REVIEW

Role of pirfenidone in TGF-β pathways and other inflammatory pathways in acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: a theoretical perspective

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Abstract

Background Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes pulmonary injury or multiple-organ injury by various pathological pathways. Transforming growth factor-beta (TGF-β) is a key factor that is released during SARS-CoV-2 infection. TGF-β, by internalization of the epithelial sodium channel (ENaC), suppresses the anti-oxidant system, downregulates the cystic fibrosis transmembrane conductance regulator (CFTR), and activates the plasminogen activator inhibitor 1 (PAI-1) and nuclear factor-kappa-light-chain-enhancer of activated B cells (NF-kB). These changes cause inflammation and lung injury along with coagulopathy. Moreover, reactive oxygen species play a significant role in lung injury, which levels up during SARS-CoV-2 infection.

Drug Suggestion Pirfenidone is an anti-fibrotic drug with an anti-oxidant activity that can prevent lung injury during SARS-CoV-2 infection by blocking the maturation process of transforming growth factor-beta (TGF-β) and enhancing the protective role of peroxisome proliferator-activated receptors (PPARs). Pirfenidone is a safe drug for patients with hypertension or diabetes and its side effect tolerated well.

Conclusion The drug as a theoretical perspective may be an effective and safe choice for suppressing the inflammatory response during COVID-19. The recommendation would be a combination of pirfenidone and *N*-acetylcysteine to achieve maximum benefit during SARS-CoV-2 treatment.

Keywords TGF-β signaling · Pathology of COVID-19 · Pirfenidone mechanism of action · COVID-19 therapy · SARS-CoV-2 mechanism

Abbreviations

ACE2	Angiotensin-converting enzyme
AEC	Alveolar epithelial cells
AFC	Alveolar fluid clearance
ALI	Acute lung injury
ANG-II	Angiotensin-II
ARDS	Acute respiratory distress syndrome
ARE	Anti-oxidant redox elements
Bach1	BTB domain and CNC homolog 1
BVR	Biliverdin reductase
CFTR	Cystic fibrosis transmembrane conductance regulator
CO	Carbon monoxide
CORM-2	Carbon monoxide-releasing molecule-2
DAD	Diffuse alveolar damage
ECM	Extracellular matrix
ENaC	Epithelial sodium channel
ELF	Epithelial lining fluid
GSH	Glutathione
HLF	Human lung fibroblast
HMGB1	High mobility group box-1
HO-1	Heme oxygenase-1
HOCL	Hypochlorous acid

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KEAP1	NRF2/Kelch-like ECH-associated protein 1
KLF4	Krüppel-like factor-4
IFN-γ	Interferon-gamma receptor
IKK	IκB kinase
IL-1R	Interleukin receptor 1
IPF	Idiopathic pulmonary fibrosis
LAP	Latency-associated protein
LMTK2	Lemur tyrosine kinase 2
LTBP	Latent TGF-β-binding protein
NAC	N-Acetylcysteine
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-kB	Nuclear factor-kappa
NLRP3	NLR family pyrin domain-containing 3
NOX4	NADPH oxidase4
Nrf2	Nuclear factor erythroid 2-related factor 2
MAP3K7IP2 or TAB2	Mitogen-activated protein kinase kinase kinase 7-interacting protein 2
MAP3K7 or TAK1	Mitogen-activated protein kinase kinase kinase 7
miR-145	Mir-145 microRNA
MLF	Mouse long fibroblasts
PA	Phosphatidic acid
PAI-1	Plasminogen activator inhibitor 1
PC	Phosphatidylcholine
PIP5K1α	Phosphatidylinositol-4-phosphate 5-kinase type-1 alpha
PIP4P	Phosphatidylinositol 4-phosphate
PI3K	NADPH oxidase4
PLD	Phospholipase D
PPARs	Peroxisome: proliferator-activated receptors
PtdIns (4,5) P2	Phosphatidylinositol 4,5-bisphosphate
RAGE	Receptor for advanced glycation end products
ROS	Reactive oxygen species
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIRT1	Sirtuin 1
TGF-β	Transforming growth factor-beta
Tgfbr1	Transforming growth factor-beta receptor 1
TLR2	Toll-like receptor 2
TRPV4	Transient receptor potential cation channel subfamily V member 4
tPA	Tissue plasminogen activator
TNFR	Tumor necrosis factor receptor
TXNIP	Thioredoxin-interacting protein

Introduction

Coronavirus disease (COVID-19) originated in Wuhan, Hubei Province, Central China, and quickly spread worldwide [1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of COVID-19. Infection of the respiratory system by the virus results in pneumonia and acute respiratory distress syndrome (ARDS) [2]. ARDS is a potent factor associated with the mortality of COVID-19 patients. Currently, no specific therapeutic regimen has been approved for COVID-19, and all current treatments are merely supportive [3].

COVID-19 is divided into three stages. The early stage is the incubation period, which occurs along with asymptomatic or asymptomatic periods. In the second stage, non-severe symptomatic illness is observed. The period of infection in 80% of the patients is completed in this stage. The last stage is the hyperinflammatory phase in which cytokine storm and diffuse alveolar damage (DAD) are seen [4].

DAD is a histological hallmark for the acute phase of ARDS [5] and is divided into two phases. In the acute phase, oedema and hyaline membrane formation are observed. Furthermore, interstitial fibrosis can be seen in this stage. In the organizing phase, interstitial fibrosis and alveolar hyperplasia are noted [6]. Formation of active myofibroblasts increases the accumulation of extracellular matrix (ECM), prevents alveolar re-epithelization, and causes the destruction of normal lung construction, thereby resulting in lung fibrosis. Overexpression and release of growth factors and cytokines by the injured cells and immune cells ensue when lung fibrosis occurs [7, 8]. Theoretically, this article attempts to provide a rational treatment to prevent or reducing lung injury by COVID19 infection.

Pathological role of TGF-β in SARS-CoV-2 infection

One of the critical growth factors released during SARS-CoV-2 infection is the transforming growth factor-beta (TGF-β), which also plays a vital role in lung fibrosis. SARS-CoV-2 infection in some patients causes acute respiratory distress syndrome (ARDS), and TGF-β is an essential factor that accumulates in the lavage fluid of patients with ARDS and can be used as a therapeutic target [7, 9–11].

Literature evidence shows three possibilities for the relationship between SARS-CoV-2 and TGF-β. The first possible mechanism is the release of TGF-β from immune cells such as neutrophils or injured cells due to SARS-CoV-2 infection [7, 12, 13]. Also, several data from other studies assert that the mRNA expression of TGF-β [14] and the protein level of TGF-β increase in severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) infected-lung cells [7] as well as in the plasma and lung tissues during the early phase of the infection [15, 16]. Recently, researchers have demonstrated similar results for SARS-CoV-2. The expression of the mRNA of TGF-β and TGF-β protein and its signaling pathway activity increases in SARS-CoV-2 infection [17–19].

A second possible mechanism is that the SARS viruses (SARS-CoV-1 and SARS-CoV-2) upregulate TGF-β by downregulating angiotensin-converting enzyme 2(ACE2) receptor via the interaction of the spike protein with ACE2. In the subsequent downregulation of ACE2, the level of angiotensin-II (ANG-II) increases. Furthermore, ANG-II enhances intracellular SMAD2 and SMAD4. Thus, TGF-β/SMAD signaling activity is hiked [7, 20–23].

The nucleocapsid (N) protein of SARS-CoV-2 may be considered as a third possibility. The N protein of SARS-Covid-1 causes a surge in the signaling pathway activity of TGF-β by interaction with SMAD3. Eventually, the SMAD3-P300 complex is formed, and now, SMAD3 is ready for interaction with SMAD4 [24, 25]. Data analysis by Chatterjee et al. revealed that the N protein of SARS-Covid-1 contains 423 amino acids, and that SARS-CoV-2 has 420 amino acids. Therefore, the pairwise identity between the N proteins of SARS-Covid and SARS-CoV-2 is around 88.3% [26]. Consequently, as a possible hypothesis, it is not surprising if the N protein of SARS-CoV-2 causes the formation of a complex of SMAD-p300; however, more research is needed. For an enhanced understanding of the role of TGF-β in SARS-CoV-2 infection, we divided the mechanism of TGF-β into two parts. The first pathway describes the upregulation of reactive oxygen species (ROS) by TGF-β and its effects, and the second pathway includes the non-ROS pathways.

Pathways dependent on reactive oxygen species

A) Internalization of the epithelial sodium channel (ENaC)

In the early stage of SARS, patients experience lung oedema. It has been found that the epithelial sodium channel (ENaC) transfers sodium ions from outside the alveolar cells (alveolar space). Along with sodium ions, fluid also enters the cells. Thus, ENaC controls the oedema in the lung. During this activity, sodium potassium pump on the other side of the cells transfers the fluid in the cytoplasm of the cells to the interstitial space [27, 28]. TGF-β, by internalization of αβγ ENaC from the membranes of the alveolar epithelial cells, causes pulmonary oedema. The signaling pathways perform the internalization of ENaC via transforming growth factor-beta receptor 1 (Tgfbr1)/SMAD2/3 at the end of this pathway. This process produces a high concentration of ROS, which results in the internalization of ENaC [9]. TGF-β can be released from different cells (immune cells or epithelial cells) as a latent complex that is deactivated and stored in the ECM. This complex contains the active domain of TGF-β as well as the latency-associated protein (LAP) and latent TGF-β-binding protein (LTBP). LTBP makes the connection between the complex and ECM. LAP covers the active domain of TGF-β, which can be released by the matrix metalloproteinase and integrins αvβ6 and αvβ8 [9, 29–33]. After the active domain of TGF-β is released, its interaction with TGFβR1 activates the receptor. TGFβR1 phosphorylates SMAD2/3. Furthermore, SMAD2/3 activates phospholipase D (PLD) [9, 34]. Owing to the activity of PLD, phosphatidylcholine (PC) is hydrolyzed and gets converted to phosphatidic acid (PA) and choline [9, 35]. PA induces the activity of phosphatidylinositol-4-phosphate 5-kinase type-1 alpha (PIP5K1α) [9], whose kinase activity converts phosphatidylinositol 4-phosphate (PIP4P) to phosphatidylinositol 4,5-bisphosphate (PtdIns (4,5) P2) [8, 9]. PtdIns (4,5) P2 exhibits two activities; the first activity is a positive feedback by the activation of PLD1 [9, 36], and the second activity is the over-regulation of NADPH oxidase4 (NOX4) activity. NOX4 generates ROS. It has been observed that ROS reduces the surface stability of the beta subunit of ENaC, which causes the internalization of βENaC. PLD1 inhibits the expression of alpha subunits of ENaC. Additionally, the cell loses its ability to absorb extracellular sodium, and hence, alveolar oedema occurs [9, 28]. The upregulation of NOX4 via TGF-β signaling is not limited to SMAD/PLD pathways. One of the non-SMAD signaling pathways of TGF-β is the activation of phosphatidylinositol 3-kinase (PI3K) via interaction with TGFβR2. PI3K signaling affects several genes, and one of them is the NADPH oxidase 4 (NOX4) gene. Besides, in SMAD signaling of TGF-β, SMAD2 enhances the expression of NOX4 by interacting with the transcriptional factors [37, 38] (Fig. 1).

B) Suppressing the anti-oxidant system

Suppressing the anti-oxidant systems by TGF-β is another way of upregulating the ROS. Oxidative stress plays a pathological role in lung inflammation and acute lung injury (ALI). Anti-oxidants provide defence against oxidative stress. One of the lung's critical anti-oxidants, which

is downregulated by TGF-β, is glutathione (GSH). GSH is an anti-oxidant with multiple functions in the cells. Among all the functions, anti-oxidant defence of GSH is the most significant one [39]. GSH exhibits anti-oxidant properties by reducing hydrogen peroxide and lipid peroxide when GSH cysteine gets oxidized during peroxidase activity. Downregulation of GSH has been observed in fibrotic disease, including cystic fibrosis and acute respiratory distress syndrome (ARDS) [31, 32, 40–44]. In the epithelial lining fluid (ELF) of the lower respiratory tract, GSH is the first defence factor against oxidative stress. Inhalation of GSH is an effective treatment for various pulmonary diseases [45]. In lung inflammation, the neutrophils release hypochlorous acid (HOCl), which reacts with GSH secreted by the epithelial cells. Thus, GSH protects the epithelial cells from HOCl [32]. In COVID-19, intravenous or oral administration of GSH is an effective treatment for cytokine storm syndrome and respiratory distress. This therapeutic effect of GSH is achieved by inhibiting TNF-α-induced NF-kappaB activation [46]. Furthermore, GSH exerts activity against viruses such as herpes by blocking their replication [47]. However, the antiviral activity of GSH on SARS-CoV-2 has not been confirmed till date. Glutamate and cysteine, in the presence of gamma-glutamylcysteine synthetase, get converted to gamma-glutamylcysteine. In the next step, gamma-glutamyl-cysteine is in turn converted to GSH due to glutathione synthase activity [48]. Arsalane et al. have shown that the potent inhibitory effect of TGF-β1 on gamma-glutamylcysteine synthetase inhibits the synthesis of GSH in the lung epithelial cell line A549 [49]. Regeneration of GSH is done by glutathione reductase. Glutathione disulphide, nicotinamide adenine dinucleotide phosphate (NADPH), and hydrogen ion in the presence of glutathione reductase yield two molecules of GSH and one molecule of NADP+ [50] (Fig. 2).

Reactive oxygen species non-dependent pathways

This section discusses other cell and tissue injuries that are not dependent on reactive oxygen pathways.

A) Downregulation of cystic fibrosis transmembrane conductance regulator (CFTR)

TGF-β can downregulate cystic fibrosis transmembrane conductance regulator (CFTR), which is located in the alveolar epithelial cell membrane. CFTR is a cAMP-regulated chloride channel which causes either secretion or absorption of chloride ions [28, 51, 52]. Its activity is critical in preventing pulmonary oedema [53]. In the alveolar epithelial cells (type I and II), it has been observed that CFTR channels, in the presence of cAMP, open and upregulate alveolar fluid clearance (AFC). The absence or lack of CFTR causes the failure of cAMP-stimulated fluid clearance from the distal air space; therefore, pulmonary oedema occurs. Inhibition of CFTR in mouse and human cells by molecules such as glibenclamide causes increased cAMP-stimulated fluid accumulation due to the inability of the CFTR to transfer chloride ions to the cells [53–57]. However, a single study has shown that paracellular transport between the alveolar epithelial cells is a significant way of transporting chloride [55].

Another role of CFTR is the secretion of bicarbonate from the epithelial cells either directly or indirectly [58]. Bicarbonate acts as a protective factor by controlling the pH of the lung. The airway becomes acidic, which causes lung injury. The acidic pH allows the easy development of viral infection. Besides, bicarbonate has chelating properties and can interact with calcium ion, which is an essential factor in the development of viral infections (when it is a free ion). Furthermore, bicarbonate, by decreasing calcium ion as a free ion and preventing lung acidification, protects the lung [59]. The normal pH of the airway surface liquid (ASL) is controlled by CFTR by transporting bicarbonate. Alkalization of ASL normalizes the pH [60]. Downregulation of CFTR also increases the expression of the high mobility group box-1 (HMGB1). HMGB1 interacts with Toll-like receptor 2 (TLR2), Toll-like receptor 4, and receptor for advanced glycation end products (RAGE), which activates nuclear factor (NF)-κB. In the subsequent activation of NF-κB, pro-inflammatory cytokines are released. Inflammation pathways that are mediated by HMGB1 cause lung injury. This role of HMGB1 is important, as suggested for COVID-19 therapy [61]. More literature evidence regarding the relationship between CFTR and endothelial cells support this fact. Erfinanda et al. found that CFTR downregulation causes increased permeability of endothelial cells as well as enhanced permeability to chloride and calcium ions through deactivation of WNK lysine deficient protein kinase 1(WNK 1) and activation of transient receptor potential cation channel subfamily V member 4 (TRPV4) [62]. WNK1 acts as a regulatory factor for several ion channels and exerts an inhibitory effect on TRPV4 via decreased surface expression [63]. Based on studies performed by Erfinanda, it could be concluded that CFTR dysfunction causes downregulation of WNK1. A mechanism that can explain this phenomenon is, when CFTR disability or lower expression happens, intracellular chloride ion concentration increases due to decreased secretion of the ions [64]. The high chloride concentration causes inhibition of WNK1 [65]. However, no study has directly proved this phenomenon. TRPV4 expression has been reported in smooth muscle cells in the pulmonary aorta and artery and vascular endothelium. Its function is to cause an influx of calcium ions into the cells. The activity of TRPV4 results in dysfunction of the alveolar-capillary barrier and thereby increases its permeability. A possible

mechanism of TRPV4 is increasing calcium and nitric oxide (NO) levels, which cause vasodilation and moderate vascular permeability [66–68]. Cruz has explained the mechanism by which TGF-β inhibits CFTR in the bronchial epithelial cells. TGF-β, after interacting with the TGF-beta receptor, stimulates intracellular trafficking of lemur tyrosine kinase 2 (LMTK2) via Rab11, which causes LMTK2 to be recycled and shifted to the cell membrane where LMTK2 phosphorylates serine-737 of CFTR and inhibits CFTR activity [69]. Kabir et al. have reported the effect of TGF-β on the expression of CFTR. The data showed that TGF-β elevates the level of mir-145 microRNA (miR-145) in the alveolar epithelial cells (AECs), which inhibits CFTR mRNA translation, affects the stability, and reduces the availability of the F508del CFTR substrate for lumacaftor corrector [70]. miR-145, by direct interaction with human CFTR mRNA at position 427–437, the 1557 bp long 3′-UTR (untranslated region), reduces the expression of CFTR protein [71, 72]. Supporting evidence has been found in the research by Mitash et al., which demonstrated that TGF-β1 causes an increased expression of CFTR inhibitors such as a miR-143 and miR-145 [73].

Yang established the role of miR-145 in lung fibroblasts and the formation of stress fibers. Overexpression of miR-145 has been reported in the pulmonary fibroblasts due to the activity of TGF-β1. Activation of miR145 by TGF-β1 causes the activation of the latent form of TGF-β1. The roles of miR-145 in the fibroblasts are the upregulation of alpha-smooth muscle actin (α-SMA), moderate formation of focal and fibrillar adhesion, and increase in contractility [74]. Similar data published by Wei et al. have indicated that miR-145 is upregulated in human lung fibroblasts (HLF) via TGF-β [75]. Similar reports revealing the overexpression of α-SMA in fibrotic diseases such as lung fibrosis have been published by Whyte et al. and Ju. et al. [76, 77]. A possible mechanism of enhancing α-SMA by miR-145 is the inhibition of Krüppel-like factor-4 (KLF4), a negative regulator of α-SMA [78–80]. However, more information on the relationship between miR-145 and TGF-β1 is required, since sufficient data indicate that miR-145 plays a negative role in TGF-β1 via the inhibition of SMAD3 [81, 82] (Fig. 3).

B) TGF-β activates plasminogen activator inhibitor 1 (PAI-1)

In the fibrosis of lung tissue, there is an increase in ECM production as well as a decrease in ECM degradation. One of the significant factors that inhibit ECM degradation is plasminogen activator inhibitor 1 (PAI-1), which has a negative regulatory effect on plasminogen. Plasminogen, by its proteolytic activity, degrades ECM; however, due to the increased expression and activity of PAI-1, the proteolytic activity of plasminogen and ECM degradation decrease. A

negative regulatory effect on plasminogen by PAI-1 occurs via the direct inhibition of tissue plasminogen activator (tPA), which prevents the conversion of plasminogen to the active form plasmin. Another role of plasminogen is its fibrinolytic property that prevents thrombosis. Thus, PAI-1 upregulation leads to the risk of thrombosis. The potency of PAI-1 activity in thrombosis is sufficient for the use PAI-1 inhibitors as therapeutic agents for thrombosis treatment [7, 83–85]. Furthermore, investigations have confirmed the critical role of PAI-1 in pulmonary coagulopathy [86, 87]. PAI-1 upregulation occurs in patients with SARS-CoV-1 infection due to TGF-β overexpression, which has been reported by Whyte. [76]. The same result has been obtained with regard to the elevation of PAI-1 in critically-ill and non-critically-ill patients with SARS-CoV-2 infection [88]. However, PAI-1 is a single factor for coagulopathy during SARS-CoV-2[89] (Fig. 4).

C) TGF-β activates nuclear factor-kappa-light-chain-enhancer of activated B cells (NF-kB)

NF-kB contains five members, namely NF-kB1 (P50), NF-kB2, RelA (P65), RelB, and c-Rel. In the normal condition, NF-kB along with nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor (IκB) forms inactive complexes in the cytoplasm of cells [90, 91]. In SARS-CoV-2 infection, NF-kB upregulates various cytokines and chemokines via the activity of angiotensin II receptor type-1 (AT1R), Toll-like receptor 4 (TLR4), interleukin receptor 1 (IL-1R), IL-6R, IL-18R, and interferon-gamma receptor (IFN-γ) [92]. TGF-β, by activation of tumor necrosis factor receptor (TNFR)-associated factor 6(TRAF6), indirectly causes increased activity of NF-kB [93]. Yamashita et al. have shown the interaction of TGF-β with TGF-β receptor and that TRAF6 binds to TβRI and TβRII, with a slightly higher tendency toward the latter [94]. Binding of TRAF6 to activated TGF-β receptors increases the K63-linked polyubiquitination of TRAF6 [95]. The polyubiquitinated TRAF6 interacts with mitogen-activated protein kinase kinase kinase 7-interacting protein 2 (MAP-3K7IP2 or TAB2) and through it, connects with mitogen-activated protein kinase kinase kinase 7 (MAP3K7 or TAK1) [96, 97]. Formation of the complex of TRAF6 with TAK1 is necessary for the activation of TAK1. TAK1 activated IκB kinase (IKK) causes the activation of NF-kB by the disassociation of NF-kB/IκB complex [97–99] (Fig. 5).

Therapeutic role of pirfenidone by inhibition of TGF-β

Pirfenidone (5-methyl-1-phenyl-2-[1H]-pyridone) has anti-fibrotic, anti-inflammatory, and anti-oxidant properties. These properties of pirfenidone are due to its downregulatory effects on pro-inflammatory cytokines [100]. Pirfenidone-mediated anti-fibrotic processes occur in the lung, kidney, and liver [101]. One of the effective mechanisms of pirfenidone is the downregulation of TGF-β in gene expression at the transcriptional and translational levels [102]. This downregulatory effect is due to the direct inhibition of furin by pirfenidone [103–105]. TGF-β is synthesized in a precursor form in the cell, which needs furin activity for maturation [106]. Pre-pro-TGF-β contains a signal peptide (pre-region), latency-associated peptide (LAP, which is also called the pro-region), and TGF-β region. This complex, during the second stage, loses its pre-region. The other parts of the complex undergo dimerisation with another complex and low glycosylation to get converted to low glycosylated pro-TGF-β. In the next step, this low glycosylated pro-TGF-β is high glycosylated. The high glycosylated pro-TGF-β, with the cleavage activity of furin, gets converted to high glycosylated latent TGF-β and is then secreted from the cells [107–109]. Pirfenidone (by blocking furin) inhibits the conversion of high glycosylated pro-TGF-β to high glycosylated latent TGF-β (Fig. 6).

Protective role of peroxisome proliferator-activated receptors (PPARs) in SARS-CoV-2

PPARs contain PPARα, PPARβ/δ. and PPARγ, which are nuclear hormone receptors. One of the effects of PPARs is the suppression of inflammatory pathways [110]. PPARs, by moderating the activity of IκBα, inhibit the NF-κB signaling pathway [111]. Agonistic effects of compounds such as pioglitazone on PPAR-γ investigated during COVID-19 have suggested that they might serve as novel therapeutic options for SARS-CoV-2 infection [112, 113]. Krönke et al. have confirmed that the activity of PPAR-γ and PPARα upregulate the expression of heme oxygenase-1 (HO-1) in human vascular cells [114]. Cho et al. have performed investigations on human pulmonary alveolar epithelial cells, which imply that activated PPAR-γ, by interacting with PPAR response element (PPRE), increases the transcription of HO-1 [115]. HO-1 degrades free heme to carbon monoxide, biliverdin and ferrous iron [116]. The importance of the discussion on HO-1 is that hemolysis, hemoptysis, or rhabdomyolysis has been reported in patients with COVID-19, which increase the free-heme level. A high level of free heme stimulates

inflammatory, thrombosis and oxidative pathways [117]. One of the main reasons for hyper inflammation in elderly patients with SARS-CoV-2 infection is the low expression of HO-1 [118]. On the other hand, metabolites of heme by HO-1 have a protective role in the lung tissue. CO, one of the metabolites of heme, has anti-oxidant and anti-inflammatory effects. Investigations have alluded that CO lowers the production of cytokines in vitro. In vivo, CO acts as a protective agent in oxidative and inflammatory lung injury [119, 120]. The anti-inflammatory activity of CO is effective in preventing lung injury by decreasing TNF-α, IL-1β, and IL-6 [121]. Biliverdin also gets converted to bilirubin, an anti-oxidant, via biliverdin reductase (BVR) [117]. Conversely, Jiang, L et al. have shown that carbon monoxide-releasing molecule (CORM)-2 causes the upregulation of HO-1, which inhibits thioredoxin-interacting protein (TXNIPrx)/NLR family pyrin domain-containing 3 (NLRP3) inflammasome pathway in LPS-induced acute lung injury [122]. In the presence of ROS, TXNIP is disassociated from Trx and binds to NLRP3. Interaction of TXNIP with NLRP3 causes the activation of NLRP3. It has been noted that HO-1, by decreasing ROS, inhibits the activation of NLRP3 [123]. By reducing the activity of NLRP3, maturation of IL-1β is disturbed [124]. In SARS-CoV-2 infection, mature IL-1β induces the inflammation of pulmonary tissue and fibrosis [125] (Fig. 7).

Therapeutic role of pirfenidone by PPARs and HO-1 in SARS-CoV-2 infection

Sandoval-Rodriguez et al. have established that prolonged-release pirfenidone upregulates PPAR-α signaling and sirtuin 1 (SIRT1) via in vivo and in vitro studies performed in HepG2 cells of mice. Molecular docking analysis has also confirmed that pirfenidone is an agonistic ligand for PPAR-α [126]. However, the study investigated the positive regulatory effect of pirfenidone in HepG2 cells. These results and data support the hypothesis of pirfenidone's agonistic effect on PPAR-α, which acts as an anti-inflammatory drug in pulmonary inflammation. Gutiérrez-Cuevas et al. have highlighted the role of prolonged-release pirfenidone as a potential cardioprotective agent via the overexpression of PPARα and PPARγ protein levels [111]. This research on pirfenidone's positive regulatory effect on hepatic and cardiac cells has shown the other pirfenidone mechanism. However, further studies are required to confirm the relationship between pirfenidone and PPARs, especially in lung inflammation.

Therapeutic role of pirfenidone by regulation of nuclear factor erythroid 2-related factor 2 (Nrf2)/BTB domain and CNC homolog 1 (Bach1)

Another function of pirfenidone is the regulation of Nrf2/Bach1 by inhibition of TGF-β1 activity. Nrf2, by binding to anti-oxidant redox elements (ARE), enhances the expression of anti-oxidants. ARE, by the action on particular genes, directly induces high anti-oxidant levels, HO-1 [127, 128]. ARE's impact is not limited, but this was not discussed as the scope of the article did not permit it. Nrf2 downregulates cytokine expression by blocking NF-kB signaling [128]. One possible inhibition mechanism is related to the dissociation of the Nrf2/Kelch-like ECH-associated protein 1 (KEAP1) complex, which allows KEAP1 to inhibit IKK beta [129]. Thus, Nrf2 is an essential factor for controlling

inflammation. Liu et al. have shown in bleomycin-induced mice with pulmonary fibrosis that pirfenidone increases the anti-oxidant expression through the regulation of Nrf2/Bach1. Bach1 is a transcription regulator protein that inhibits the interaction of Nrf2 with ARE due to its competitive inhibitory effect. Liu et al. have proven this in mouse lung fibroblasts (MLF) stimulated by TGF-β1. It has been observed that the mRNA expressions of Nrf2 and HO-1 were low, even though the mRNA expression of Bach1 was high. Pirfenidone (by inhibiting TGF-β1 activity) prevents the downregulation of Nrf2 and HO-1 and upregulation of Bach1 [130]. Liu et al. have found similar results in the lung tissues of mice with BLM (bleomycin)-induced pulmonary fibrosis after pirfenidone administration. It is worth mentioning that these two experiments though different show the same result [130] (Fig. 7).
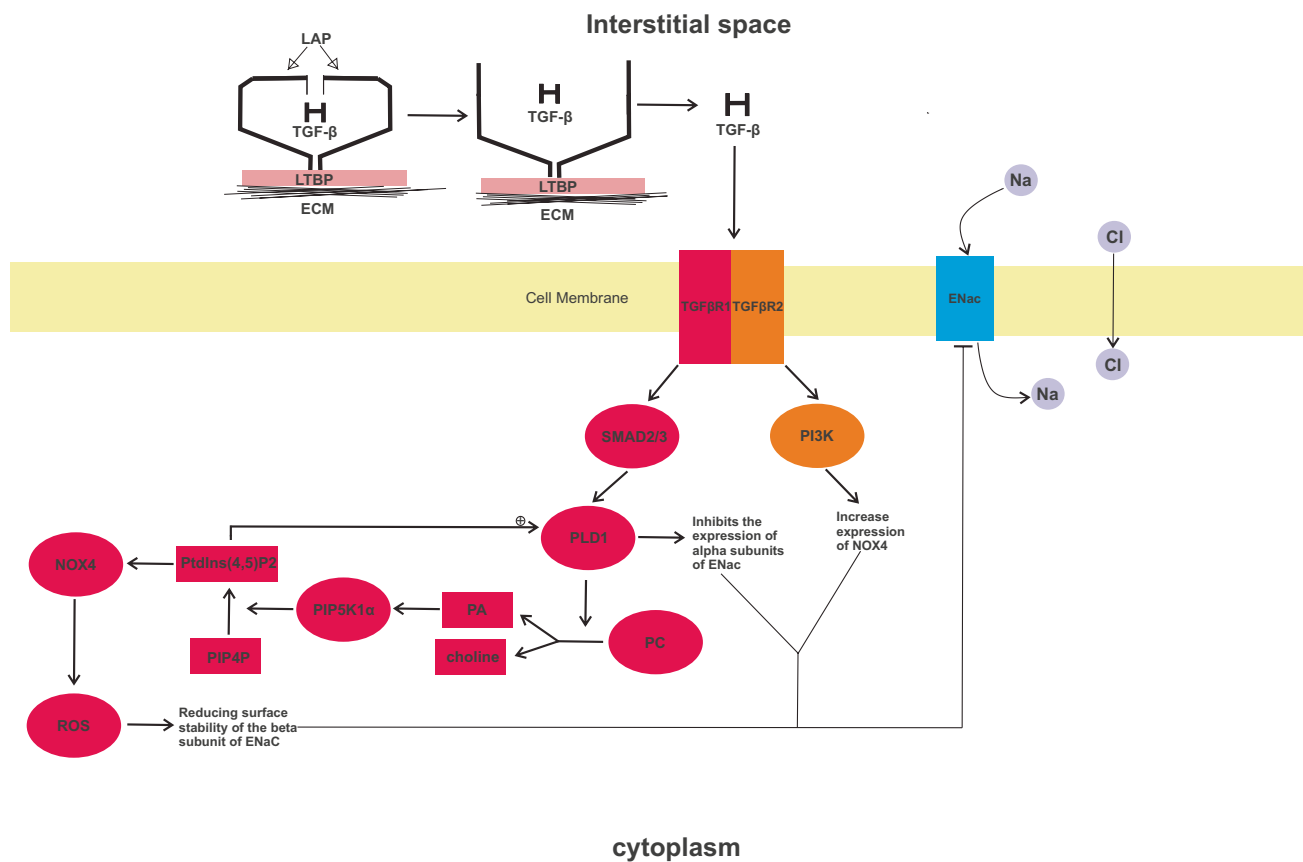

Fig. 1 Transforming growth factor-beta (TGF-β) is activated and released from latency-associated protein (LAP) cover due to inflammatory response [31]. TGF-β binds to transforming growth factor-beta receptor (TGFβR1 and TGFβR2). TGFβR1 by SMAD2/3 causes the activation of phospholipase D (PLD1) [9, 34]. PLD1 decomposes phosphatidylcholine (PC) to phosphatidic acid (PA), and choline [9, 35]. PA activates phosphatidylinositol-4-phosphate 5-kinase type-1 alpha (PIP5K1α) [9]. PIP5K1α converts phosphatidylinositol 4-phosphate (PIP4P) to phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5) P2) [8, 9]. PtdIns(4,5) P2, by activation of NADPH oxidase4 (NOX4), increases ROS production. ROS causes internalization of the epithelial sodium channel (EnaC) [9, 28]. In addition, PtdIns(4,5) P2 has a positive feedback by activation of PLD1 [9, 36]. Also, TGFβR2 by co-operation with PI3K, increases the expression of NOX4 [37]. PLD1 too, by the effect on the gene, decreases the expression of the alpha subunit of EnaC. Non-functional EnaC causes pulmonary oedema [9]
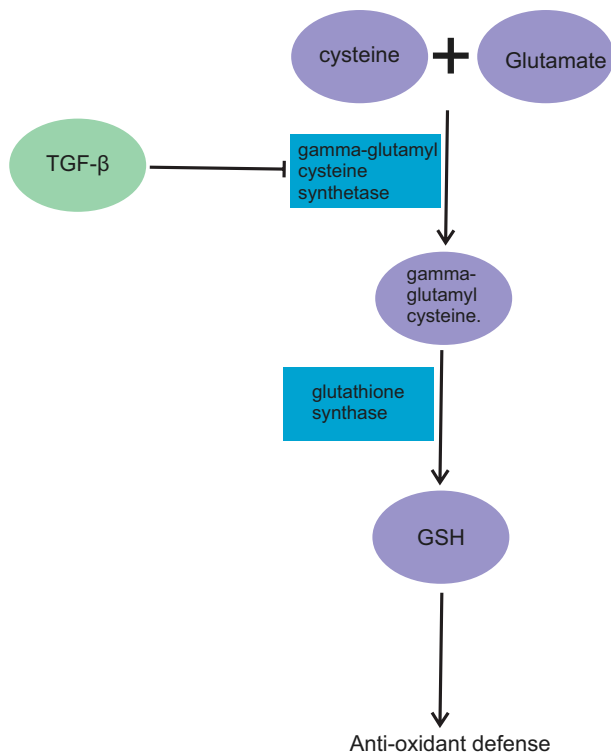
Fig. 2 Transforming growth factor-beta (TGF-β) inhibits gamma-glutamylcysteine synthetase. Owing to this inhibition, glutathione (GSH) synthesis pathway is blocked and GSH level is decreased. Without anti-oxidant defence, lung cells are exposed to more injury [48]
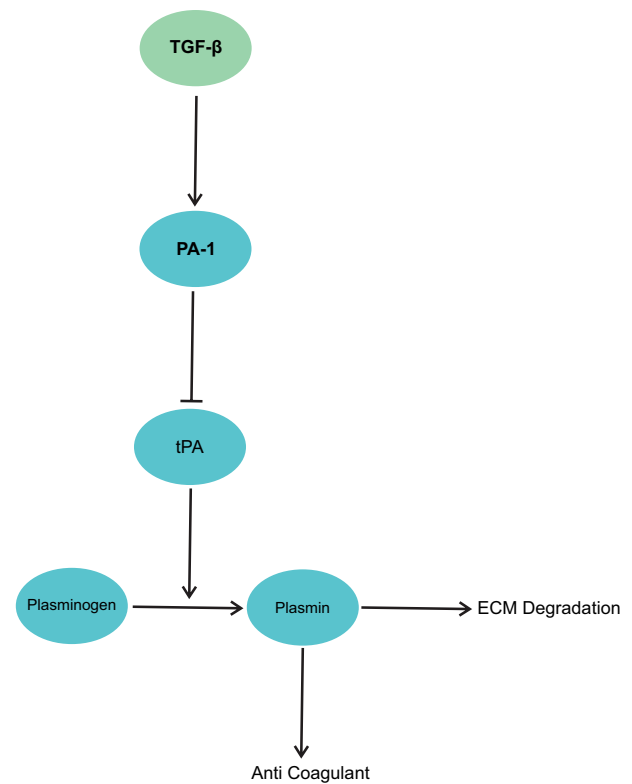
Fig. 4 Transforming growth factor-beta (TGF-β) activates plasminogen activator inhibitor 1 (PAI-1). PAI-1 inhibits tissue plasminogen activator (tPA) and prevents the conversion of plasminogen to plasmin. This effect of TGF-β on PAI-1 may cause coagulopathy [83]

Combination therapy with *N*-acetylcysteine (NAC)

NAC is a potent drug used in the treatment for COVID-19 due to the synthesis of GSH; besides, it exerts direct
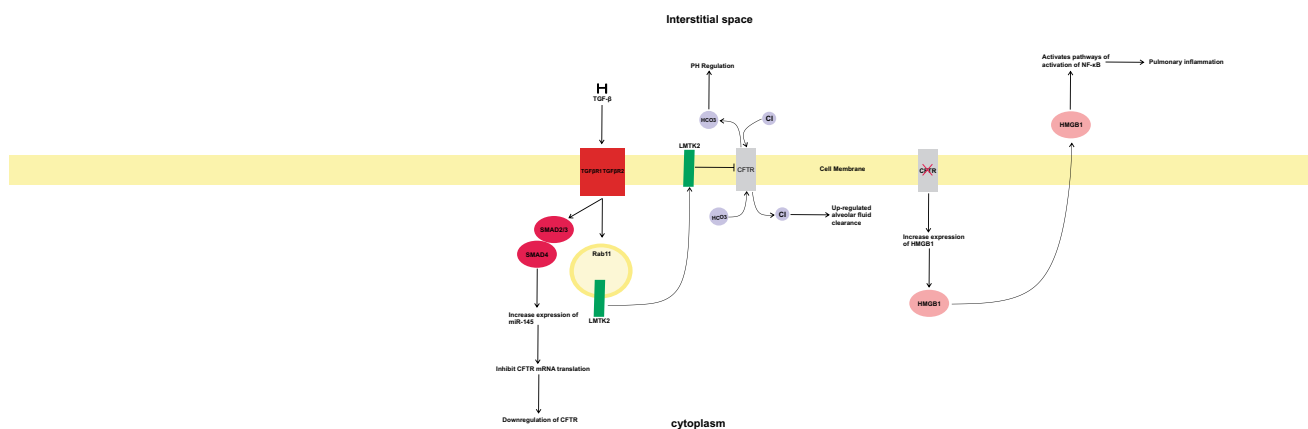

Fig. 3 Active transforming growth factor-beta (TGF-β) activates the transforming growth factor-beta receptor (TGFβR1 and TGFβR2). TGFβR1 and TGFβR2 downregulate the expression of CFTR [70]. On the other hand, co-operation with Rab11 causes the activation of lemur tyrosine kinase 2 (LIMK2). LIMK2 internalizes CFTR [69]. Non-functional CFTR cannot transfer chloride, and pulmonary oedema ensues [53]. Conversely, bicarbonate ions are unable to enter the cells, and the pH may be disturbed [58–60]
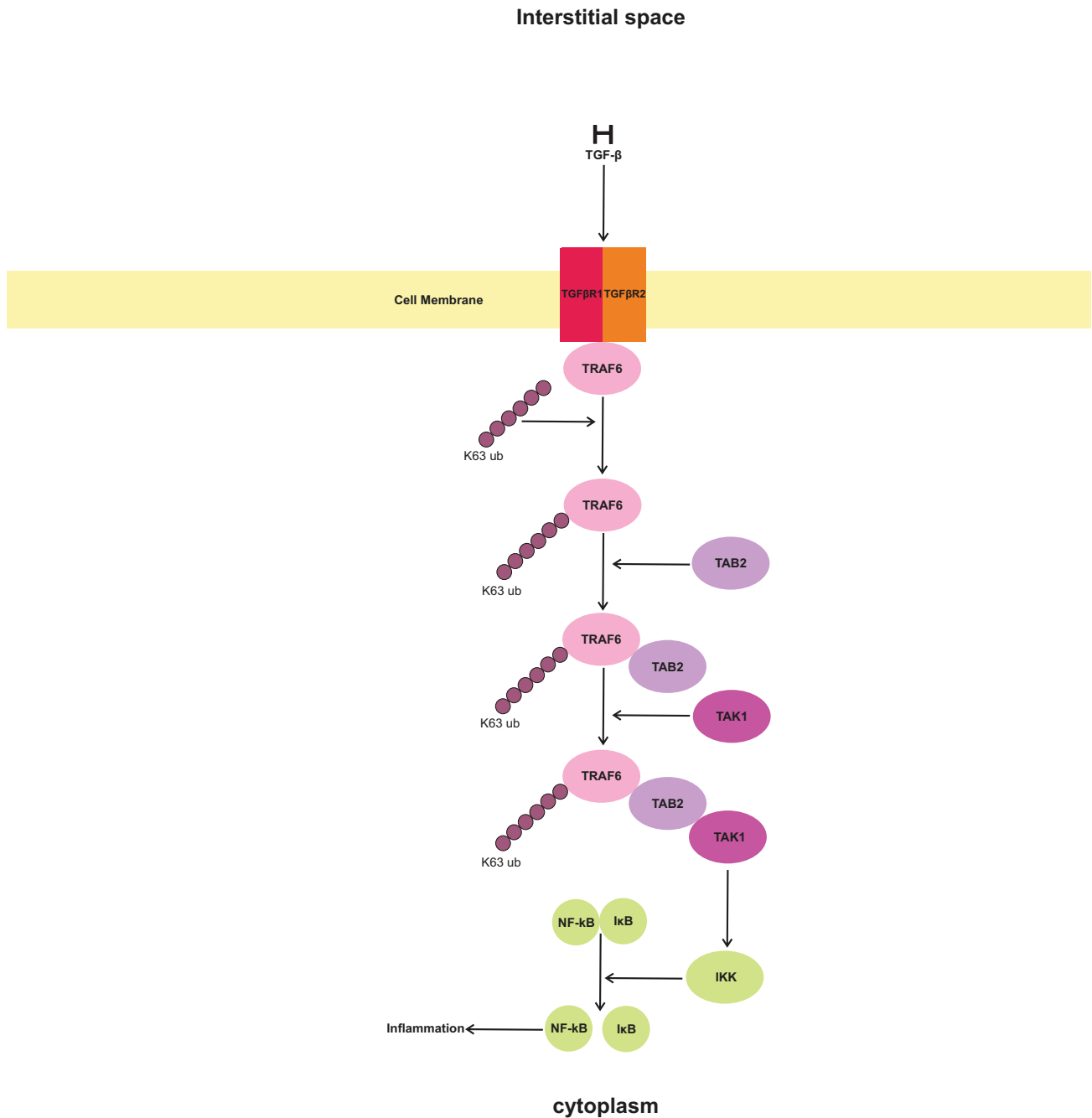
Interstitial space

Fig. 5 Active transforming growth factor-beta (TGF-β) activates the transforming growth factor-beta receptor (TGFβR1 and TGFβR2). TGFβR1 and TGFβR2 cause activation of TRAF6, which subsequently activates TAB2. TAB2 activates TAK1, which in stimulates IκB kinase (IKK) and increases the dissociation of nuclear factor-kappa (NF-kB) from IκB. The free form of NF-kB is active and shows an inflammatory response [93–99]
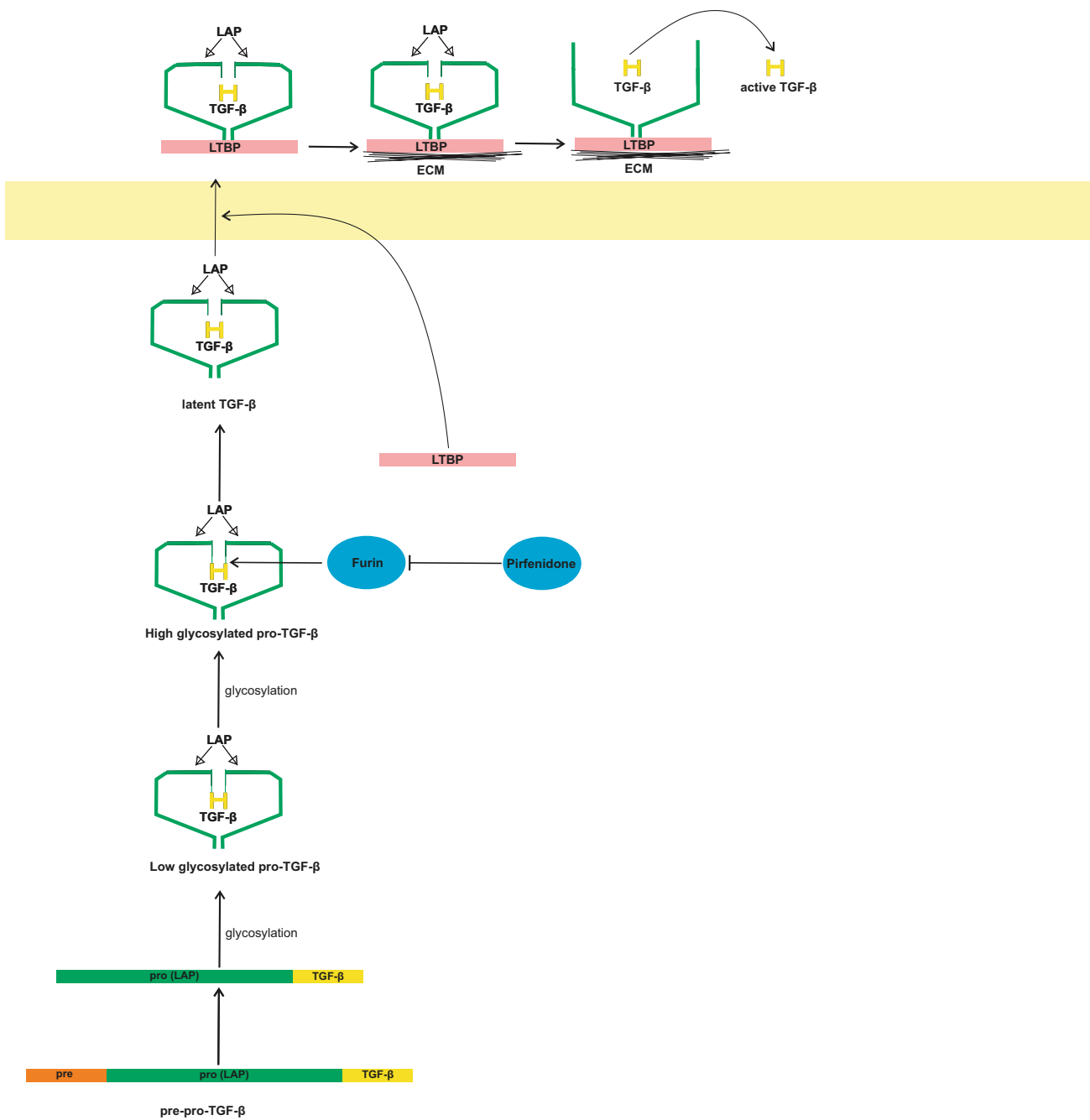
Fig. 6 Pre-pro transforming growth factor-beta (TGF-β) loses its pre-region and gets converted to pro-TGF-β. Pro TGF-β, under glycosylation, gets converted to low glycosylated pro-TGF-β. The low glycosylated pro-TGF-β again undergoes glycosylation and is converted to high glycosylated pro-TGF-β. Furin cleaves the active region of TGF-β from the pro-region and forms latent TGF-β [106–109]. Pirfenidone at this stage stops the maturation of TGF-β by inhibition of furins [103–105]. After the formation of latent TGF-β, it is secreted outside the cells and the active form of TGF-β is released as shown in Fig. 1
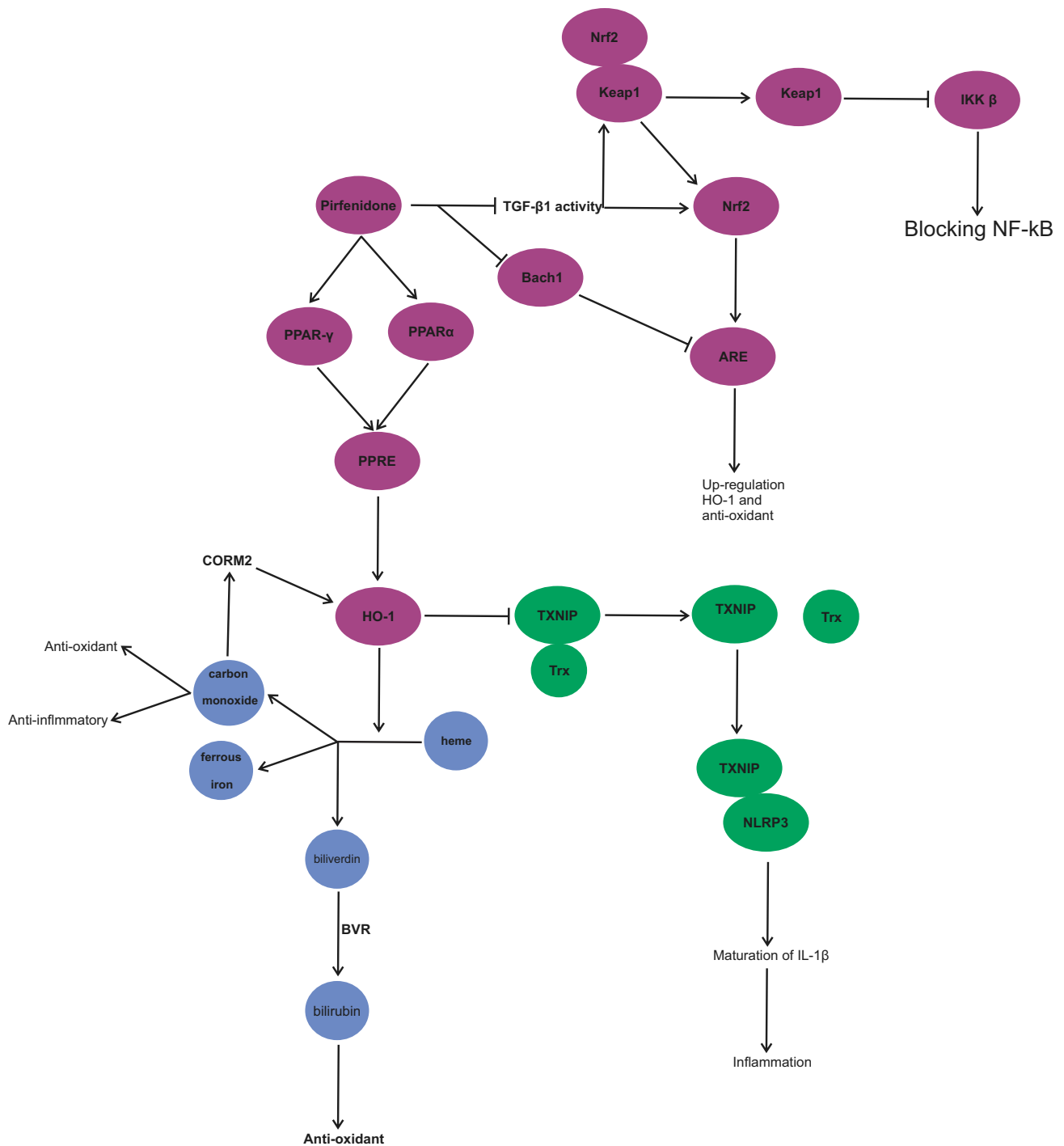
Fig. 7 Pirfenidone activates peroxisome proliferator-activated receptors (alpha and gamma) (PPAR) [111, 126]. PPAR activates PPAR response element (PPRE). PPRE increases the activity of heme oxygenase-1 (HO-1) [115]. HO-1 coverts free heme to carbon monoxide (CO), ferrous iron, and biliverdin [116]. CO shows anti-oxidant and anti-inflammatory effects [119, 120]. Biliverdin is converted to bilirubin with the help of biliverdin reductase (BVR) and shows anti-oxidant activity [117]. Furthermore, pirfenidone, by blocking BTB domain and CNC homolog 1 (Bach1) increases the activity of anti-oxidant redox elements (ARE). ARE upregulates the expression of HO-1 and anti-oxidant system. By blocking TGF-β, pirfenidone indirectly activates nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 activates ARE. Pirfenidone indirectly causes the dissociation of Nrf2 from KEAP1. The dissociated KEAP1 inhibits IκB kinase (IKK) and blocks the activity of nuclear factor-kappa (NF-kB) [127–130]

anti-oxidant activity and improves T-cell response [131]. As a hypothesis, we suggest the combination of NAC and pirfenidone to be a potent and effective in the treatment of patients with COVID-19. Studies by Shi et al. have revealed that the combination of these two drugs in patients with IPF did not affect the safety or efficacy when compared with single therapy of pirfenidone [132]. However, further investigation is needed in patients with COVID-19.

Adverse events and drug–drug interaction of pirfenidone

Gastrointestinal upset (nausea, vomiting, diarrhea, decreased appetite, and constipation) and skin reaction (photosensitivity and rash) are the possible side effects of pirfenidone. Gastrointestinal adverse effects happened at the early stage of treatment and disappeared after the first 6 months. However, photosensitivity is more varied during the treatment period. During therapy, monitoring liver function is required [133–135]. Contraindication of therapy includes hypersensitivity to the drug and severe liver or kidney disease. Drug interactions that lead to increased serum concentration and increased drug toxicity should also be avoided. Grapefruit juice, fluvoxamine, fluoxetine, ciprofloxacin, paroxetine, amiodarone, and propafenone increase the bioavailability of pirfenidone [136]. Adverse reaction of drug during IPF treatment in several clinical research papers tolerated well [135, 137]. However, further studies are needed on other possible side effects of pirfenidone on COVID-19 patients.

Scope of treatment

Due to a lack of sufficient clinical information on the use of pirfenidone in COVID-19 patients, initiation of pirfenidone treatment was not specified. However, based on the uncertain response of patients to COVID-19 infection (due to severity of illness, age, and other risk factors) and chronic pulmonary involvement of patients after a period of disease [138, 139], we suggested that the best treatment period is from the beginning of the infection for at least 14 days or longer. This hypothesis is about the duration of treatment given according to the behavior of COVID-19 infection and the mechanism of drug action. To date, several clinical trials regarding the possible treatment of COVID-19 by pirfenidone are still under investigation.

Discussion

Pirfenidone, by inhibiting TGF-β, agonistic activity on PPARs and regulation of Nrf2/BTB domain and CNC homolog 1 (Bach1) increase the anti-oxidant activity of the body, reducing lung fibrosis and inflammation, and exert a potential therapeutic effect on COVID-19. The drug can reduce pulmonary oedema, decrease cytokine storm, and help lung tissue fight against inflammation pathways. For better results, it should be combined with N-acetylcysteine for maximum efficiency. Pirfenidone is well tolerated in a long course of treatment and it can be good choice for supportive treatment of COVID-19 during the infection period or even during recovery period. However, all these data are theoretical and need clinical research to prove the hypothesis.

Declarations

Conflict of interest The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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