



Review

SARS-CoV-2 Spike Protein and Lung Vascular Cells

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing the current pandemic of coronavirus disease 2019 (COVID-19), and COVID-19 vaccines focus on its spike protein. However, in addition to facilitating the membrane fusion and viral entry, the SARS-CoV-2 spike protein promotes cell growth signaling in human lung vascular cells, and patients who have died of COVID-19 have thickened pulmonary vascular walls, linking the spike protein to a fatal disease, pulmonary arterial hypertension (PAH). In addition to SARS-CoV spike proteins, gp120, the viral membrane fusion protein of human immunodeficiency virus (HIV), has been reported to promote cell signaling, and long-term surviving HIV-positive patients have a high incidence of developing PAH. This article describes the findings of the SARS-CoV-2 spike protein affecting lung vascular cells and explains how the spike protein possibly increases the incidence of PAH. Since the SARS-CoV-2 spike protein will be administered to millions of people as COVID-19 vaccines, it is critical to understand the biological effects of this protein on human cells to ensure that it does not promote long-term adverse health consequences.

Keywords: cell signaling; coronavirus; COVID-19; lung; pulmonary hypertension; SARS-CoV-2; spike protein; vaccine; vascular; viral membrane fusion protein

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing the current pandemic of coronavirus disease 2019 (COVID-19) [1,2]. To date, over 75 million people have been infected with this virus, and COVID-19 has killed 1.7 million patients worldwide. In addition to the serious health problems, the pandemic has affected people economically, as well as sociologically, and the world awaits the means to end this horrible situation.

As a respiratory virus, the SARS-CoV-2 infection promotes severe pneumonia and acute respiratory distress syndrome [3]. One characteristic feature of the SARS-CoV-2 infection is, however, that elderly patients with pre-existing cardiovascular diseases are particularly susceptible to developing severe conditions and, ultimately, suffering death, while younger individuals only exhibit mild symptoms or are even asymptomatic when infected [2,4]. This feature of SARS-CoV-2 distinguishes it from other respiratory viruses and may serve as a basis for its profound effects. Thus, understanding these aspects of the disease could be crucial to developing therapeutic strategies to treat COVID-19.

Viruses use their membrane fusion proteins to bind to host cell receptors to facilitate entry into the cells through the process of membrane fusion and release the viral genomes [5]. The viral membrane fusion protein of SARS-CoV-2 is the spike protein, which is a Class I viral membrane fusion protein [2,6]. The host cell receptor of the SARS-CoV-2 spike protein for viral membrane fusion has been identified as angiotensin-converting enzyme 2 (ACE2) [7]. In humans, ACE2 (EC 3.4.17.23) normally functions as a

peptidase enzyme that cleaves angiotensin II into angiotensin (1-7) [8]. Many cell types, including those in the lungs, arteries, heart, kidneys, and intestines, express ACE2 [9].

The SARS-CoV-2 spike protein consists of two subunits: Subunit 1 (S1) that contains the ACE2 receptor-binding domain (RBD) and Subunit 2 (S2) that participates in viral cell membrane fusion [3,6]. Since it is a well-conserved and exposed region of the virus, the spike protein has been used as the molecule to acquire immunity in the COVID-19 vaccines.

In addition to facilitating the viral entry and serving as the antigen for the vaccines, the SARS-CoV-2 spike protein elicits cell signaling in human host cells without the rest of the virus [10]. The SARS-CoV-2 spike protein is potent in affecting human pulmonary artery smooth muscle cells (PASMCs) and human pulmonary artery endothelial cells; pM levels of the recombinant protein were able to activate cell signaling. Thus, the spike protein of this virus can influence the host cells, and some of these actions could cause the pathogenesis of some diseases (Figure 1).

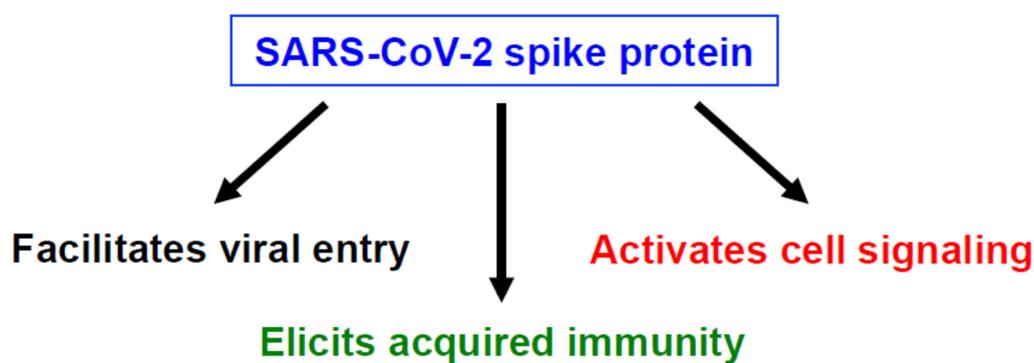


Figure 1. Actions of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein. In addition to facilitating SARS-CoV-2 entry into host cells and serving as the basis for the vaccine development, the spike protein of this virus also influences the host cells by activating cell signaling. Spike protein-mediated cell signaling could have adverse consequences.

This review article describes the finding of the SARS-CoV-2 spike protein affecting lung vascular cells and explains how the spike protein (as a component of SARS-CoV-2, as well as the molecule used in COVID-19 vaccines) possibly increases the incidence of a fatal disease, pulmonary arterial hypertension (PAH). This article also surveys the literature to describe the current knowledge of the roles of the membrane fusion proteins of other viruses in eliciting cell signaling without the rest of the viral components to help tackle the problem of SARS-CoV-2. We conclude that it is critical to understand the biological actions of the SARS-CoV-2 spike protein in affecting human cells and possibly promoting long-term adverse health consequences, given that this protein will be administered to millions and possibly billions of people as vaccines.

2. The SARS-CoV-2 Spike Protein Activates Cell Signaling in Lung Vascular Cells

In the studies published by Suzuki et al. [10], cultured human PASMCs were treated with the recombinant full-length S1 subunit (Val16—Gln690) of the SARS-CoV-2 spike protein. As shown in Figure 2, the full-length S1 subunit of SARS-CoV-2 at a concentration as low as 130 pM strongly activated the phosphorylation of a mitogen-activated protein kinase kinase (MEK) at the Ser217 and Ser221 residues [10]. The kinetics of MEK phosphorylation promoted by the SARS-CoV-2 spike protein were consistently found to be transient, with a peak at 10 min. This fast activation suggests that it may be a receptor-mediated cell-signaling event. The recombinant SARS-CoV-2 spike protein also activated MEK in human pulmonary artery endothelial cells [10].

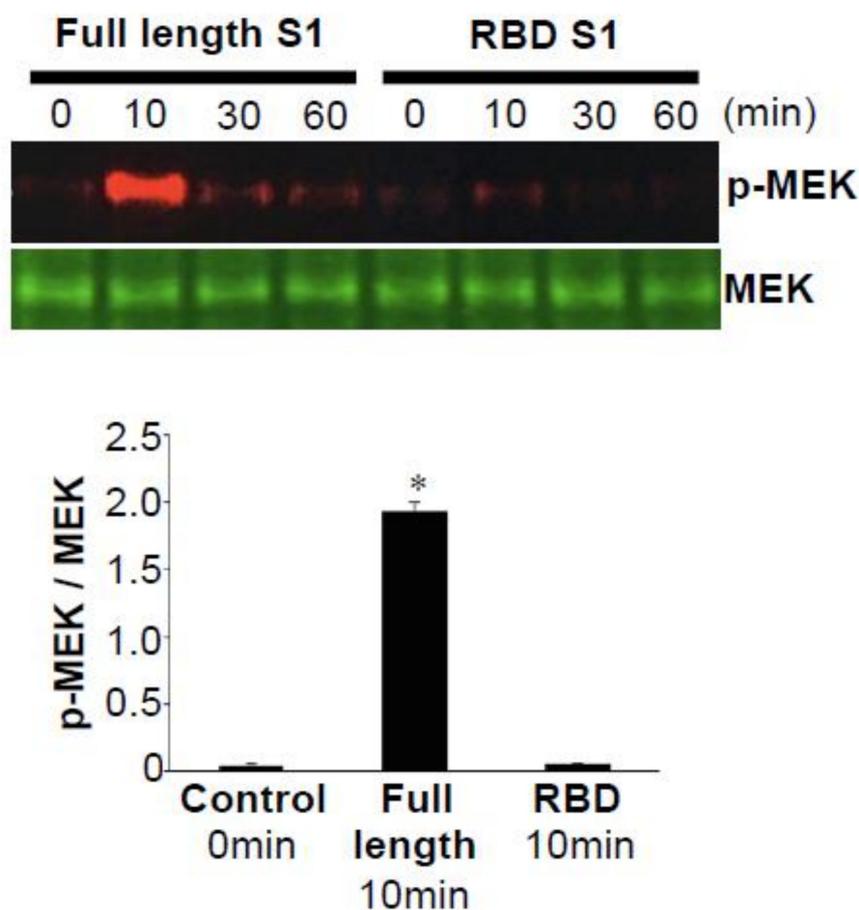


Figure 2. Full-length SARS-CoV-2 spike protein subunit 1 (S1), but not the receptor-binding domain (RBD)-only containing S1 protein, activates mitogen-activated protein kinase kinase (MEK) phosphorylation. Human pulmonary artery smooth muscle cells (PASMCs) were treated with the recombinant full-length S1 subunit (Val16—Gln690) or the RBD region of the S1 subunit (Arg319—Phe541). Cell lysates were prepared and subjected to Western blotting using antibodies against phosphorylated MEK (p-MEK) and the MEK protein. The bar graph represents means \pm SEM. * Significantly different from the 0-min control at $p < 0.05$ (Taken from Suzuki et al. [10]).

These results demonstrate that the SARS-CoV-2 spike protein without the rest of the virus can elicit cell signaling—specifically, the activation of the MEK/extracellular signal-regulated kinase (ERK) pathway—in human lung vascular smooth muscle and endothelial cells. The MEK/ERK pathway is a well-known cell growth mechanism [11]. Thus, this SARS-CoV-2 spike protein-mediated cell growth signaling may promote the thickening of pulmonary vessels in COVID-19. Consistently, we found that the pulmonary vessel walls of postmortem lung tissues from COVID-19 patients who died of acute respiratory distress syndrome were significantly thickened compared with those of H1N1 influenza patients who died of the same syndrome [10]. These results provide a possible link between the SARS-CoV-2 spike protein and PAH.

3. Pathology of PAH

PAH is a fatal disease without a cure that can affect both males and females of any age, including children [12,13]. It is a progressive disease, and by the time patients are diagnosed, the thickening of the pulmonary vascular walls has often already occurred. Increased resistance in the pulmonary circulation places strain on the right ventricle, which leads to right heart failure and death [12,13]. The median overall survival for patients with PAH is 2.8 years from the time of diagnosis (three-year

survival: 48%) without treatment [14,15]. Even with currently available therapies, the prognosis remains poor, with a three-year survival of PAH patients reported to be only 58–75% [16–19].

4. Do the Membrane Fusion Proteins of Other Viruses Also Activate Cell Signaling in Host Cells without the Rest of the Viral Components?

As we surveyed the literature using keywords including phosphorylation, protein kinase, calcium, cAMP, and phospholipase, it was surprising to find that the recombinant viral membrane fusion proteins of SARS-CoV-1 and human immunodeficiency virus (HIV) were the only ones reported to elicit cell signaling in host cells. By contrast, an extensive literature search did not find publications showing that the recombinant viral membrane fusion proteins of other viruses, including Middle East respiratory syndrome-related coronavirus (MERS-CoV), Ebola, Zika, West Nile, dengue, herpes, and influenza, elicit cell signaling events in cultured host cells.

SARS-CoV (now known as SARS-CoV-1) caused the SARS outbreak in 2002–2004 [20]. Its spike protein, which is 76–78% identical to that of SARS-CoV-2 [21], has been reported to activate the casein kinase II-dependent activation of the ERK pathway through ACE2 in the A549 human lung epithelial cell line [22]. This pathway activated by the SARS-CoV-1 spike protein also caused the upregulation of monocyte chemoattractant protein-1 (MCP-1) expression and release. This study demonstrated that ACE2 (which is a peptidase, not a cell signaling receptor) could also act as a receptor for cell signaling only in response to the spike protein, reinforcing the idea that host human lung cells are sufficiently sensitive to be affected by this viral membrane fusion protein. In addition, MCP-1 has been shown to be increased in PAH patients [23], providing another link between the spike protein and PAH.

The treatment of human brain microvascular endothelial cells with the recombinant HIV-1 gp120 protein was found to activate signal transducer and activator of transcription 1 (STAT1) and induce interleukin (IL)-6 and IL-8 secretion [24]. These gp120-induced IL-6 and IL-8 secretion processes were inhibited by a STAT1 inhibitor [24]. Similarly, gp120 activated the STAT3/IL-6 axis of cell signaling in human monocyte-derived dendritic cells [25]. Hioe et al. [26] reported that HIV-1 gp120 was sufficient to trigger leukocyte function antigen-1 (LFA-1) activation in T cells in a CD4-dependent manner.

The HIV gp120 protein has also been shown to activate cell signaling without the rest of the HIV components in pulmonary vascular cells, as described below, and this viral membrane fusion protein appears to play a crucial role in the development of PAH.

5. HIV Increases the Incidence of PAH

HIV-associated PAH occurs in approximately one out of 200 HIV-infected patients [27–30], which is 100–500 times higher than the prevalence of PAH in individuals without HIV. A large prospective study of 7648 patients with HIV from 2004 to 2005—after the introduction of potent antiretroviral therapy (ART)—showed a prevalence of right-heart catheterization-confirmed HIV-PAH of 0.5% [30]. These findings are similar to those studies performed before the use of effective ART. Thus, ART does not seem to have altered the prevalence of HIV-associated PAH, providing evidence for the direct role of HIV and/or its molecular components in promoting the pathogenesis of PAH.

The viral membrane fusion protein of HIV, gp120, has been shown to activate the cell signaling pathway for the induction of tissue factor expression through chemokine receptors, protein kinase C, mitogen-activated protein kinases, and reactive oxygen species in human vascular smooth muscle cells [31]. Since the tissue factor has been implicated in the pathogenesis of PAH [32,33], these findings provided a possible link between gp120 and PAH. Figure 3 shows our experimental results demonstrating that recombinant HIV gp120 at 80 pM activated MEK in human PASMCs. Additionally, in human PASMCs, gp120 at 100 pM increased the intracellular calcium and induced cell growth [34]. These effects of gp120 were inhibited by an inhibitor of CCR5, a coreceptor for cellular HIV entry, providing the causal link between gp120 signaling and PASMC growth. Further, in CCR5-deficient mice, hypoxic pulmonary hypertension was abrogated [34]. In human lung microvascular endothelial cells, gp120 promoted apoptosis and the secretion of endothelin-1, which mediate the development of

PAH [35]. These results suggest that the membrane fusion protein of HIV, gp120, likely participates in the development of PAH.

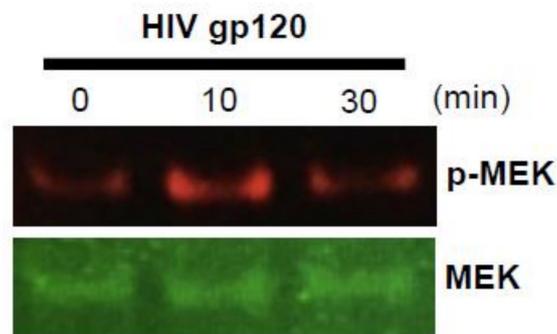


Figure 3. HIV gp120 activates MEK phosphorylation. Human PSMCs (purchased from ScienCell Research Laboratories, Carlsbad, CA, USA) were treated with the recombinant HIV gp120 (Glu31-Arg494, Accession# AAT67478.1; Catalog # 40403-V08H, SinoBiological, Wayne, PA, USA) at 80 pM. Cell lysates were prepared and subjected to Western blotting using antibodies against phosphorylated MEK (p-MEK) and the MEK protein (Cell Signaling Technology, Danvers, MA, USA).

6. Are Individuals Infected with SARS-CoV-2 Predisposed to Developing PAH?

Our results showed that the SARS-CoV-2 spike protein activated cell growth signaling in human PSMCs and in human pulmonary artery endothelial cells and that patients who died of COVID-19 exhibited thickened pulmonary vascular walls [10], which may suggest that the spike protein predisposes individuals infected with SARS-CoV-2 to develop PAH in the future. SARS-CoV-2 infection and exposure to the spike protein may trigger cell signaling events, promoting the pathogenesis of PAH in both patients who survived severe COVID-19 conditions, as well as individuals who exhibited mild symptoms or even were asymptomatic. Coincidentally, the only other virus whose membrane fusion protein has been shown to activate cell signaling (i.e., HIV) also predisposes infected patients to develop PAH. Further experimentations and careful clinical observations for PAH in relation to SARS-CoV-2 and COVID-19 are, thus, warranted.

7. COVID-19 Vaccines and PAH

COVID-19 vaccines currently under consideration, including RNA vaccines (BNT162b2 and mRNA-1273) [36–38], viral vector-based vaccines (AZD1222 and Ad26.COV2.S) [39,40], and recombinant protein (NVX-CoV2373) [41], all introduce the SARS-CoV-2 spike protein into the human body. Whether the spike protein elicits cell signaling in host cells and exerts adverse events such as promoting PAH is a question raised in response to the experimental results in cultured cells [10]. RNA and viral vector-based vaccines use human host cells to produce the spike protein; thus, the intracellular spike protein will be produced. The intracellular effects of this foreign molecule on human cells have not been defined.

BNT162b2 encodes the full-length spike protein of SARS-CoV-2 with two proline mutations [36,37], while its sister vaccine BNT162b1 encodes only the RBD of the SARS-CoV-2 spike protein, trimerized by the addition of a T4 fibrin foldon domain [42,43]. Our experiments also revealed that, in contrast to the full-length S1 subunit (Val16—Gln690) of the SARS-CoV-2 spike protein that activated cell signaling, the S1 RBD (Arg319—Phe541) did not elicit such events [10]. Figure 2 shows that, while the full-length S1 subunit strongly activated MEK, the S1 RBD did not activate MEK in human PSMCs [10]. Similarly, the full-length S1 subunit, but not the RBD-only containing S1 subunit, of the SARS-CoV-2 spike protein promoted the phosphorylation of MEK in human pulmonary artery endothelial cells [10]. Thus, the RBD-only protein such as the one encoded by the BNT162b1 vaccine may not impose the risk of developing PAH. Further work is needed to understand the effects of various SARS-CoV-2

spike protein segments on human host cells in order to develop the most efficacious and safe vaccines without long-term adverse consequences.

8. Conclusions

This analysis suggests that the SARS-CoV-2 spike protein and HIV gp120 have the capacity to trigger cell biological events that may lead to the development of pulmonary vascular remodeling and, perhaps, clinically significant PAH, a fatal condition. Given the observations that cells sensitively respond to the spike protein at pM concentrations in cultured cells [10], it is likely that the SARS-CoV-2 spike protein not only facilitates the viral entry and serves to acquire immunity as an antigen for vaccines but, also, targets host cells and may exert adverse effects (Figure 4). Further experiments should be performed to address the possible effects of the SARS-CoV-2 spike protein on developing PAH. The effects of the SARS-CoV-2 spike protein on the cells of other tissues/organs, such as those of the systemic vasculature, heart, and brain, should also be investigated. Given that this protein will be administered as vaccines to millions and possibly billions of people, it is critical to understand the extracellular and intracellular effects of the SARS-CoV-2 spike protein on human cells that may promote long-term adverse health consequences.

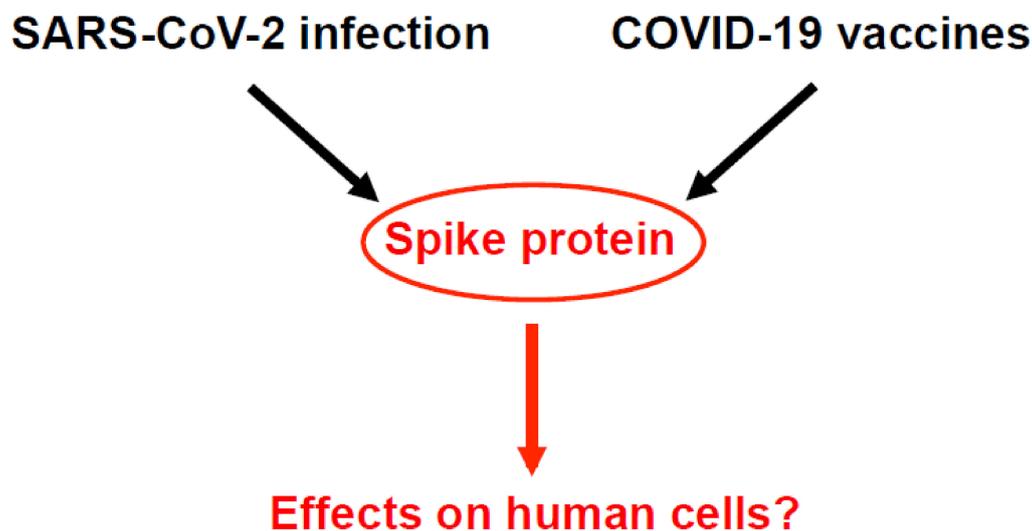


Figure 4. What are the consequences of the SARS-CoV-2 spike protein? Through SARS-CoV-2 infection and the COVID-19 vaccination, the spike protein is about to become a part of the human body. Some vaccines will also produce the intracellular spike protein in human cells. We need to know the biological actions of this protein that is new to human physiology.

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