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SARS-CoV-2 Spike protein enhances ACE2 expression via facilitating Interferon effects in bronchial epithelium

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ABSTRACT

Objective: In this study, we focused on the interaction between SARS-CoV-2 and host Type I Interferon (IFN) response, so as to identify whether IFN effects could be influenced by the products of SARS-CoV-2.
Methods: All the structural and non-structural proteins of SARS-CoV-2 were transfected and overexpressed in the bronchial epithelial cell line BEAS-2B respectively, and typical antiviral IFN-stimulated gene (ISG) *ISG15* expression was detected by qRT-PCR. RNA-seq based transcriptome analysis was performed between control and Spike (S) protein-overexpressed BEAS-2B cells. The expression of *ACE2* and IFN effector JAK-STAT signaling activation were detected in control and S protein-overexpressed BEAS-2B cells by qRT-PCR or/and Western blot respectively. The interaction between S protein with STAT1 and STAT2, and the association between JAK1 with downstream STAT1 and STAT2 were measured in BEAS-2B cells by co-immunoprecipitation (co-IP).
Results: S protein could activate IFN effects and downstream ISGs expression. By transcriptome analysis, over-expression of S protein induced a set of genes expression, including series of ISGs and the SARS-CoV-2 receptor ACE2. Mechanistically, S protein enhanced the association between the upstream JAK1 and downstream STAT1 and STAT2, so as to promote STAT1 and STAT2 phosphorylation and ACE2 expression.
Conclusion: SARS-CoV-2 S protein enhances ACE2 expression via facilitating IFN effects, which may help its infection.

1. Introduction

The coronavirus disease 2019 (COVID-19), one of the most severe global pandemics, has killed over 1.7 million people around the world [1,2]. Up to March 21 2021, more than 123 million people have been confirmed to be infected with a single-strand positive-sense RNA virus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which caused COVID-19 [3,4]. Due to the phylogenetic similarity of coronavirus, two transmissible diseases were seen before caused by the severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, which shows about 80% and 50% sequence identity with SARS-CoV-2, respectively [5,6]. These three coronaviruses belong to the *betacoronavirus* genus, and the genome of SARS-CoV-2 ranges from 26 to 32 kilobases in length and encodes three kinds of proteins, including the 4 structural proteins (Spike (S), E, N, M), 15 non-structural proteins (Nsp1-10, Nsp12-16) and 7 accessory proteins (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8b, ORF9b) [7]. Human host factor angiotensin converting enzyme 2 (ACE2), a critical tissue protective component during severe acute lung injury, is the receptor for both SARS-CoV and

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; MERS-CoV, Middle East respiratory syndrome coronavirus; S protein, Spike protein; ISGs, IFN-stimulated genes; ACE2, angiotensin converting enzyme 2; dACE2, delta ACE2; Nsp, non-structural protein; ORF, open reading frame; STAT, signal transducer and activator of transcription; JAK, Janus kinase.

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SARS-CoV-2, and the S protein of SARS-CoV-2 has been experimentally shown to bind ACE2 for viral entry [8,9]. Current single cell RNA sequencing (scRNA-seq) to elucidate SARS-CoV-2 invaded host cell types based on ACE2 expression have determined that nasal Goblet cells, bronchial Club cells, type II pneumocytes, and ileal absorptive enterocytes are infected [10], which are correlated with the symptoms and transmission route of COVID-19.

Host innate immune response, triggered by the infection of viruses, is the robust first line to identify and eliminate the invading pathogen-associated molecular patterns (PAMPs) [11]. Subsequent production of cytokines initiates two general antiviral programs. On one hand, a set of proinflammatory cytokines and chemokines trigger inflammation and recruitment of specific subsets of leukocytes. On the other hand, transcriptional induction of Type I interferons (IFN) promotes the downstream expression of IFN-stimulated genes (ISGs), which directly inhibits viral replication and causes apoptosis of virus-infected cells [12,13]. However, viruses may also invade host IFN system for their survival, and excessive IFN production may cause immune disorder and tissue damage. The potential interaction between SARS-CoV-2 and host IFN antiviral system has attracted much attention now.

As to SARS-CoV and MERS-CoV, studies have determined that proteins encoded by these coronaviruses had diverse impact on IFN production and effects to facilitate the virus evasion against host innate immunity. For instance, SARS-CoV Nsp1 inhibits IFN-dependent signal transducer and activator of transcription (STAT) 1 phosphorylation; SARS-CoV open reading frame (ORF) 3b, ORF6, and N protein can inhibit IFN-β production and only ORF6 protein confines the nuclear translocation of STAT1; MERS-CoV ORF8b and N protein play a pivotal role in the suppression of type I IFN production [14-17]. As for SARS-CoV-2, Nsp1, Nsp3, Nsp6, Nsp12, Nsp13, Nsp14, ORF3, ORF6 and M protein antagonize type I IFN production through several different approaches, whereas Nsp2 and S protein promote SeV-induced IFN-β promoter activation [18,19]. SARS-CoV-2 Nsp1 and Nsp6 inhibit IFN-induced activation and translocation of STAT1/STAT2 [19]. Furthermore, a recent study found a ISGs upregulation signature and impaired type I IFN production in myeloid and peripheral dendritic cells from COVID-19 infected patients through CITE-seq single cell RNA sequencing and bulk RNA sequencing analysis, which might be induced by low dose type I IFN production in the lung caused by SARS-CoV-2 early infection [20]. Therefore, the understanding of the interaction between SARS-CoV-2 and host IFN response at different infection stages is crucial for clarifying the virus evasion mechanisms, which is still uncomplete up to now.

The current literature for the roles of IFN in COVID-19 is still somewhat controversial. A study reported that the IFN production and activity was weakened in severe and critical COVID-19 patients, in contrast, the IFN response in mild-to-moderate patients was relatively strong [12]. However, another recent study using scRNAseq to profile the immunophenotypes of patients' PBMCs, and found a positive association between viral load, disease severity, serum IFN-α, and the expression of ISGs, implicating that IFN-α may promote severe COVID-19 progression [21]. Moreover, a current study indicated that the receptor ACE2 expression could be upregulated by both IFN-α and IFN-γ in human upper airway basal cells, which may facilitate the viral entry [10]. Researchers demonstrated that high-dose Type I IFN had partial antiviral effects against both SARS-CoV and MERS-CoV in cell culture, animal experiments, and clinically, for instance, IFN-β treatment could not completely reduce SARS-CoV titre and antigen production except for releasing virus-induced cytopathogenic effects [22,23]. Especially, MERS-CoV is much more sensitive to the antiviral function of IFN-β than SARS-CoV is [24]. Accordingly, timely and sufficient IFN production upon coronavirus infection may be important for the antiviral response. Although the roles of IFN in the pathogenesis and progression of COVID-19 are still somewhat controversial, the antagonizing inhibitors against proinflammatory cytokines including the inhibition of IL-6, IL-6R, JAK2 and GM-CSF, have been determined to have promising therapeutic efficacy in restraining inflammation in severe COVID-19 patients [25-28]. Thus, SARS-CoV-2 may have developed its own strategy to conquer host IFN antiviral system, which needs intensive investigation.

In order to identify whether IFN effects could be influenced by the products of SARS-CoV-2, including the structural and non-structural proteins, we screened all the products of SARS-CoV-2 in the regulation of type I IFN effects in bronchial epithelial cell line BEAS-2B, and found that the S protein could significantly enhance IFN effects. Interestingly, S protein could activate IFN effects and downstream ISGs expression. By transcriptome analysis, overexpression of S protein induced a set of genes expression, including series of ISGs and the SARS-CoV-2 receptor ACE2. Thus, we intend to investigate the role and mechanism for the induced *ACE2* expression by S protein in this study, so as to elucidate the potential mechanism for the infection of SARS-CoV-2.

2. Materials and methods

2.1. Cell lines and transfection

BEAS-2B and HEK293T cell lines were obtained from cell bank of Chinese Academy of Sciences (Shanghai, China). These cell lines have been authenticated using STR profiling and tested for mycoplasma contamination once per two to three weeks using MycoFree Kit by Genechem (Shanghai, China). BEAS-2B cells were cultured in RPMI 1640 with 10% Fetal Bovine Serum (FBS), and HEK293T cells were cultured in DMEM with 10% FBS as previously described [29]. Cells were seeded at 10^5/ml into culture plates and grew to density about 50% in the next day, and then were transfected with plasmids using jetPRIME transfection reagent (114-15, Polyplus-transfection, France) according to the manufacturer's protocol as we described previously [29]. For transfection concentration, 0.25 μg DNA with 0.5 μl jetPRIME was diluted into 50 μl jetPRIME buffer for per well of 24-well plate and 1 μg DNA with 2 μl jetPRIME was diluted into 200 μl jetPRIME buffer for per well of 6-well plate respectively. Cells were harvested 24 hours after transfection and the cell density reached about 90%.

2.2. Reagents

Antibodies specific to STAT1 (14994), STAT2 (72604), phospho-STAT1 (9167), phospho-STAT2 (4441), JAK1 (3344), and horseradish peroxidase-coupled secondary antibodies (7074 and 7076) were from Cell Signaling Technology (Danvers, MA). Antibodies specific to β-actin (A5441) and Flag tag (F1804) were from Sigma-Aldrich (St. Louis, MO). Antibody specific to V5 tag (ab9116) and C-terminal ACE2 (15348) was from Abcam (Cambridge, UK). Protease inhibitor cocktail (539134-1SML) and the STAT1 inhibitor (Fludarabine, 100 μg/mL) were from Calbiochem (Darmstadt, Germany). Recombined human IFN-α-2b was purchased from Kawin Technology (Beijing, China). After transfection, cells were stimulated with 1000 IU/mL human IFN-α-2b for the indicated time as described previously [30]. All the SARS and SARS-CoV-2 protein constructs were constructed and determined to be successfully expressed as we described previously [31].

2.3. RNA isolation and Real-time PCR analysis

Total RNA was extracted from human cell lines using RNAiso Plus reagent (Takara, Dalian, China) following the standard protocol. Real-time quantitative RT-PCR (qRT-PCR) analysis was performed using LightCycler 2.0 (Roche, Switzerland) and SYBR RT-PCR kit (Takara) as previously described [32,33]. For gene expression analysis, qPCR primers were human *ISG15* (forward: 5'- CGC AGA TCA CCC AGA AGA TCG -3', reverse: 5'- TTC GTC GCA TTT GTC CAC CA -3'); human long *ACE2* (forward: 5'- CAA GAG CAA ACG GTT GAA CAC -3', reverse: 5'- AGA CTA CAA TGA GAG GCT CTG GG -3'); human *dACE2* (forward: 5'- GTG AGA GCC TTA GGT TGG ATT C -3', reverse: 5'- TAA GGA TCC TCC

CTC CTT TGT -3'); human total ACE2 (forward: 5'- TGG GAC TCT GCC ATT TAC TTA C -3', reverse: 5'- CCC AAC TAT CTC TCG CTT CAT C-3'); internal control human *β-actin* (forward: 5'-GGC GGC ACC ACC ATG TAC CCT-3', reverse: 5'-AGG GGC CGG ACT CGT CAT ACT-3'). The relative expression level of the individual genes was normalized to that of internal control by using $2^{-\Delta\Delta Ct}$ cycle threshold method in each sample [34,35]. The efficiency of each pair primers was determined.

2.4. RNA-seq

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) as we previously described [32]. And then each sample was qualified and quantified by GBI-Shenzhen using Nano Drop and Agilent 2100 bioanalyzer (Thermo Fisher Scientific, MA, USA), with appropriate RIN value of 10.0. Afterwards, mRNA library was constructed and validated on the Agilent Technologies 2100 bioanalyzer by GBI-Shenzhen using Oligo(dT)-attached magnetic beads. Subsequently, DNA nanoballs were

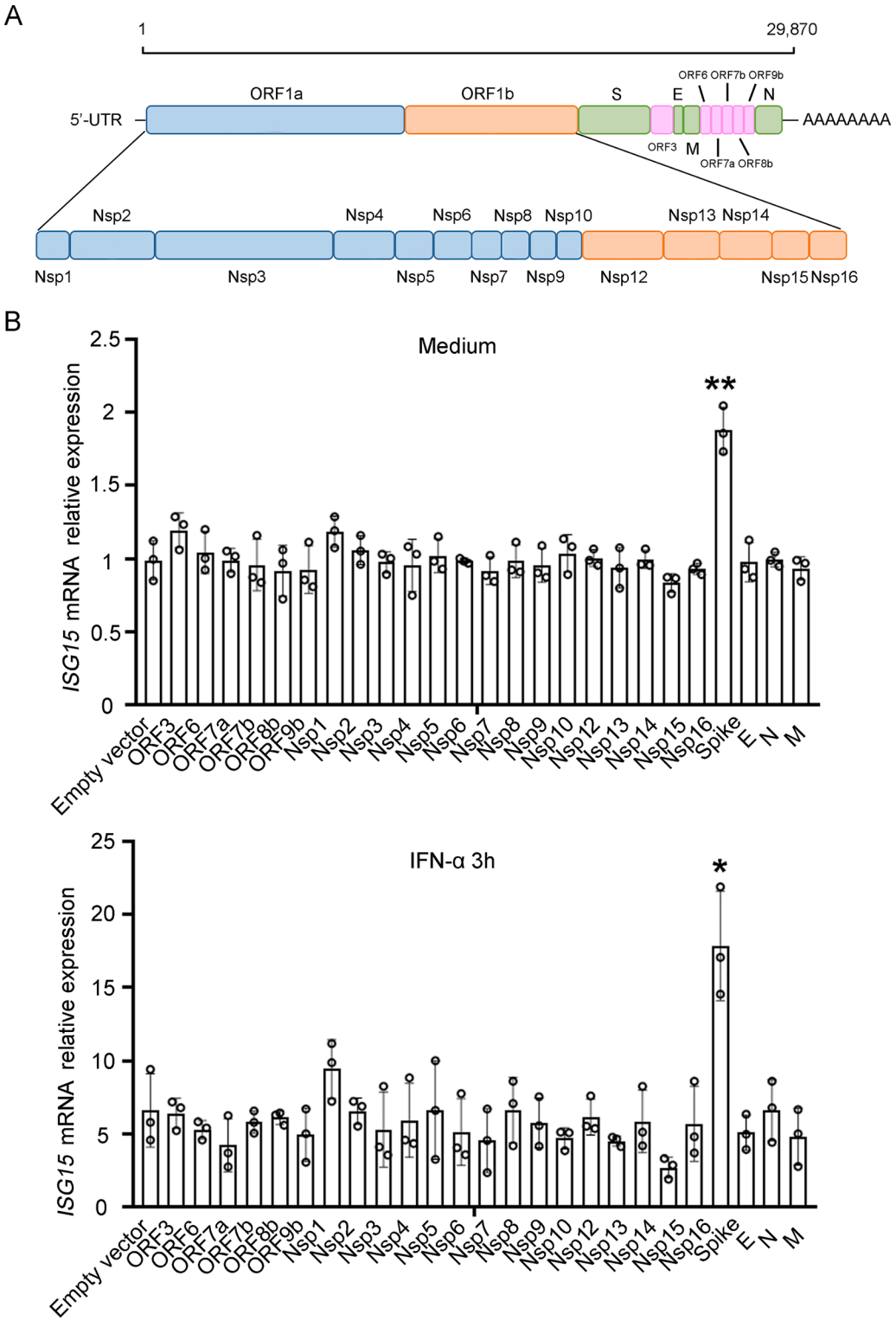

Fig. 1. SARS-CoV-2 S protein promotes ISG15 expression.
Note: A, Schematic diagrams of the SARS-CoV-2 genome, including ORF3, ORF6, ORF7a, ORF7b, ORF8b, ORF9b, Nsp1, Nsp2, Nsp3, Nsp4, Nsp5, Nsp6, Nsp7, Nsp8, Nsp9, Nsp10, Nsp12, Nsp13, Nsp14, Nsp15, Nsp16, S, E, N, and M protein; B, BEAS-2B cells were transfected with empty vector, SARS-CoV-2 structural, non-structural, or accessory protein constructs respectively, and then treated with IFN-α for 3 hours or not. The expression of *ISG15* was detected by qRT-PCR; Data are shown as mean ± SD (n=3). $^{*}P< 0.05$; $^{**}P< 0.01$.

loaded into the patterned nanoarray, single end 50 bases reads were generated on BGIseq500 platform (GBI-Shenzhen, China), resulting in 23.85 million total clean reads per library. The clean reads were mapped to the reference genome using HISAT2 (v2.0.4) with 95.81% of mappability to the human genome, and then expression level of gene was calculated by RSEM (v1.2.12). Genes with expression values above 2 were used for subsequent analysis and differentially expressed genes were screened with|log2Ratio|≥0.5. The differentially expressed genes were analyzed by KEGG mapper-Search pathway (https://www.kegg.jp/kegg/tool/map_pathway1.html).

2.5. Western blot and Immunoprecipitation

Cells were lysed on ice with cell lysis buffer (Cell Signaling Technology) supplemented with protease inhibitor cocktail (Calbiochem) at a ratio of 1:200. Protein concentrations of the extracts were measured with bicinchoninic acid (BCA) assay (Pierce). Equal amount of the extracts was loaded to SDS-PAGE and transferred onto nitrocellulose membrane for immunoblot analysis as described previously [36,37]. As for immunoprecipitation, equal amount of cell lysate samples was added with 50 μl anti-Flag agarose conjugate suspension (approx. 5 μl agarose/bed volume) and incubated for 2 hours at 4 °C with gentle mixing. Then immunoprecipitated complexes were collected by centrifugation at 3,000 g for 2 minutes and washed with 1 mL washing buffer by resuspension for at least 3 times. Each pellet was resuspended in 25 μL loading buffer, and heated at 95°C for 5 minutes. IP samples were then subjected to SDS-PAGE and transferred to nitrocellulose membrane for immunoblot analysis as described previously [36,37]. Protein levels were quantified using Image J software.

2.6. Statistical analysis

Data are shown as mean ± SEM of three independent experiments. Statistical comparisons between experimental groups were analyzed by unpaired Student's *t*-test in GraphPad Prism 8.0, and a two-tailed $P<0.05$ was taken to indicate statistical significance.

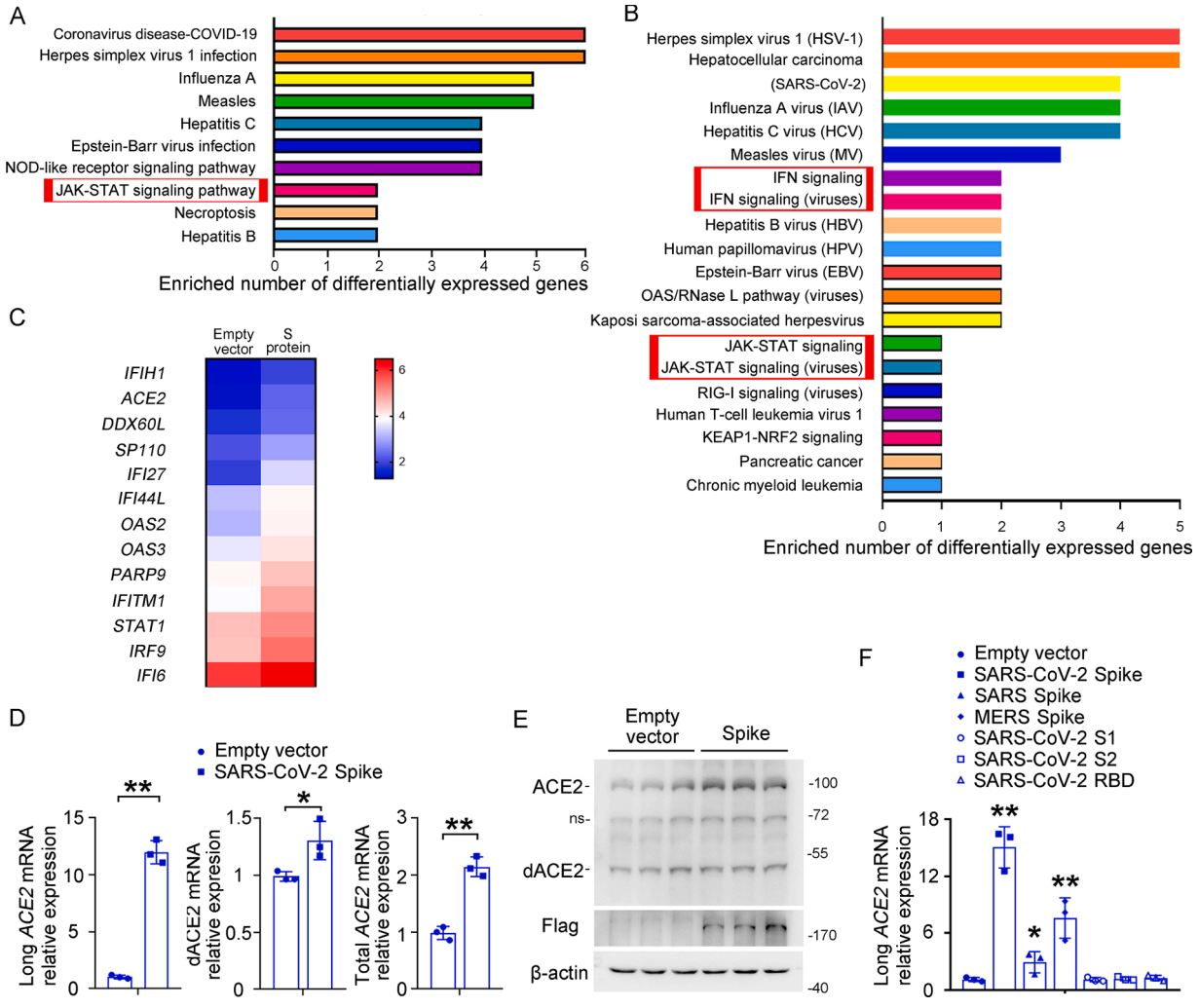

Fig. 2. SARS-CoV-2 S protein enhances its receptor ACE2 expression.

Note: A-B, KEGG pathway (A) and network (B) enrichment analysis of differentially expressed genes between BEAS-2B cells transfected with S protein and empty vector; C, Heatmap summarizing the differentially expressed ISGs (log_2-transformed expression value) in the transcriptome analysis between BEAS-2B cells transfected with S protein or empty vector; D, BEAS-2B cells were transfected with empty vector, or SARS-CoV-2-Spike protein constructs as indicated, and then the expression of long *ACE2, dACE2* and total *ACE2* was detected by qRT-PCR; E, BEAS-2B cells were transfected with empty vector or S protein, and the expression of long ACE2 and dACE2 was detected by Western blot using C-terminal anti-ACE2 antibody. β-actin was used as internal control; F, BEAS-2B cells were transfected with empty vector, SARS-CoV-2 Spike, SARS Spike, MERS Spike, SARS-CoV-2 S1, SARS-CoV-2 S2, or SARS-CoV-2 RBD protein constructs as indicated, and then the expression of long *ACE2* was detected by qRT-PCR; Data are shown as mean ± SD (n=3), or typical photographs of one representative experiment from three independent experiments. *$P<$ 0.05; **$P<$ 0.01.

3. Results

3.1. SARS-CoV-2 S protein increases the expression of ISGs

In order to investigate whether the products of SARS-CoV-2, including the structural and non-structural proteins, could influence the effect of type I IFN, we first transfected and overexpressed the proteins of SARS-CoV-2 respectively in the bronchial epithelial cell line BEAS-2B, and chose IFN-α-induced expression of typical antiviral ISG *ISG15* as the readout. By screening, the results showed that IFN-α-induced ISG15 expression could be enhanced by SARS-CoV-2 structural S protein both with and without IFN-α stimulus (Fig. 1A-B), suggesting that S protein could activate IFN-α effector signaling in bronchial epithelial cells. Therefore, we intended to focus on the roles of S protein-enhanced IFN effects in bronchial epithelium and the corresponding molecular mechanisms.

3.2. SARS-CoV-2 S protein enhances the expression of ISGs and the receptor ACE2

As SARS-CoV-2 S protein could activate the expression of some ISGs, we next performed the RNA-seq based transcriptome analysis between control and S protein-overexpressed BEAS-2B cells to elucidate the genes that S protein could activate. The 54 differentially expressed genes were assigned to various annotation in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and network, and the data revealed that the differentially expressed genes could be enriched to COVID-19 SARS-CoV-2 and the antiviral signaling including IFN signaling and downstream JAK-STAT signaling (Fig. 2A-B). In agreement with the induced expression of *ISG15* by S protein, a set of ISGs were also determined to be induced by the overexpression of S protein (Fig. 2C), suggesting that S protein could activate IFN effector signaling. Interestingly, among the induced genes by S protein, we found that the expression of *ACE2*, the receptor of SARS-CoV-2, was also significantly increased by SARS-CoV-2 S protein (Fig. 2C). Together with a recent report determining that IFN treatment directly induced *ACE2* expression in primary human nasal epithelial cells [10], we presumed that SARS-CoV-2 S protein may activate IFN effector signaling to induce *ACE2* expression for its entry.

Recently, several studies demonstrated a new truncated isoform of *ACE2*, named *deltaACE2* (*dACE2*), whose translation product lacked 356 amino acids at N-terminal as compared to that of full length ACE2 (long ACE2) [38-40]. dACE2 protein is unlikely to facilitate the entry of SARS-CoV-2, as result of the deficiency in the domain required for SARS-CoV-2 binding [38-40]. Therefore, the induced long *ACE2, dACE2* and total *ACE2* levels by SARS-CoV-2 S protein were then examined in bronchial epithelial BEAS-2B cells, and the increased long ACE2 expression was the most markedly and significantly (Fig. 2D). Overexpression of S protein enhanced the expression of long *ACE2* at both mRNA and protein levels, while has little effect on dACE2 protein expression (Fig. 2D-E). Therefore, we focused on the long ACE2 isoform, and found that long *ACE2* expression was commonly upregulated by S protein of the three respiratory syndrome coronavirus, including SARS-CoV-2, SARS-CoV and MERS-CoV, and SARS-CoV-2 S protein had the most marked and significant effect (Fig. 2F). Moreover, the truncates of SARS-CoV-2 S protein, including S1, S2, and RBD domains, were also transfected respectively, and none of these truncates could induce the expression of long *ACE2* (Fig. 2F), suggesting that only the intact structure of S protein could enhance long *ACE2* expression. Taken together, these data determine that SARS-CoV-2 S protein induces the expression of a set of ISGs and the receptor *ACE2*, and the induced long *ACE2* may be involved in the infection of SARS-CoV-2.

3.3. S protein enhanced ACE2 expression by activating IFN effector signaling

The mechanism responsible for S protein-induced *ACE2* expression was then investigated. As SARS-CoV-2 S protein was determined here to activate ISGs expression and a recent research reported that IFN could drive *ACE2* expression in primary human nasal epithelial cells [10], we examined that whether S protein could induce *ACE2* expression by activating IFN effector JAK-STAT signaling. The phosphorylation and activation of STAT1 and STAT2 were analyzed in S protein-overexpressed BEAS-2B cells, and we found that S protein could enhance the phosphorylation of STAT1 at tyrosine 701 and STAT2 at tyrosine 690, thus contributing for their activation (Fig. 3A-B). Furthermore, using Fludarabine to inhibit STAT1 activation, the induced long *ACE2* expression by S protein overexpression was abrogated in BEAS-2B cells (Fig. 3C). Together, we conclude that SARS-CoV-2 S protein could induce the receptor long *ACE2* expression by activating IFN effector JAK-STAT signaling.

3.4. SARS-CoV-2 S protein activated STAT1 and STAT2 via promoting their association with upstream JAK1

We next investigated the molecular mechanism responsible for the SARS-CoV-2 S protein-prompted STAT1 and STAT2 phosphorylation. The possible interaction between S protein with STAT1 and STAT2 was then examined, and we found that exogeneous STAT1 and STAT2 could both co-immunoprecipitated with SARS-CoV-2 S protein using co-immunoprecipitaion (co-IP) analysis in HEK293T cells overexpressed with the tagged proteins (Fig. 4A), suggesting the potential direct interaction between S protein with STAT1 and STAT2. The endogenous interaction between S protein with STAT1 and STAT2 was also confirmed using co-IP in bronchial epithelial BEAS-2B cells (Fig. 4B). Furthermore, to identify the corresponding domains within STAT1 and STAT2 responsible for their interaction with S protein, the truncates of STAT1 and STAT2 were constructed and co-transfected into BEAS-2B cells with V5 tagged-S protein. Co-IP analysis determined that SARS-CoV-2 S protein interacted with the N-terminal domain of STAT1 and N-terminal domain of STAT2 respectively (Fig. 4C). Together, these data suggest that SARS-CoV-2 S protein could directly interact with STAT1 and STAT2 through their N-terminal domains.

In order to illustrate how the activation between S protein with STAT1 and STAT2 enhances their phosphorylation, we first examined the activation of upstream catalytic kinase Janus kinase (JAK) 1, which directly phosphorylated downstream STAT1 and STAT2. However, overexpression of S protein in BEAS-2B cells did not significantly influence the phosphorylation of JAK1, which is corresponding for its catalytic activation (data not shown). Since JAK1 directly associated with downstream STAT1 and STAT2 to function the catalytic role, we then presumed that SARS-CoV-2 S protein might influence this association. Through co-IP analysis, we found that overexpression of S protein enhanced the association between JAK1 with downstream STAT1 and STAT2 (Fig. 4D). Thus, we conclude that SARS-CoV-2 S protein facilitates the activation of STAT1 and STAT2 through reinforcing their interaction with upstream JAK1, which then induces the receptor *ACE2* expression and may participate in the infection process of SARS-CoV-2.

4. Discussion

In this study, we found that SARS-CoV-2 S protein could activate IFN effector signaling to enhance the receptor *ACE2* expression in human bronchial epithelial cell line BEAS-2B, which may be involved in the viral infection. For the potential interaction between SARS-CoV-2 and host IFN response, previous studies determined the inhibitory roles of Nsp1, Nsp6, Nsp7, Nsp13, Nsp14, ORF3a, M, ORF6, ORF7a, and ORF7b proteins on ISRE promoter activity in HEK293T cells treated with IFN-α, while S protein had no effect [19]. This disparity may result from the different cells used in these studies, and bronchial epithelium is one of the host cells for the entry of SARS-CoV-2, which may better reflect the process of SARS-CoV-2 infection. However, as all these data of SARS-CoV-2's effects on IFN response were analyzed *in vitro*, further

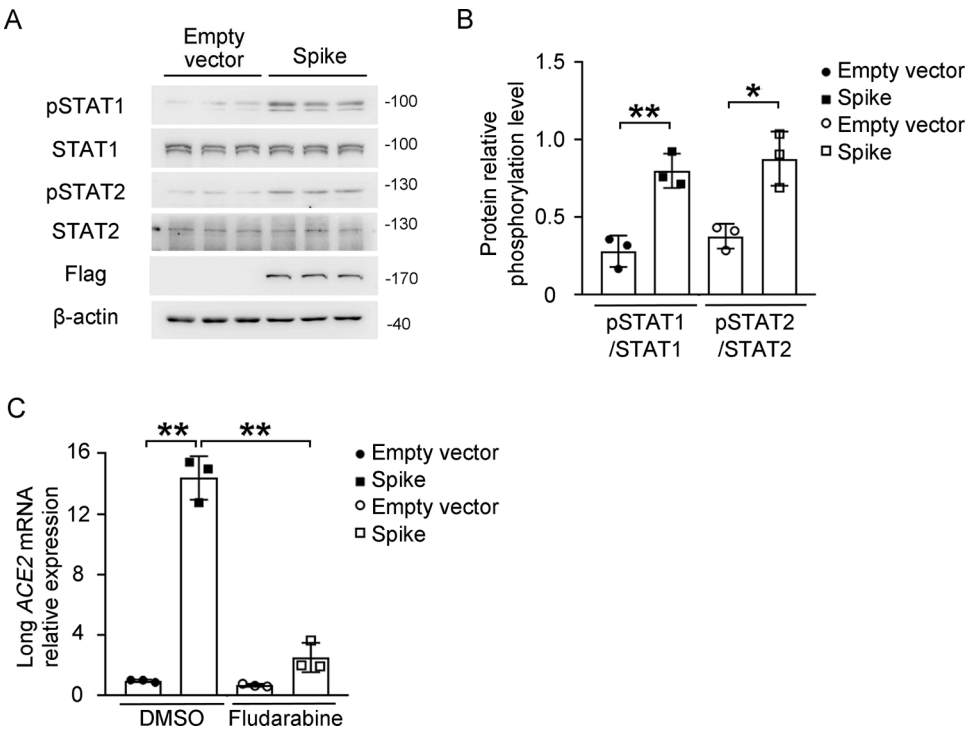

Fig. 3. SARS-CoV-2 S protein activated IFN effector JAK-STAT1/2 signaling.
Note: A, BEAS-2B cells were transfected with empty vector or S protein without IFN-α stimulus. The phosphorylation of STAT1 and STAT2 was examined by Western blot. β-actin was used as internal control; B, Quantification of the percentage of STAT1/2 phosphorylation from Western blot in A; C, BEAS-2B cells were transfected with empty vector or S protein, and then treated with DMSO or STAT inhibitor Fludarabine as indicated without IFN-α stimulus. The expression of *ACE2* was detected by qRT-PCR; Data are shown as mean ± SD or typical photographs of one representative experiment from three independent experiments. *$P< 0.05$; **$P< 0.01$.

experiments in the primary bronchial epithelial cells or in the *in vivo* SARS-CoV-2 infection models are more crucial and necessary for the better understanding of the interaction between SARS-CoV-2 products and host IFN response.

SARS-CoV-2 S protein was found here to promote its receptor *ACE2* expression in bronchial epithelium BEAS-2B cells, which may then assist virus entry. A recent research identified the target cells of SARS-CoV-2 through using single-cell RNA-sequencing to elucidate which cells could express ACE2, and found that *ACE2* was expressed in lung type II pneumocytes, ileal absorptive enterocytes, nasal goblet secretory cells, and bronchial epithelium BEAS-2B cells [10], which might be invaded by SARS-CoV-2. Simultaneously, another study confirmed that SARS-CoV-2 entry-associated genes can be co-expressed in specific respiratory, corneal and intestinal epithelial cells, highlighting their potential role in initial viral infection, spread and clearance [41]. Hence, *ACE2* expression level may determine the efficiency of SARS-CoV-2 infection to host cells, and the increased ACE2 expression by S protein may further help its invasion. Thus, the roles of SARS-CoV-2 protein following viral infection should be reconsidered, especially in the *in vivo* model of SARS-CoV-2 infection. Moreover, owing to the effects of SARS-CoV-2 S protein on promoting its receptor *ACE2* expression in bronchial epithelium BEAS-2B cells, the safety of mRNA vaccines encoding the stabilized SARS-CoV-2 S protein may raise concerns. Especially considering people who are not sensitive to vaccination by mRNA vaccine encoding the SARS-CoV-2 S protein, they may be more susceptible to SARS-CoV-2 infection because of increased receptor ACE2 expression. Therefore, there's a need for an adequate assessment of safety and risk of mRNA vaccine encoding the SARS-CoV-2 S protein.

Mechanistically, SARS-CoV-2 S protein enhances the phosphorylation of STAT1 and STAT2 through strengthening their interaction with upstream kinase JAK1, which activates *ACE2* expression. This phenomenon is in consistent with the results of a previous study, which demonstrates that S protein promotes IFN-β promoter activity mediated by SeV infection and RIG-I activation in HEK293T cells [18]. Thus, SARS-CoV-2 S protein may promote both IFN production in innate immune cells and IFN effects in the epithelium. In the epithelium, the activation of IFN effects mediated by S protein leads to the upregulation of *ACE2* expression, which may facilitate viral entry. For the potential roles of S protein in innate immune cells, whether the enhancement of IFN promoter activity and then IFN production exist in the $CD169^+$ macrophages of COVID-19 patients need to be further examined, as $CD169^+$ macrophages are the major SARS-CoV-2-infected tissue-resident macrophages [42]. Moreover, the roles of the potential promoted type I IFN production in SARS-CoV-2 infection are still not fully determined, whether this effect helps viral invasion, enhances host antiviral response, or even initiate immune disorders still need further investigation *in vivo*. As recent studies determining IFN-inducible expression of nonfunctional dACE2 not long ACE2 [38-40], our findings provided that long ACE2 was increased by SARS-CoV-2 S protein through STAT1/2 activation independent on IFN-α, and the mechanisms for IFN-α-induced dACE2 and S protein-induced long ACE2 still need further investigation in details.

As mentioned above, type I IFN is recognized as the most important antiviral cytokine for the clearance of viral infection, inhibition of viral replication, and induction of apoptosis in virus-infected cells [43]. Although several studies have suggested the low levels of IFN-I or IFN-III and high levels of chemokines or IL-6 signatures in the serum samples of COVID-19 patients [44], recent clinical data also determined a possible positive correlation between viral load, IFN-α levels and the disease severity of COVID-19 patients, with high ISGs expression and high serum IFN-α levels during ICU stage [21]. Moreover, studies demonstrated that most immune cells from patients with severe COVID-19 showed a strong type I IFN response through single cell RNA sequencing analysis [45, 46]. Together with our data that SARS-CoV-2 S protein activating IFN effector signaling to induce *ACE2* expression, the roles of type I IFN production in SARS-CoV-2 infection and COVID-19 progression still need intensive investigation, especially in the different time periods post viral infection and different disease severity of COVID-19.

In conclusion, SARS-CoV-2 S protein facilitates IFN effects signaling to induce the expression of ISGs including its receptor ACE2, by activating STAT1 and STAT2 via promoting their association with upstream kinase JAK1, which may assist viral entry in bronchial epithelium.

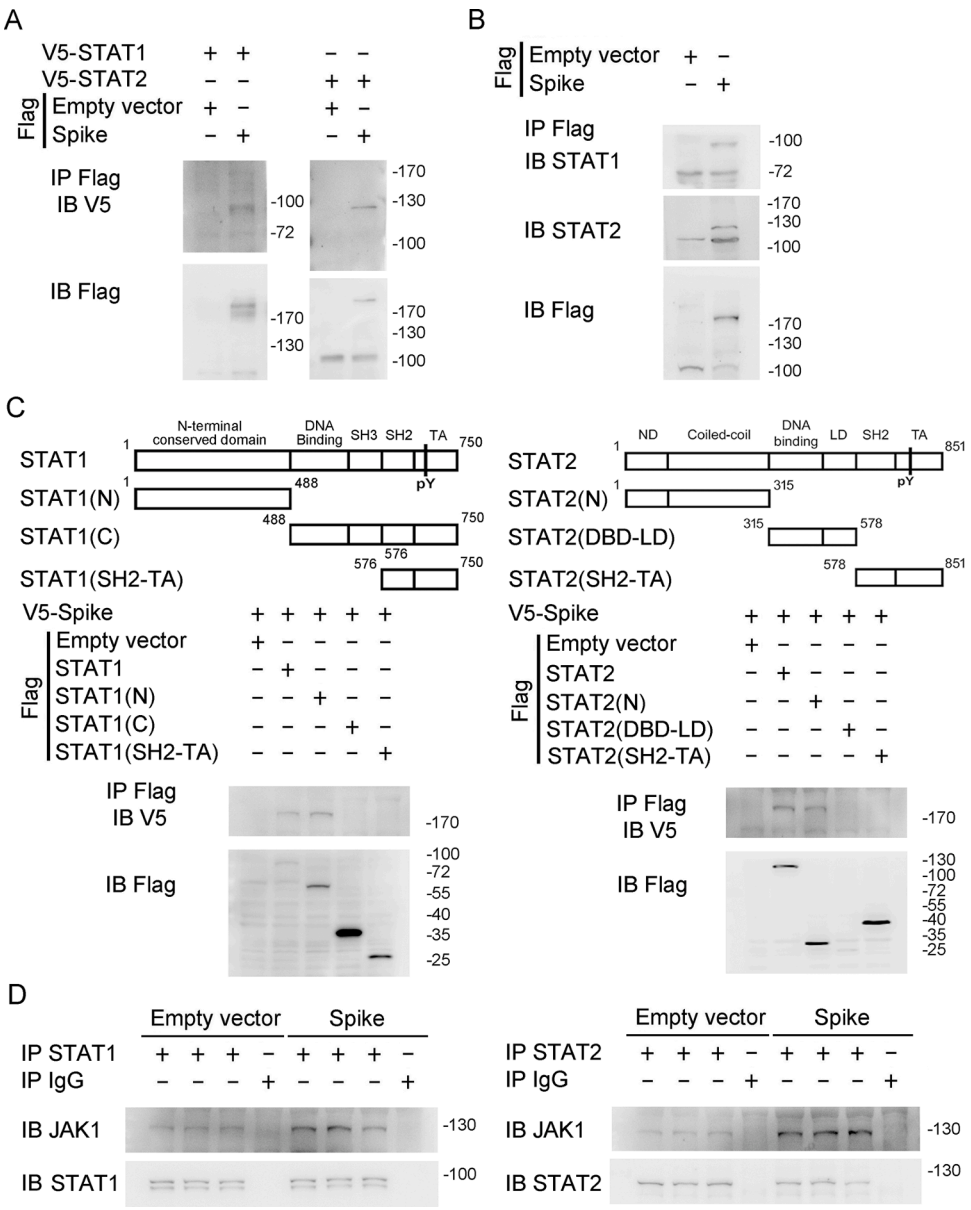

Fig. 4. SARS-CoV-2 S protein promoted the activation of STAT1 and STAT2 through enhancing their association with JAK1.
Note: A, Association of Flag-tagged STAT1 and STAT2 with V5-tagged S protein was determined using co-IP in the transfected HEK293T cells; B, Co-IP examination of the interaction between STAT1, STAT2, and Flag-tagged S protein was determined in BEAS-2B cells; C, Wild-type or fragments of STAT1 and STAT2 were constructed as indicated. Association of Flag-tagged STAT1 and STAT2 with V5-tagged S protein was determined using co-IP in the transfected HEK293T cells; D, Co-IP examination of the interaction between STAT1, STAT2, and JAK1 was determined in BEAS-2B cells transfected with S protein or empty vector; Data are shown as typical photographs of one representative experiment, similar results were obtained in three independent experiments.

Authors' Contributions

JH and YZ designed the project and wrote the manuscript. YZ, MW and YL performed experiments and analysed the data; PW and PZ provided the SARS-CoV-2 structural, non-structural, and accessory protein constructs; ZY, SW, LZ, CZ, ZL, NL and YY participated in the data preparation and analysis. All authors have read and approved the final manuscript. Ye Zhou, Mu Wang and Yunhui Li contributed equally to this work.

Data Availability

The accession number of the RNA-seq data reported in this paper is SRA: SRX9324012.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.imlet.2021.06.008.

References

[1] R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, H. Song, B. Huang, N. Zhu, Y. Bi, X. Ma, F. Zhan, L. Wang, T. Hu, H. Zhou, Z. Hu, W. Zhou, L. Zhao, J. Chen, Y Meng, J. Wang, Y. Lin, J. Yuan, Z. Xie, J. Ma, W.J. Liu, D. Wang, W. Xu, E. C. Holmes, G.F. Gao, G. Wu, W. Chen, W. Shi, W. Tan, Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding, Lancet 395 (10224) (2020) 565–574.

[2] H. Ouassou, L. Kharchoufa, M. Bouhrim, N.E. Daoudi, H. Imtara, N. Bencheikh, A. Elbouzidi, M. Bnouham, The pathogenesis of Coronavirus Disease 2019 (COVID-19): evaluation and prevention, J. Immunol. Res. 2020 (2020), 1357983.

[3] P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, Y. Zhu, B. Li, C. L. Huang, H.D. Chen, J. Chen, Y. Luo, H. Guo, R.D. Jiang, M.Q. Liu, Y. Chen, X. R. Shen, X. Wang, X.S. Zheng, K. Zhao, Q.J. Chen, F. Deng, L.L. Liu, B. Yan, F. X. Zhan, Y.Y. Wang, G.F. Xiao, Z.L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (7798) (2020) 270–273.

[4] Y. Cao, B. Su, X. Guo, W. Sun, Y. Deng, L. Bao, Q. Zhu, X. Zhang, Y. Zheng, C. Geng, X. Chai, R. He, X. Li, Q. Lv, H. Zhu, W. Deng, Y. Xu, Y. Wang, L. Qiao, Y. Tan, L. Song, G. Wang, X. Du, N. Gao, J. Liu, J. Xiao, X.D. Su, Z. Du, Y. Feng, C. Qin, C. Qin, R. Jin, X.S. Xie, Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells, Cell 182 (1) (2020) 73–84.

[5] K.G. Andersen, A. Rambaut, W.I. Lipkin, E.C. Holmes, R.F. Garry, The proximal origin of SARS-CoV-2, Nat. Med. 26 (4) (2020) 450–452.

[6] E. Mahase, Coronavirus COVID-19 has killed more people than SARS and MERS combined, despite lower case fatality rate, BMJ 368 (2020) 641.

[7] A. Wu, Y. Peng, B. Huang, X. Ding, X. Wang, P. Niu, J. Meng, Z. Zhu, Z. Zhang, J. Wang, J. Sheng, L. Quan, Z. Xia, W. Tan, G. Cheng, T. Jiang, Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China, Cell Host Microbe 27 (3) (2020) 325–328.

[8] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T. S. Schiergens, G. Herrler, N.H. Wu, A. Nitsche, M.A. Müller, C. Drosten, S. Pöhlmann, SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Cell 181 (2) (2020) 271–280.

[9] D. Wrapp, N. Wang, K.S. Corbett, J.A. Goldsmith, C-L. Hsieh, O. Abiona, B. S. Graham, J.S. McLellan, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation, Science 367 (6483) (2020) 1260–1263.

[10] C.G.K. Ziegler, S.J. Allon, S.K. Nyquist, I.M. Mbano, V.N. Miao, C.N. Tzouanas, Y. Cao, A.S. Yousif, J. Bals, B.M. Hauser, J. Feldman, C. Muus, M.H. Wadsworth 2nd, S.W. Kazer, T.K. Hughes, B. Doran, G.J. Gatter, M. Vukovic, F. Taliaferro, B. E. Mead, Z. Guo, J.P. Wang, D. Gras, M. Plaisant, M. Ansari, I. Angelidis, H. Adler, J.M.S. Sucre, C.J. Taylor, B. Lin, A. Waghray, V. Mitsialis, D.F. Dwyer, K. M. Buchheit, J.A. Boyce, N.A. Barrett, T.M. Laidlaw, S.L. Carroll, L. Colonna, V. Tkachev, C.W. Peterson, A. Yu, H.B. Zheng, H.P. Gideon, C.G. Winchell, P.L. Lin, C.D. Bingle, S.B. Snapper, J.A. Kropski, F.J. Theis, H.B. Schiller, L-E. Zaragosi, P. Barbry, A. Leslie, H-P. Kiem, J.L. Flynn, S.M. Fortune, B. Berger, R.W. Finberg, L. S. Kean, M. Garber, A.G. Schmidt, D. Lingwood, A.K. Shalek, J. Ordovas-Montanes, SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues, Cell 181 (5) (2020) 1016–1035.

[11] S. Lee, R. Channappanavar, T-D. Kanneganti, Coronaviruses: innate immunity, inflammasome activation, inflammatory cell death, and cytokines, Trends Immunol 41 (12) (2020) 1083–1099.

[12] D. Blanco-Melo, B.E. Nilsson-Payant, W.C. Liu, S. Uhl, D. Hoagland, R. Møller, T. X. Jordan, K. Oishi, M. Panis, D. Sachs, T.T. Wang, R.E. Schwartz, J.K. Lim, R. A. Albrecht, B.R. tenOever, Imbalanced host response to SARS-CoV-2 drives development of COVID-19, Cell 181 (5) (2020) 1036–1045.

[13] X. Cao, COVID-19: immunopathology and its implications for therapy, Nat. Rev. Immunol. 20 (5) (2020) 269–270.

[14] M.G. Wathelet, M. Orr, M.B. Frieman, R.S. Baric, Severe acute respiratory syndrome coronavirus evades antiviral signaling: role of nsp1 and rational design of an attenuated strain, J. Virol. 81 (21) (2007) 11620–11633.

[15] S.A. Kopecky-Bromberg, L. Martínez-Sobrido, M. Frieman, R.A. Baric, P. Palese, Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF6, and nucleocapsid proteins function as interferon antagonists, J. Virol. 81 (2) (2007) 548–557.

[16] M. Frieman, B. Yount, M. Heise, S.A. Kopecky-Bromberg, P. Palese, R.S. Baric, Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane, J. Virol. 81 (18) (2007) 9812–9824.

[17] L.R. Wong, Z.W. Ye, P.Y. Lui, X. Zheng, S. Yuan, L. Zhu, S.Y. Fung, K.S. Yuen, K. L. Siu, M.L. Yeung, Z. Cai, P.C. Woo, K.Y. Yuen, C.P. Chan, D.Y. Jin, Middle east respiratory syndrome coronavirus ORF8b accessory protein suppresses type I IFN expression by impeding HSP70-dependent activation of IRF3 kinase IKKε, J. Immunol. 205 (6) (2020) 1564–1579.

[18] X. Lei, X. Dong, R. Ma, W. Wang, X. Xiao, Z. Tian, C. Wang, Y. Wang, L. Li, L. Ren, F. Guo, Z. Zhao, Z. Zhou, Z. Xiang, J. Wang, Activation and evasion of type I interferon responses by SARS-CoV-2, Nat. Commun. 11 (1) (2020) 3810.

[19] H. Xia, Z. Cao, X. Xie, X. Zhang, J.Y. Chen, H. Wang, V.D. Menachery, R. Rajsbaum, P-Y. Shi, Evasion of type I interferon by SARS-CoV-2, Cell Rep 33 (1) (2020), 108234.

[20] P.S. Arunachalam, F. Wimmers, C.K.P. Mok, R.A.P.M. Perera, M. Scott, T. Hagan, N. Sigal, Y. Feng, L. Bristow, O.T-Y. Tsang, D. Wagh, J. Coller, K.L. Pellegrini, D. Kazmin, G. Alaaeddine, W.S. Leung, J.M.C. Chan, T.S.H. Chik, C.Y.C. Choi, C. Huerta, M.P. McCullough, H. Lv, E. Anderson, S. Edupuganti, A.A. Upadhyay, S. E. Bosinger, H.T. Maecker, P. Khatri, N. Rouphael, M. Peiris, B. Pulendran, Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans, Science 369 (6508) (2020) 1210–1220.

[21] L. Wei, S. Ming, B. Zou, Y. Wu, Z. Hong, Z. Li, X. Zheng, M. Huang, L. Luo, J. Liang, X. Wen, T. Chen, Q. Liang, L. Kuang, H. Shan, X. Huang, Viral invasion and type I interferon response characterize the immunophenotypes during COVID-19 infection, SSRN: https://ssrn.com/abstract=3564998 or https://doi.org/10.2139/ssrn.3564998.

[22] J. Cinatl, B. Morgenstern, G. Bauer, P. Chandra, H. Rabenau, H.W. Doerr, Treatment of SARS with human interferons, Lancet 362 (9380) (2003) 293–294.

[23] E. Kindler, V. Thiel, F. Weber, Interaction of SARS and MERS coronaviruses with the antiviral interferon response, Adv. Virus Res. 96 (2016) 219–243.

[24] F. Zielecki, M. Weber, M. Eickmann, L. Spiegelberg, A.M. Zaki, M. Matrosovich, S. Becker, F. Weber, Human cell tropism and innate immune system interactions of human respiratory coronavirus EMC compared to those of severe acute respiratory syndrome coronavirus, J. Virol. 87 (9) (2013) 5300–5304.

[25] A. Vanderheiden, P. Ralfs, T. Chirkova, A.A. Upadhyay, M.G. Zimmerman, S. Bedoya, H. Aoued, G.M. Tharp, K.L. Pellegrini, C. Manfredi, E. Sorscher, B. Mainou, J.L. Lobby, J.E. Kohlmeier, A.C. Lowen, P-Y. Shi, V.D. Menachery, L. J. Anderson, A. Grakoui, S.E. Bosinger, M.S. Suthar, Type I and type III interferons restrict SARS-CoV-2 infection of human airway epithelial cultures, J. Virol. 94 (19) (2020) e00920–e00985.

[26] D. Wu, X.O. Yang, TH17 responses in cytokine storm of COVID-19: an emerging target of JAK2 inhibitor Fedratinib, J. Microbiol. Immunol. Infect. 53 (3) (2020) 368–370.

[27] J.J. Chen, L.N. Zhang, H. Hou, L. Xu, K. Ji, Interleukin-6 signaling blockade treatment for cytokine release syndrome in COVID-19, Exp. Ther. Med. 21 (1) (2021) 24.

[28] A. Bonaventura, A. Vecchié, T.S. Wang, E. Lee, P.C. Cremer, B. Carey, P. Rajendram, K.M. Hudock, L. Korbee, B.W.V. Tassell, L. Dagna, A. Abbate, Targeting GM-CSF in COVID-19 pneumonia: rationale and strategies, Front. Immunol. 11 (2020) 1625.

[29] Q. Zheng, J. Hou, Y. Zhou, Z. Li, X. Cao, The RNA helicase DDX46 inhibits innate immunity by entrapping m^6A-demethylated antiviral transcripts in the nucleus, Nat. Immunol. 18 (10) (2017) 1094–1103.

[30] Y. Yang, Y. Zhou, J. Hou, C. Bai, Z. Li, J. Fan, I.O.L. Ng, W. Zhou, H. Sun, Q. Dong, J.M.F Lee, C-M. Lo, K. Man, Y. Yang, N. Li, G. Ding, Y. Yu, X. Cao, Hepatic IFIT3 predicts interferon-α therapeutic response in patients of hepatocellular carcinoma, Hepatology 66 (1) (2017) 152–166.

[31] J. Zhang, R. Cruz-Cosme, M. Zhuang, D. Liu, Y. Liu, S. Teng, P.H. Wang, Q. Tang, A systemic and molecular study of subcellular localization of SARS-CoV-2 proteins, Signal Transduct. Target. Ther. 5 (1) (2020) 269.

[32] Y. Han, Q. Liu, J. Hou, Y. Gu, Y. Zhang, Z. Chen, J. Fan, W. Zhou, S. Qiu, Y. Zhang, T. Dong, N. Li, Z. Jiang, H. Zhu, Q. Zhang, Y. Ma, L. Zhang, Q. Wang, Y. Yu, N. Li, X. Cao, Tumor-induced generation of splenic erythroblast-like Ter-cells promotes tumor progression, Cell 173 (3) (2018) 634–648.

[33] Z. Li, Y. Zhou, L. Zhang, K. Jia, S. Wang, M. Wang, N. Li, Y. Yu, X. Cao, J. Hou, MicroRNA-199a-3p inhibits hepatic apoptosis and hepatocarcinogenesis by targeting PDCD4, Oncogenesis 9 (10) (2020) 95.

[34] Q. Zheng, J. Hou, Y. Zhou, Y. Yang, X. Cao, Type I IFN-inducible downregulation of microRNA-27a feedback inhibits antiviral innate response by upregulating Siglec1/TRIM27, J. Immunol. 196 (3) (2016) 1317–1326.

[35] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCt method, Methods 25 (4) (2001) 402–408.

[36] Q. Zheng, J. Hou, Y. Zhou, Y. Yang, B. Xie, X. Cao, Siglec1 suppresses antiviral innate immune response by inducing TBK1 degradation via the ubiquitin ligase TRIM27, Cell Res 25 (10) (2015) 1121–1136.

[37] J. Hou, Y. Zhou, Y. Zheng, J. Fan, W. Zhou, I.O.L. Ng, H. Sun, L. Qin, S. Qiu, J.M. F. Lee, C-M. Lo, K. Man, Y. Yang, Y. Yang, Y. Yang, Q. Zhang, X. Zhu, N. Li, Z. Wang, G. Ding, S-M. Zhuang, L. Zheng, X. Luo, Y. Xie, A. Liang, Z. Wang, M. Zhang, Q. Xia, T. Liang, Y. Yu, X. Cao, Hepatic RIG-I predicts survival and interferon-α therapeutic response in hepatocellular carcinoma, Cancer Cell 25 (1) (2014) 49–63.

[38] O.O. Onabajo, A.R Banday, M.L. Stanifer, W. Yan, A. Obajemu, D.M. Santer, O. Florez-Vargas, H. Piontkivska, J.M. Vargas, T.J. Ring, C. Kee, P. Doldan, D. L. Tyrrell, J.L. Mendoza, S. Boulant, L. Prokunina-Olsson, Interferons and viruses induce a novel truncated ACE2 isoform and not the full-length SARS-CoV-2 receptor, Nat. Genet. 52 (12) (2020) 1283–1293.

[39] K.W. Ng, J. Attig, W. Bolland, G.R. Young, J. Major, A.G. Wrobel, S. Gamblin, A. Wack, G. Kassiotis, Tissue-specific and interferon-inducible expression of nonfunctional ACE2 through endogenous retroelement co-option, Nat. Genet. 52 (12) (2020) 1294–1302.

[40] C. Blume, C.L. Jackson, C.M. Spalluto, J. Legebeke, L. Nazlamova, F. Conforti, J-M. Perotin, M. Frank, J. Butler, M. Crispin, J. Coles, J. Thompson, R.A. Ridley, L.S. N. Dean, M. Loxham, S. Reikine, A. Azim, K. Tariq, D.A. Johnston, P.J. Skipp, R. Djukanovic, D. Baralle, C.J. McCormick, D.E. Davies, J.S. Lucas, G. Wheway, V. Mennella, A novel ACE2 isoform is expressed in human respiratory epithelia and is upregulated in response to interferons and RNA respiratory virus infection, Nat. Genet. 53 (2) (2021) 205–214.

[41] W. Sungnak, N. Huang, C. Bécavin, M. Berg, R. Queen, M. Litvinukova, C. Talavera-López, H. Maatz, D. Reichart, F. Sampaziotis, K.B. Worlock, M. Yoshida, J. L. Barnes, SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes, Nat. Med. 26 (5) (2020) 681–687.

[42] Z. Feng, B. Diao, R. Wang, G. Wang, C. Wang, Y. Tan, L. Liu, C. Wang, Y. Liu, Y. Liu, Z. Yuan, L. Ren, Y. Wu, Y. Chen, The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) directly decimates human spleens and lymph nodes, medRxiv doi: https://doi.org/10.1101/2020.03.27.20045427.

[43] A. Park, A. Iwasaki, Type I and type III interferons - induction, signaling, evasion, and application to combat COVID-19, Cell Host Microbe 27 (6) (2020) 870–878.
[44] G. Chen, D. Wu, W. Guo, Y. Cao, D. Huang, H. Wang, T. Wang, X. Zhang, H. Chen, H. Yu, X. Zhang, M. Zhang, S. Wu, J. Song, T. Chen, M. Han, S. Li, X. Luo, J. Zhao, Q. Ning, Clinical and immunological features of severe and moderate coronavirus disease 2019, J. Clin. Invest. 130 (5) (2020) 2620–2629.
[45] J.Y. Zhang, X.M. Wang, X. Xing, Z. Xu, C. Zhang, J.W. Song, X. Fan, P. Xia, J.L. Fu, S.Y. Wang, R.N. Xu, X.P. Dai, L. Shi, T.J.Jiang L..Huang, M. Shi, Y. Zhang, A. Zumla, M. Maeurer, F. Bai, F.S. Wang, Single-cell landscape of immunological responses in patients with COVID-19, Nat. Immunol. 21 (9) (2020) 1107–1118.
[46] J.S. Lee, S. Park, H.W. Jeong, J.Y. Ahn, S.J. Choi, H. Lee, B. Choi, S.K. Nam, M. Sa, J.S. Kwon, S.J. Jeong, H.K. Lee, S.H. Park, S.H. Park, J.Y. Choi, S.H. Kim, I. Jung, E. C. Shin, Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19, Sci. Immunol. 5 (49) (2020) eabd1554.