



SARS-CoV-2 S1 protein activates human cardiac fibroblasts by priming NLRP3 inflammasomes through NF- κ B signaling in an ACE2-dependent way and promotes cardiac fibrosis | 1

Cardiovascular complications, including myocardial injury, heart failure, arrhythmias, and coagulation disorders, can manifest not only during the acute phase of COVID-19 but also in long-COVID-19 or post-acute COVID-19 (PACS) syndrome. In this study, the authors from Taiwan investigated the effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein on cultured human cardiac fibroblasts (CFs) and the molecular mechanisms underlying cardiac fibrosis induced by SARS-CoV-2 S protein. They noted that Cao et al. discovered that the SARS-CoV-2 S protein promotes cardiac fibrosis in obese mice. Specifically, these results suggest that S protein causes myocardial contractile impairment by inducing long-term aberrancies in the cardiac transcriptional signatures of mitochondrial respiratory chain genes. (Mol. Metab. 2023, 74, 101756)

<https://www.sciencedirect.com/science/article/pii/S221287782300090X?via%3Dihub>

The genome of SARS-CoV-2 encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. The S protein, composed of subunits S1 and S2, appears to be a major pathogenic factor contributing to the unique pathogenesis of SARS-CoV-2. The receptor binding domain (RBD) of the S1 subunit recognizes human angiotensin-converting enzyme 2 receptor (ACE2) and is responsible for attachment to host cells. Evidence also suggests that the S protein can independently induce cardiovascular complications by binding to cell membrane receptors, resulting in inflammation and cell and tissue damage.

The NOD-, LPR-, and pyrin-domain-containing protein 3 (NLRP3) is a component of intracellular pattern recognition receptors that detects a broad range of microbial motifs, endogenous danger signals, and environmental irritants, resulting in the formation and activation of NLRP3 inflammasome and release of the proinflammatory cytokines interleukin (IL)-1 β and IL-18. Toll-like receptor 4 (TLR4) belongs to pattern recognition receptors that recognize conserved pathogen-associated molecular patterns, thus representing the first line of defense against infection. It plays a pivotal role in the immune response to bacterial lipopolysaccharide (LPS) and other danger signals. Previous studies have shown that the SARS-CoV-2 S protein interacts with and activates TLR4 and its downstream signaling, triggering inflammatory responses in immune cells, the lungs, and the brain. The SARS-CoV-2 S protein can activate the NLRP3 inflammasome in LPS-primed microglia through nuclear factor kappa-B (NF- κ B) signaling in an ACE2-dependent manner. NF- κ B can regulate the expression of hundreds of genes involved in inflammation, immunity, proliferation, and cell death.

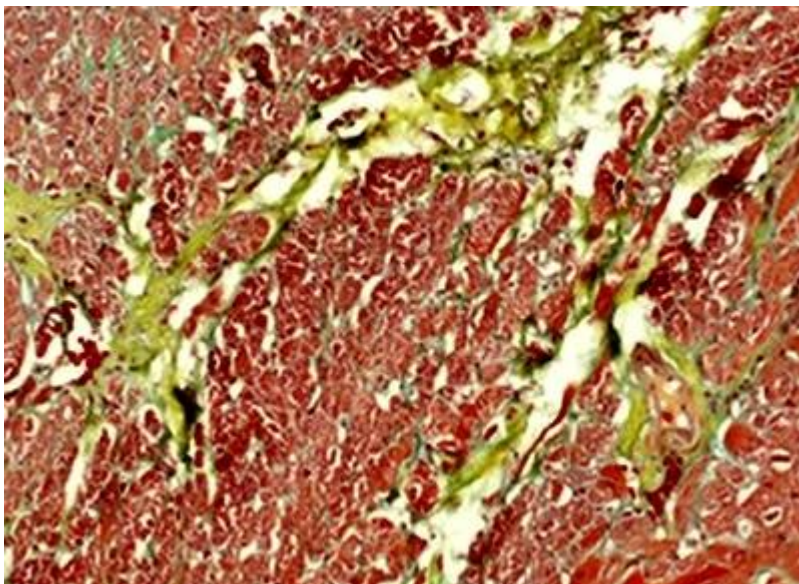
Several studies have shown signs of non-ischemic myocardial fibrosis in patients diagnosed with PASC, exceeding its prevalence in the normal adult population.

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<https://discovermednews.com/non-ischemic-myocardial-fibrosis-in-long-covid-patients/> The exact mechanisms involved in cardiac fibrosis, including the activation of CFs, as crucial cells, and the interaction of the S protein with NLRP3 and TLR4, remain poorly understood. The TLR4 and NLRP3 inflammasomes are highly involved in cardiac fibrosis development in response to inflammatory signals and pathogens.

As the SARS-CoV-2 S protein can induce TLR4 and NLRP3 inflammasome activation, the authors hypothesized that modulation of NLRP3 and TLR4 activity could attenuate the SARS-CoV-2 S protein-induced inflammatory response and associated cardiac injury.



Cardiac fibrosis

About the study

Researchers employed various methodologies, including scratch assays, Western blotting, and immunofluorescence, to assess the migration, fibrosis signaling, mitochondrial calcium levels, reactive oxygen species (ROS) production, and cell morphology of cultured human CFs treated with the SARS-CoV-2 subunit S1 for 24 hours with or without an anti-ACE2 neutralizing antibody, a TLR4 blocker, or an NLRP3 inflammasome inhibitor. Human atrial fibroblasts were used as these cells exhibit greater secretory activity and reactivity, making them more suitable for studying the mechanisms of cardiac fibrosis.

The impact of the S1 protein on the activation of CFs was assessed by scratch assay. The



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mechanisms by which the S1 protein activates CFs were analyzed through the expression of transforming growth factor β 1 (TGF- β 1) at both the protein and mRNA levels. To investigate whether ACE2 receptors mediate the effects of the S1 protein on CFs, the cells were treated with an anti-ACE2 antibody before adding the S1 protein.

The roles of NLRP3 and TLR4 in activating CFs were investigated by treating cells with an NLRP3 inflammasome inhibitor, MCC950, and a TLR4 inhibitor, TAK-242. The involvement of NF- κ B in activating CFs by the S1 protein was investigated by treating cells with an NF- κ B inhibitor, Bay 11-7082. The authors also assessed the activation of IL-1 β , a marker of NF- κ B and NLRP3 inflammasome activation.

Results

The expression of myofibroblast markers was examined in a scratch assay, which showed that exposure to the S1 protein significantly enhanced migration, but not the proliferation of human CFs.

The S1 protein enhanced the expression of collagen 1, α -smooth muscle actin, TGF- β 1, phosphorylated SMAD2/3 (the downstream targets of TGF- β 1), IL-1 β , and NF- κ B. According to these results, S1 protein directly induces the expression of key fibrotic markers, which play crucial roles in promoting fibrotic processes within cardiac tissues.

Also, the S1 protein enhanced ROS production; however, it did not affect mitochondrial morphology or mitochondrial calcium content.

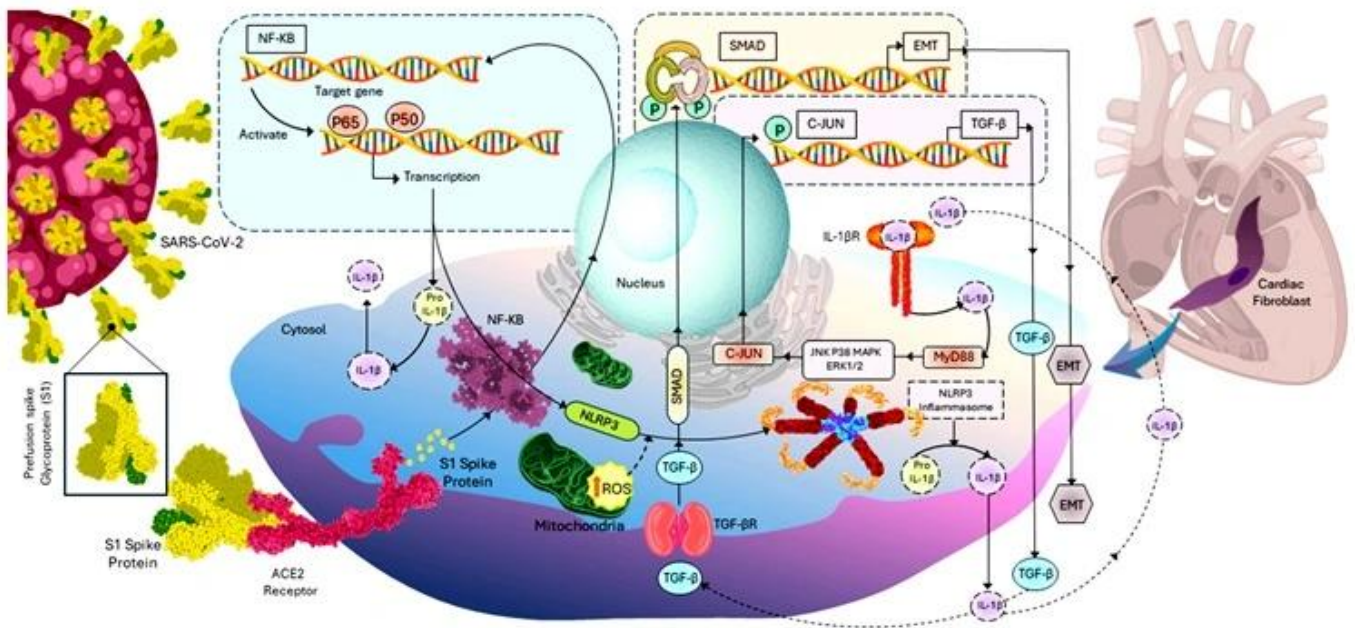
Treatment of CFs with NLRP3 inhibitor (MCC950) completely inhibited the effects of the S1 protein: prevented the migration of CFs and S1 protein-induced overexpression of collagen 1, TGF- β 1, and phosphorylated SMAD2/3. Similarly, an NF- κ B inhibitor (Bay 11-7082) effectively prevented the migration of CFs and suppressed the increase in expression of pro-COL1A1, TGF- β 1, and IL-1 β , induced by S1 protein. The BAY 11-7082 also reversed the effect of the S1 protein on SMAD2/3 phosphorylation. According to the authors, these results show that NF- κ B mediates the activation of CFs induced by the S1 protein.

In contrast, the TLR4 inhibitor (TAK-242) did not exhibit inhibitory effects on these processes.

Treatment of CFs with an anti-ACE2 neutralizing antibody effectively attenuated the effects of the S1 protein on collagen 1 and TGF- β 1 expression and prevented the CFs migration enhanced by the S1 protein. This suggests that the observed effects of the S1 protein on

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CFs are ACE2-dependent.



Original illustration from Van Tin H, et al. *Cells* 2024, 13, 1331.

Conclusion

This study showed that the S1 protein activates human CFs by priming NLRP3 inflammasomes through NF- κ B signaling in an ACE2-dependent way. Researchers emphasized that the molecular mechanisms underlying the effects of the S1 protein on CFs and the development of cardiac fibrosis are as follows: the S1 protein binds to ACE2 and activates intracellular NF- κ B signaling. ROS accumulation triggers NLRP3 inflammasome activation and IL-1 β production. IL-1 β binds to its receptors, initiating signaling cascades that upregulate the transcription of TGF- β 1. These processes ultimately lead to cardiac fibrosis.

These results highlight the critical roles of NF- κ B and NLRP3 inflammasome signaling pathways in the profibrotic effects of S1 protein in CFs. Given the known role of NF- κ B in regulating inflammatory and fibrotic processes, the authors suggested that targeting this signaling pathway may offer promising therapeutic approaches to prevent or treat cardiac fibrosis associated with COVID-19.



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