: A pathological review for clinicians, Respir. Med. 176 (2021), 106239.
[95] S. Sanjabi, S.A. Oh, M.O. Li, Regulation of the Immune Response by TGF-β: From Conception to Autoimmunity and Infection, Cold Spring Harb Perspect Biol 9 (2017), a022236.
[96] A. Ghazavi, A. Ganji, N. Keshavarzian, S.R. Cytokine, Undefined. Cytokine profile and disease severity in patients with COVID-19, Elsevier, 2021.
[97] W. Chen, A potential treatment of COVID-19 with TGF-β blockade, Int. J. Biol. Sci. 16 (2020) 1954.
[98] L. He, et al., Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: Relation to the acute lung injury and pathogenesis of SARS, J. Pathology 210 (2006) 288–297.
[99] X.M. Meng, D.J. Nikolic-Paterson, H.Y. Lan, TGF-β: the master regulator of fibrosis, Nat. Rev. Nephrology 2016 12:6 12, 325–338 (2016).
[100] M.O. Li, R.A. Flavell, TGF-β: A Master of All T Cell Trades, Cell 134 (2008) 392–404.
[101] T.A. Wynn, T.R. Ramalingam, Mechanisms of fibrosis: therapeutic translation for fibrotic disease, Nat. Med. 2012 18:7 18, 1028–1040 (2012).
[102] K.M. Loh, R. van Amerongen, R. Nusse, Generating Cellular Diversity and Spatial Form: Wnt Signaling and the Evolution of Multicellular Animals, Dev. Cell 38 (2016) 643–655.
[103] O. Oren, B.D. Smith, Eliminating Cancer Stem Cells by Targeting Embryonic Signaling Pathways, Stem Cell Reviews and Reports 2016 13:1 13, 17–23 (2016).
[104] D. Newman, W. Sills, … K. H.-… of H. & & 2016, undefined. Expression of WNT5A in idiopathic pulmonary fibrosis and its control by TGF-β and WNT7B in human lung fibroblasts. journals.sagepub.com 64, 99–111 (2016).
[105] S. Giacoppo, et al., Aberrant expression of β-catenin in CD4+ T cells isolated from primary progressive multiple sclerosis patients, Neurosci. Lett. 653 (2017) 159–162.

[106] E.Y. Choi, et al., Wnt5a and Wnt11 as acute respiratory distress syndrome biomarkers for severe acute respiratory syndrome coronavirus 2 patients, Eur. Respir. J. 56 (2020).
[107] B. Shen, et al., Proteomic and Metabolomic Characterization of COVID-19 Patient Sera, Cell 182 (2020) 59–72.e15.
[108] C. Niehrs, S.P. Acebron, Mitotic and mitogenic Wnt signalling, EMBO J. 31 (2012) 2705–2713.
[109] R. Nusse, H. Clevers, Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities, Cell 169 (2017) 985–999.
[110] C.P. Petersen, P.W. Reddien, Wnt Signaling and the Polarity of the Primary Body Axis, Cell 139 (2009) 1056–1068.
[111] J. Villar, H. Zhang, A.S. Slutsky, Lung Repair and Regeneration in ARDS: Role of PECAM1 and Wnt Signaling, Chest 155 (2019) 587–594.
[112] A. Vallée, Y. Lecarpentier, R. Guillevin, J.N. Vallée, Interactions between TGF-β1, canonical WNT/β-catenin pathway and PPAR γ in radiation-induced fibrosis, Oncotarget 8 (2017) 90579.
[113] A. Vallée, Y. Lecarpentier, TGF-β in fibrosis by acting as a conductor for contractile properties of myofibroblasts, Cell & Biosci. 2019 9:1 9, 1–15 (2019).
[114] M. Shtutman, et al., The cyclin D1 gene is a target of the β-catenin/LEF-1 pathway, National Acad Sciences 96 (1999) 5522–5527.
[115] S. Angers, R.T. Moon, Proximal events in Wnt signal transduction, Nat. Rev. Mol. Cell Biol. 2009 10:7 10, 468–477 (2009).
[116] T.C. He, et al., Identification of c-MYC as a Target of the APC Pathway, Science 1979 (281) (1998) 1509–1512.
[117] I.M. Moya, G. Halder, Hippo–YAP/TAZ signalling in organ regeneration and regenerative medicine, Nat. Rev. Mol. Cell Biol. 20 (2018 20:4 2018,) 211–226.
[118] A. Genevet, N. Tapon, The Hippo pathway and apico–basal cell polarity, Biochem. J. 436 (2011) 213–224.
[119] B. Zhao, et al., Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control, Genes Dev. 21 (2007) 2747–2761.
[120] S. Liu, et al., Comprehensive assessment of peripheral blood TCRβ repertoire in infectious mononucleosis and chronic active EBV infection patients, Ann. Hematol. 96 (2017) 665–680.
[121] Gustavo Garcia, Jr. *et al.* Hippo Signaling Pathway Activation during SARS-CoV-2 Infection Contributes to Host Antiviral Response. *bioRxiv* (2022) doi:10.1101/2022.04.07.487520.
[122] C.B.V. Paula, de et al. COVID-19: Immunohistochemical Analysis of TGF-β Signaling Pathways in Pulmonary Fibrosis, Int. J. Mol. Sci. 2022, Vol. 23, Page 168 23, 168 (2021).
[123] X. Sun, et al., Cytokine storm intervention in the early stages of COVID-19 pneumonia, Cytokine Growth Factor Rev. 53 (2020) 38–42.
[124] E. Prompetchara, C. Ketloy, T. Palaga, Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic, Asian Pac. J. Allergy Immunol. 38 (2020) 1–9.
[125] V.J. Thannickal, G.B. Toews, E.S. White, J.P. Lynch, F.J. Martinez, Mechanisms of Pulmonary Fibrosis. (2004) 395–417, https://doi.org/10.1146/annurev.med.55.091902.103810 55.
[126] B.C. Willis, R.M. DuBois, Z. Borok, Epithelial origin of myofibroblasts during fibrosis in the lung, Proc. Am. Thorac. Soc. 3 (2006) 377–382.
[127] I. Grgic, J.S. Duffield, B.D. Humphreys, The origin of interstitial myofibroblasts in chronic kidney disease, Pediatr. Nephrol. 2011 27:2 27, 183–193 (2011).
[128] J. Zavadil, E.P. Böttinger, TGF-β and epithelial-to-mesenchymal transitions, Oncogene 2005 24:37 24, 5764–5774 (2005).
[129] G. Gabbiani, The myofibroblast in wound healing and fibrocontractive diseases, J. Pathol. 200 (2003) 500–503.
[130] P.J. Sime, K.M.A. O'reilly, Fibrosis of the lung and other tissues: new concepts in pathogenesis and treatment, Clin. Immunol. 99 (2001) 308–319.
[131] K.K. Kim, et al., Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix, National Acad Sciences (2006).
[132] I.Y.R. Adamson, L. Young, D.H. Bowden, Relationship of alveolar epithelial injury and repair to the induction of pulmonary fibrosis, Am. J. Pathol. 130 (1988) 377.
[133] B. Piersma, R.A. Bank, M. Boersema, Signaling in fibrosis: TGF-β, WNT, and YAP/TAZ converge, Front. Med. (Lausanne) 2 (2015).
[134] B. Zhao, L. Li, K. Tumaneng, C.Y. Wang, K.L. Guan, A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCFβ-TRCP, Genes Dev. 24 (2010) 72–85.
[135] B. Zhao, et al., TEAD mediates YAP-dependent gene induction and growth control. genesdev.cshlp.org (2008) doi:10.1101/gad.1664408.
[136] T. Oka, V. Mazack, M. Sudol, Mst2 and Lats kinases regulate apoptotic function of Yes kinase-associated protein (YAP), J. Biol. Chem. 283 (2008) 27534–27546.
[137] C.Y. Liu, et al., The hippo tumor pathway promotes TAZ degradation by phosphorylating a phosphodegron and recruiting the SCFβ-TrCP E3 ligase, J. Biol. Chem. 285 (2010) 37159–37169.
[138] H. Zhang, et al., Correction: TEAD transcription factors mediate the function of TAZ in cell growth and epithelial-mesenchymal transition, J. Biol. Chem. 294 (2019) 5808.
[139] W.M. Mahoney, J.H. Hong, M.B. Yaffe, I.K.G. Farrance, The transcriptional co-activator TAZ interacts differentially with transcriptional enhancer factor-1 (TEF-1) family members, Biochem. J. 388 (2005) 217–225.
[140] A. Vassilev, K.J. Kaneko, H. Shu, Y. Zhao, M.L. DePamphilis, TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm, Genes Dev. 15 (2001) 1229–1241.
[141] A. John, C. Joseph, … G. J.-I. & 2021, undefined. COVID-19 and pulmonary fibrosis: A potential role for lung epithelial cells and fibroblasts. Wiley Online Library 302, 228–240 (2021).
[142] D. Hui, A. Z.-I. D. Clinics, 2019, undefined. Severe acute respiratory syndrome: historical, epidemiologic, and clinical features. id.theclinics.com.
[143] N.J. Simmonds, et al., Cystic fibrosis and survival to 40 years: a study of cystic fibrosis transmembrane conductance regulator function, Eur. Respir. J. 37 (2011) 1076–1082.
[144] A.W. Thille, et al., Chronology of histological lesions in acute respiratory distress syndrome with diffuse alveolar damage: a prospective cohort study of clinical autopsies, Lancet Respir Med 1 (2013) 395–401.
[145] N.E. Cabrera-Benitez, et al., Mechanical Ventilation–associated Lung Fibrosis in Acute Respiratory Distress SyndromeA Significant Contributor to Poor Outcome, Anesthesiology 121 (2014) 189–198.
[146] K.J. Myall, et al., Persistent post–COVID-19 interstitial lung disease: An observational study of corticosteroid treatment, Ann Am Thorac Soc 18 (2021) 799–806.
[147] R.J.J. van Gassel, et al., High prevalence of pulmonary sequelae at 3 months after hospital discharge in mechanically ventilated survivors of COVID-19, Am. J. Respir. Crit. Care Med. 203 (2021) 371–374.
[148] L.T. McDonald, Healing after COVID-19: Are survivors at risk for pulmonary fibrosis? Am. J. Physiol. Lung Cell. Mol. Physiol. 320 (2021) L257–L265.
[149] G. Beltramo, et al., Chronic respiratory diseases are predictors of severe outcome in COVID-19 hospitalised patients: a nationwide study, Eur. Respir. J. 58 (2021) 2004474.
[150] T.M. Drake, et al., Outcome of hospitalization for COVID-19 in patients with interstitial lung disease an international multicenter study, Am. J. Respir. Crit. Care Med. 202 (2020) 1656–1665.
[151] A.J. Esposito, et al., Increased odds of death for patients with interstitial lung disease and COVID-19: A case–control study, Am. J. Respir. Crit. Care Med. 202 (2020) 1710–1713.
[152] R. Bussani, et al., Persistence of viral RNA, pneumocyte syncytia and thrombosis are hallmarks of advanced COVID-19 pathology, EBioMedicine 61 (2020).
[153] S. Steiner, et al., HCoV- and SARS-CoV-2 Cross-Reactive T Cells in CVID Patients, Front. Immunol. 11 (2020) 3347.
[154] X. Ma, et al., Extracorporeal Membrane Oxygenation (ECMO) in Critically Ill Patients with Coronavirus Disease 2019 (COVID-19) Pneumonia and Acute Respiratory Distress Syndrome (ARDS), Med. Sci. Monit. 26 (2020) e925364–e925371.
[155] V.M. Castro, R.A. Ross, S.M. McBride, R.H. Perlis, Identifying common pharmacotherapies associated with reduced COVID-19 morbidity using electronic health records. medRxiv 2020.04.11.20061994 (2020) doi:10.1101/2020.04.11.20061994.
[156] W. Hong, et al., Celebrex adjuvant therapy on COVID-19: An experimental study. medRxiv 2020.05.05.20077610 (2020) doi:10.1101/2020.05.05.20077610.

Further reading

[58] S. Nusair, Abnormal carbon monoxide diffusion capacity in COVID-19 patients at time of hospital discharge, Eur. Respir. J. 56 (2020).