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TRANSLATIONAL PERSPECTIVE

Multiorgan Damage in Patients With COVID-19

Is the TGF-β/BMP Pathway the Missing Link?

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Coronavirus disease-2019 (COVID-19) is a viral infection that attacks the lungs, leading to severe acute respiratory syndrome (SARS) with a resultant high mortality. The highest mortality is seen in patients with multiorgan injuries, including the heart, the kidneys, and the liver (1). These derangements speak to a systemic disorder, and an effective treatment will need to address the multiplicity of effects.

Recent data showed that there are long-term deleterious effects from COVID-19. In 1 study, using cardiac magnetic resonance, 70% of patients with mild disease from COVID-19 had evidence of cardiac dysfunction and significant cardiac fibrosis up to 2 months after discharge from the hospital. Developing a treatment to stop the virus from replicating will be critical to controlling this pandemic, but treatments for multiorgan involvement will be essential in treating the widespread damage to the lungs, the heart, the kidneys, and other organs. With the highest mortality in these patients believed to be caused by rapid progression of pulmonary fibrosis, an agent that could slow the progression of fibrosis, or possibly reverse the fibrosis and restore function, could be a significant advance in the treatment of this devastating disease, for which we currently have limited treatment options. An agent that could prevent or reverse fibrosis in multiple organs could prevent patients from developing disabling chronic conditions.

Although antiviral drugs and vaccines are being developed, the current treatment for COVID-19 SARS is supportive therapy with intubation and ventilator support. The underlying pathology of this process is complex and incompletely characterized, but involves inflammation, apoptosis, and fibrosis (2). Patients with the most serious respiratory distress are characterized by severe pulmonary fibrosis, for which we have no current therapy.

The cardiac injury associated with COVID-19 has yet to be characterized in detail, but it is known that it involves direct injury to the myocardium, which leads to left ventricular dysfunction. There is now evidence of permanent damage to cardiac function in patients who have recovered from COVID-19. Similar to heart injury, the kidney injury that occurs with COVID-19 is also associated with higher mortality. The injury is believed to result from the direct toxicity of the virus to the cardiac or kidney cells, which results in apoptosis and a cytokine release phenomenon that further injures organ cells and leads to fibrosis and loss of function. Because angiotensin-converting enzyme (ACE) and ACE2 are known to be expressed in cardiac and renal tissue, it is likely that COVID-19 binds to these extracellular proteins and stimulates transforming growth factor (TGF)-β through the same Smad3 activation as in the pulmonary tissue.

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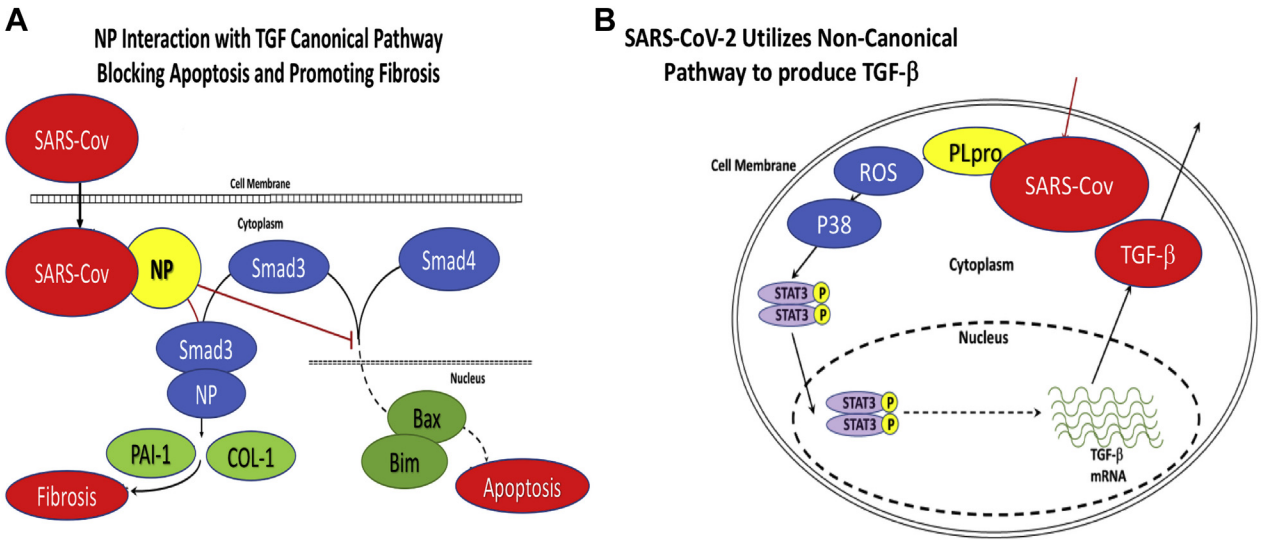
FIGURE 1 COVID (SARS-Cov) Interaction With TGF-β Signaling Pathways



(A) The severe acute respiratory syndrome-coronavirus (SARS-CoV) capsid N protein (Np) takes over the transforming growth factor (TGF)-β canonical pathway to shut down apoptosis and promote fibrosis. Np associates with Smad3 and promotes Smad3-p300 complex formation to ultimately promote tissue fibrosis. In doing so, it interferes with the association of Smad4 with Smad3, thereby blocking canonical TGF-β signaling that would lead to apoptosis with the ultimate degradation of viral and cellular RNA. Adapted with permission from Zhao et al. (3). **(B)** SARS-CoV takes over the non-canonical signaling pathway to produce and export TGF-β. The Papain-like protease (PLpro) of SARS-CoV activates the ROS/p38 MAPK/STAT3 pathway to ultimately upregulate the production and release of TGF-β1. Adapted with permission from Li et al. (6).

After the SARS epidemic in 2003, there were a number of investigations into the mechanism of organ damage produced by that viral infection. It emerged that the virus had a protein on its surface that attached to the ACE2 receptor in lung epithelial cells. This attachment was followed by internalization and intracellular replication of the virus. Subsequently, TGF-β signaling was activated through both the canonical and the non-canonical signaling pathways (3,4). The canonical pathway was activated by a nucleocapsid protein interacting with Smad3 (**Figure 1A**), and the non-canonical pathway was activated by a papain-like protein, thus upregulating TGF-β (**Figure 1B**).

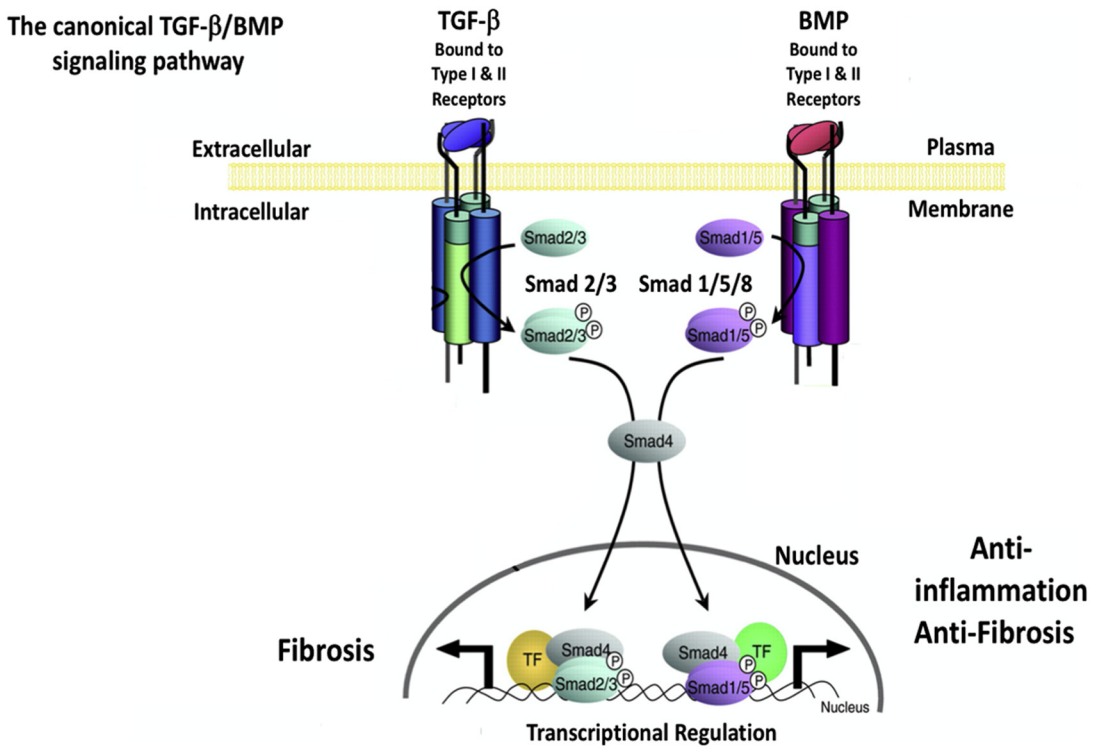
This activation of the TGF-β pathways triggered inflammation, apoptosis, and fibrosis, which led to severely damaging effects in the lungs and other tissues. Data from patients with COVID-19 showed that those patients with concomitant myocardial injury and acute kidney injury had the highest mortality and died with acute respiratory disease syndrome and irreversible pulmonary fibrosis. Thus, TGF-β induction is a significant contributor to the immediate and long-term effects of COVID-19.

Much has been written about the use of ACE inhibitors and angiotensin receptor blockers in patients with COVID-19, but little has been written about the downstream effects produced by activation of the TGF-β pathway. There is a large body of knowledge regarding the deleterious effects of TGF-β and the mild blunting of these effects by ACE inhibitors and angiotensin receptor blockers. A significant effort has been expended by pharmaceutical companies and biotech companies to develop a TGF-β inhibitor that would be useful in blunting the deleterious effects of TGF-β. As yet, there are no TGF-β inhibitors that have been successfully developed and no completed clinical trials.

The flip side of the TGF-β signaling is the bone morphogenetic protein (BMP) signaling pathway (**Figure 2**). Study of the BMP pathway has shown that activation of this pathway counters the deleterious effects of TGF-β, blocking inflammation and apoptosis and reversing fibrosis. Recently, there has been an interest in harnessing the BMP pathway to reverse the deleterious effects of TGF-β with a BMP agonist.

In the TGF-β/BMP superfamily of growth factors, BMP-7 is known to antagonize TGF-β signaling and its actions. Several lines of evidence indicate that

FIGURE 2 BMP 7/Signaling Antagonizes TGF-β–Induced Organ Fibrosis: Mechanism



BMP-7 and TGF-β1 bind to distinct type II receptors that form complexes of specific type I serine-threonine kinase receptors. BMP-7 binds specifically to activin-like kinase (ALK)-3 and ALK-6 receptors, whereas TGF-β1 binds to the ALK-5 receptor. The complexes trigger the initiation of intracellular signal streams mediated by Smad proteins. BMP-7 phosphorylates RSmads 1, 5, and 8, whereas TGF-β1 activates RSmads 2 and 3. Smad4 acts as a partner to facilitate translocation of the activated RSmads into the nucleus. The translocated heteromeric complex involving RSmads 2 and 3 modulates profibrotic genes, promoting the differentiation of fibroblasts to myofibroblasts such as α-smooth muscle actin (SMA), inducing epithelial-to-mesenchymal transition, and enhancing the deposition of collagen. Similarly, upon activation by exogenous BMP-7, the Smad1/5/8 complex with Smad4, translocates into the nucleus and blocks the nuclear accumulation of Smad3 and thereby TGF-β1 action. This phosphorylation of Smad 1/5/8 by BMP-7 results in the transcription of target genes that oppose the fibrogenic effects induced by TGF-β/Smad 2/3 signaling. At physiological concentrations, Smad6 appears to inhibit BMP signaling by interacting with BMP type I receptors and Smad7 inhibits TGF-β/activin signaling by interaction with TGF-β/activin type I receptors, which suggests that the principal functions of Smad6 and Smad7 regulate the BMP or TGF-β/activin pathways, respectively. Moreover, Smad7 suppresses fibrosis by altering TGF-β/Smad3-regulated microRNAs. Abbreviations as in Figure 1.

agonists of the BMP signaling pathway may be useful for the treatment of SARS COVID-19 pulmonary, cardiac, and kidney injuries. BMPs are phylogenetically conserved signaling molecules that belong to the TGF-β protein superfamily. BMPs signal through serine and/or threonine kinase receptors, which are composed of type I and II subtypes that are required for signal transduction. After ligand binding, they form heterotetrameric-activated receptor complexes consisting of 2 pairs of a type I and II receptor, which phosphorylate the intracellular Smad 1/5/8. The phosphorylated Smad proteins associate with the related protein Smad4, which acts as a shared partner. This complex translocates into the nucleus and participates in gene transcription with other transcription factors.

BMPs play important roles in numerous physiological processes, including cell proliferation, differentiation, apoptosis, and specification of developmental fate during embryogenesis and in adult tissues. Aberrations in the BMP signaling pathway are associated with the pathophysiology of several diseases, including osteoporosis, arthritis, pulmonary fibrosis and hypertension, cerebrovascular and cardiovascular diseases, diabetes, cancer, and kidney diseases. Genetic deletion of BMP-7 in mice led to severe impairment of kidney development, which resulted in peri-natal death. The primary sites

of BMP-7 synthesis, both during embryogenesis and in the adult, are in the kidney confined to the distal tubules, collecting ducts, and podocytes of glomeruli. BMP-7 expression decreases in several kidney disease models, including acute ischemic renal injury, tubulointerstitial fibrosis, diabetic nephropathy, and remnant kidney model. The cellular targets for BMP-7 in kidney are convoluted tubule epithelium, glomeruli, and collecting ducts.

BMP-7 has emerged as a key antifibrotic cytokine in the kidney that antagonizes the fibrotic activity of TGF-β in various animal models of chronic kidney disease. The administration of BMP-7 reverses TGF-β–induced fibrosis and epithelial-to-mesenchymal transition, inducing mesenchymal-to-epithelial transition in vitro and in vivo. It inhibits the induction of inflammatory cytokine expression, attenuates inflammatory cell infiltration, reduces apoptosis of tubular epithelial cells, and alleviates tubulointerstitial fibrosis in renal disease models. Thus, BMP-7 plays critical roles in reversing the processes that result in renal tubular damage in kidney diseases.

Accordingly, harnessing the BMP pathway would appear to hold the potential for a treatment strategy for the multiorgan injuries prevalent in patients with COVID-19. However, the naturally occurring BMP proteins have several drawbacks that preclude their use as therapeutics. They are all characterized by their ability to induce bone formation, which in soft tissue would be deleterious. They are difficult and expensive to make and likely induce antibodies to the protein. Lastly, there are natural inhibitors of BMP signaling that could block the beneficial effects being sought.

The osteogenic activity appears to be mediated through the activin-like kinase (ALK6) type I receptor. Thus, any therapeutic agent using the BMP signaling pathway necessarily needs to have no or minimal affinity for the ALK6 type I receptor while retaining the anti-inflammatory, antiapoptotic, and antifibrotic properties of BMP-7. There is a class of compounds that have been developed to activate the BMP pathway, called BMP mimetics. These compounds do not have the aforementioned limitations and meet the criteria for a potential therapeutic (5). They are resistant to inhibition by natural BMP inhibitory proteins, such as follistatin, noggin, gremlin, and chordin. These BMP mimetics have been shown to protect cardiac and renal tissue from injury arising from a variety of agents, by reversing fibrosis and restoring function. Because available evidence suggests that the triad of pathological mechanisms of inflammation, apoptosis, and fibrosis promote high mortality in patients with SARS COVID19, these BMP mimetics could be an effective agent to treat the associated multiorgan injuries.

Activation of the BMP signaling pathway is a natural and effective means of modulating the deleterious effects of TGF-β by antagonizing TGF-β signaling. Taking this TGF-β signaling aspect of the disease into account likely provides a major treatment opportunity for reducing mortality and an even more significant opportunity to reducing serious long-term morbidity.

AUTHOR DISCLOSURES

The authors all hold equity in Therapeutics by Design, LLC; and all authors have been listed as inventors on BMP mimetic patents.

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