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SARS-CoV-2 attenuates corticosteroid sensitivity by suppressing DUSP1 expression and activating p38 MAPK pathway

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ABSTRACT

The efficacy of corticosteroids and its use for the treatment of SARS-CoV-2 infections is controversial. In this study, using data sets of SARS-CoV-2 infected lung tissues and nasopharyngeal swabs, as well as *in vitro* experiments, we show that SARS-CoV-2 infection significantly downregulates DUSP1 expression. This downregulation of DUSP1 could be the mechanism regulating the enhanced activation of MAPK pathway as well as the reported steroid resistance in SARS-CoV-2 infection. Moreover, chloroquine, an off labeled COVID-19 drug is able to induce DUSP1 and attenuate MAPK pathway; and is expected to improve sensitivity to steroid treatment. However, further mechanistic studies are required to confirm this effect.

1. Introduction

Novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) outbreak is a major challenge for the whole world, and especially for clinicians. Approximately 5%–10% of the cases are critical and require admission to intensive care unit (Matthay et al., 2020). Little data exist that explain SARS-CoV-2 pathogenesis, and no pharmacological therapies of proven efficacy yet exist.

Corticosteroids were widely used during the previous outbreaks of SARS-CoV-1 and Middle East respiratory syndrome (MERS)-CoV (Arabi et al., 2018), and was associated with little reduction in mortality in patients with MERS-CoV (Arabi et al., 2018). However, they are still being used in patients with COVID-19 in addition to other treatment options (Bani-Sadr et al., 2020).

At the beginning of the pandemic, a clinical panel for managing critically ill adults with COVID-19, as well as the WHO guidance on clinical management of COVID-19, advised against the use of systemic corticosteroids, unless indicated, for managing the associated severe acute respiratory distress syndrome (ARDS) (Matthay et al., 2020; World Health, 2020). The RECOVERY Trial on dexamethasone found that low-dose (6 mg) of dexamethasone for 10 days reduced deaths by one-third in critically ill patients with COVID-19 (Horby et al., 2020). However, it is still debatable why some hospitalized COVID-19 patients are not responding to this treatment. Thus, understanding the reasons leading to corticosteroids (glucocorticoids) resistance in COVID-19 is of immediate clinical importance.

Glucocorticoids function by binding to their intracellular receptor (Glucocorticoids Receptor (GR), NR3C1-the gene encode GR), which is a ligand-inducible transcription factor. In the absence of ligand, GR resides permanently in the cytoplasm. Binding of glucocorticoids to the cytoplasmic GR (NR3C1) causes translocation of the receptor to the nucleus. However, the mechanisms by which glucocorticoids reduce expression of inflammatory genes remain poorly understood. One of the proposed mechanisms is binding of NR3C1 with the inflammatory transcription factors to recruit HDAC2 and repress transcription of inflammatory genes, or transrepression. Another mechanism is to induce transcriptional activity of multiple effector genes with anti-inflammatory potential. Among these genes is the dual specificity phosphatase, DUSP1 and glucocorticoid-induced leucine zipper (TSC22D3) to reduce activation of MAPK, NF-κB and AP-1

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transcriptional responses (Newton, 2014).

In this study, the expression levels of these glucocorticoid-dependent genes including NR3C1, HDAC2, DUSP1 and TSC22D3 following SARS-CoV-2 infection was investigated. Accordingly, assessing expression of these genes in human airway epithelial cells (HAECs) infected with SARS-CoV-2 as well as nasopharyngeal swabs of the recruited severe COVID-19 and lung autopsies of COVID-19 patients revealed that among these genes, expression level of DUSP1 is particularly downregulated in the nasopharyngeal swabs and lung tissues of COVID-19 patients. This downregulation of DUSP1 could be the mechanism regulating the enhanced activation of MAPK pathway as well as the reported steroid resistance in SARS-CoV-2 infection.

2. Materials and methods

2.1. Gene expression datasets

In this study, bioinformatic analyses was conducted to investigate differential gene expressions of steroid-dependent genes in the HAECs infected with either SARS-CoV-2 or SARS-CoV-1, nasopharyngeal swabs and lung autopsies of COVID-19 patients. Publicly available gene expression datasets deposited in National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO, http://www.ncbi.nlm.nih.gov/geo) and the European Bioinformatics Institute (EMBL-EBI, https://www.ebi.ac.uk) were used. Before data preprocessing, all data was evaluated for quality control (QC) (Galamb et al., 2009; The Tumor Analysis Best Practices Working, 2004).

The datasets included are as follow; a dataset for SARS-CoV-2 infected HAECs (GSE147507) (Blanco-Melo et al., 2020), a dataset for SARS-CoV-2 infected lung autopsies (GSE150316), a data set of COVID-19 nasopharyngeal swabs (GSE152075), 3 datasets for SARS-CoV-1 infected HAECs (GSE47960, GSE47961, and GSE47962) (Mitchell et al., 2013), a dataset of COPD airway epithelial cells (GSE56341) (Vucic et al., 2014). Furthermore, a dataset was used for assessing the effect of chloroquine on *in vitro* viral infected cells (GSE30351). Details of the datasets used are presented in supplementary Table 1. General characteristics of COPD airway epithelial cells donors used in this study was added in the supplementary Table 2.

For both SARS-CoV-2 and SARS-CoV-1 infections, the primary human airway epithelial cells were cultured at the air-liquid interface, as described previously (Blanco-Melo et al., 2020; Mitchell et al., 2013).

2.2. Nasopharyngeal swabs of COVID-19 patients

Nasopharyngeal swabs where isolated from 36 severe COVID-19 patients (average age of 48 ± 5 years), and 6 healthy individuals (average age of 45 ± 7 years). The Ethics Committee of Dubai Health Authority approved this study. Written informed consent was obtained from all study participants prior to inclusion.

2.3. Cell culture

Primary bronchial fibroblasts were isolated from endobronchial tissue biopsies obtained from 3 COPD patients (average age 65.3 ± 7.7 years), as described previously (Panariti et al., 2018). The cells were cultured at confluence in 6 well flat-bottom plates in complete media (Dulbecco's modified Eagle's media (Sigma) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml of penicillin, and 100 ng/ml streptomycin). Furthermore, the cells were treated or not with 10 μM chloroquine (Sigma) and 100 nM dexamethasone (Sigma) for 6 h. The concentration of chloroquine was chosen based on the reported potency of chloroquine at concentration of 10 μM to inhibit SARS-CoV-1 viral replication *in vitro* (Keyaerts et al., 2004; Vincent et al., 2005).

2.4. Western blot assay

The nasopharyngeal swabs were pelleted by centrifugation of 2 ml of the solution at 14,000 g for 20 min at 4 °C. The protein concentrations were measured using the BCA protein assay reagent kit (Thermo-Scientific Pierce BCA Protein Assay Kit). The cells were lysed using 10X RIPA Buffer (Abcam) after supplementation with 1x Protease Inhibitor Cocktail (Sigma-Aldrich) and 1 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich). Fifteen micrograms total proteins were separated using 10% gels. The proteins were transferred onto a nitrocellulose membrane (Bio-Rad), blocked in skimmed milk for 1 h at room temperature, incubated overnight at 4 °C with antibodies specific to p-ERK 1/2 (Cell Signaling Technology), ERK 1/2 (Cell Signaling Technology), and DUSP1/MKP1 (Cell Signaling Technology). β-Actin (Cell Signaling Technology) was used as loading controls. The blots were developed using the Clarity Western ECL Substrate (Bio-Rad) in the ChemiDoc Touch Gel Imaging System (Bio-Rad). Image Lab software (Bio-Rad) was used to detect and quantify the protein bands.

2.5. ELISA assay of IL-1β

IL-1β cytokine concentrations were determined in cell supernatant using commercially available human ELISA kit (Human IL-1β/IL-1F2, DY201, R&D). Assays were preformed following the manufacturer's instructions. All samples were measured in duplicates.

2.6. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted using Trizol (Invitrogen) according to manufacturer instructions. For cDNA amplification 5x Hot FirePol EvaGreen qRT-PCR SuperMix (Solis Biodyne) was used and RT-qPCR was performed in QuantStudio 3 Real-Time PCR System (Applied Biosystems). The following primers were used: human DUSP1, forward, 5′-3': GCCGCGCAAGTCTTCTTCCTC, and reverse, 5′-3': CAGGGACGCTAGTACTCAGGGG; human TSC22D3, forward, 5′-3': GAGCCAGCGTGGTGGCCATAG, and reverse, 5′-3': CCTTCAGGATCTCCACCTCCTC; human 18s, forward, 5′-3': TGACTCAACACGGGAAACC, and reverse, 5′-3': TCGCTCCACCAACTAAGAAC.

2.7. Data analyses

Microarray data (CEL files) were pre-processed with Robust Multi-Array Average (RMA) technique (Hughey and Butte, 2015). The raw microarray data was normalized, and log transformed. Log-transformed normalized intensities were used in Linear Models for MicroarrayArray data (*LIMMA)* to identify differentially expressed genes between cases and controls (Dudoit et al., 2002; Smyth Gordon, 2004). For RNA-seq study, the data was pre-processed using the *limma-voom* function which takes gene-level counts as its input, filter and normalize the data, and by linear modeling assess differential expression and gene set testing (Ritchie et al., 2015). The Benjamini-Hochberg correction was used for multiple testing. Expressed genes in the MAPK pathway (Fig. 2A, Figs. 2B, & Fig. 3B) were ranked by signal-to-noise ratio (|S2N|) using Gene Set Enrichment Analysis (Subramanian et al., 2005). Gene expression was analyzed using the Comparative Ct (ΔΔCt) method after normalization to the housekeeping gene 18s rRNA. All analyses were performed using R version 3.6.1. software (The R Foundation; http://www. r-project.org/) and Prism (v8; GraphPad Software). p-values <0.05 were considered significant.

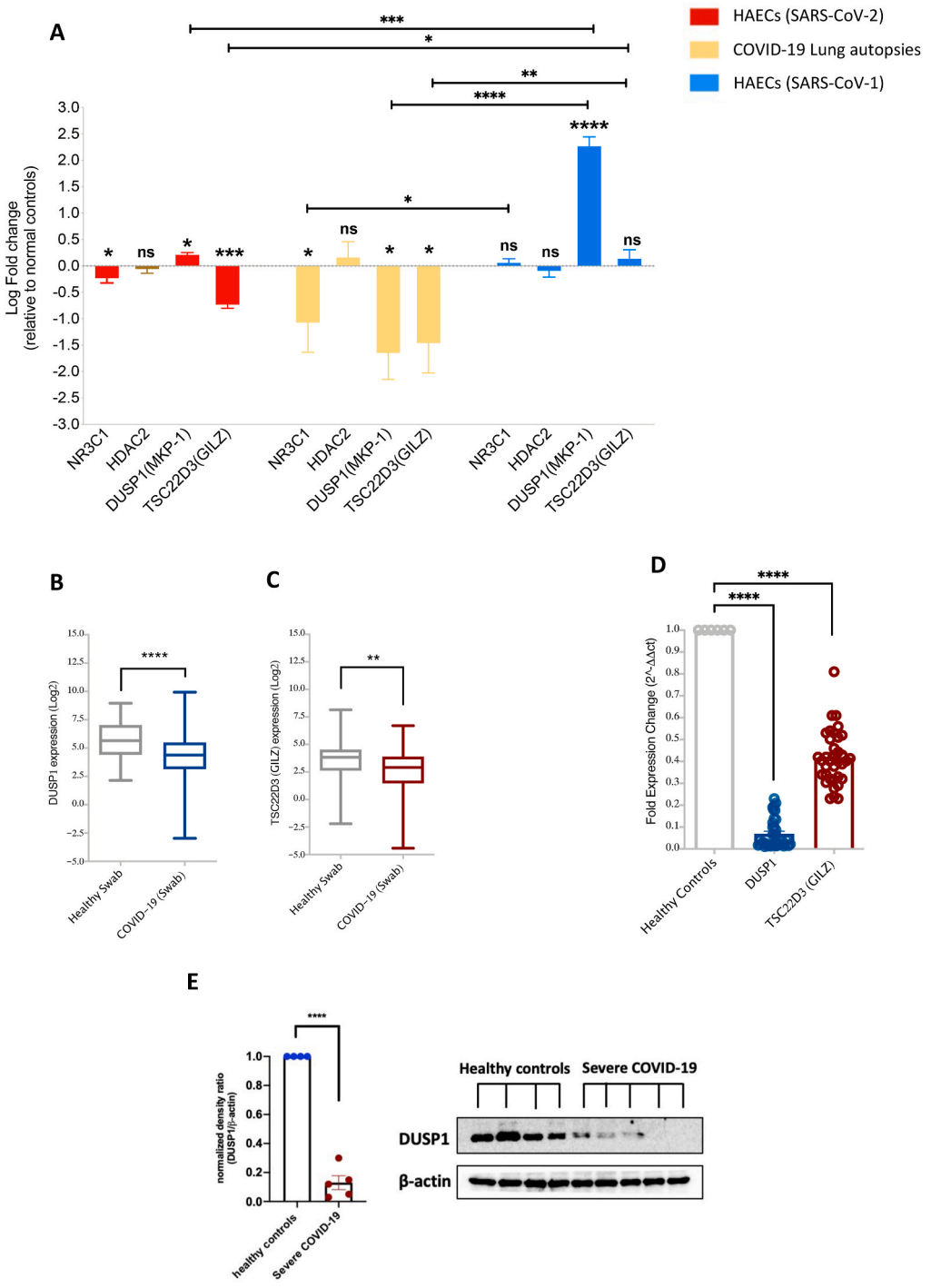

Fig. 1. SARS-CoV-2 attenuated corticosteroid sensitivity by suppressing DUSP1 Expression. (A) Log fold change (LogFC) expression of glucocorticoid-dependent genes in SARS-CoV-2 (n = 3 infected human airway epithelial cells [HAECs] vs. n = 3 mock-treated HAECs; GSE147507) or SARS-CoV-1 (n = 11 infected HAECs vs. n = 9 mock-treated HAECs; GSE47960, GSE47961, and GSE47962) infected HAECs as well as lung autopsies of COVID-19 patients (n = 17 SARS-CoV-2 infected lung vs. n = 5 healthy lung biopsies; GSE150316). The data presented shows significantly lower expression levels of DUSP1 and TSC22D3 (GILZ) in SARS-CoV-2 compared to SARS-CoV-1 infections (2 LogFC decrease with DUSP1, P = 0.0003; and 0.88 LogFC decrease with TSC22D3, P = 0.032, with SARS-CoV-2 vs SARS-CoV-1 infection, respectively). **(B and C)** Gene expression levels of DUSP1 and TSC22D3 (GILZ) in COVID-19 (n = 430) and in healthy (n = 54) nasopharyngeal swabs (GSE152075). The data shows expression levels of these genes are significantly lower in nasopharyngeal swabs from COVID-19 patients as compared to healthy controls. **(D)** Confirmation of gene expression levels of DUSP1 and TSC22D3 (GILZ), as measured by RT-qPCR, in severe COVID-19 nasopharyngeal swabs (n = 36) and in healthy nasopharyngeal swabs (n = 6). The data shows expression levels of these genes are lower in COVID-19 nasopharyngeal swabs compared to healthy nasopharyngeal swabs. **(E)** Protein expression of DUSP1 in nasopharyngeal swabs from severe COVID-19 patients (n = 5) and in healthy nasopharyngeal swabs (n = 4) that were randomly chosen from the 36 patient cohorts. The data presented shows reduced protein expression of DUSP1 in COVID-19 nasopharyngeal swabs compared to healthy nasopharyngeal swabs. Statistical test: LogFC was determined using adjusted *LIMMA* and two-way comparison was done using unpaired *t*-test or Mann-Whitney *U* test, depending on the skewness of the data. ns = non-significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

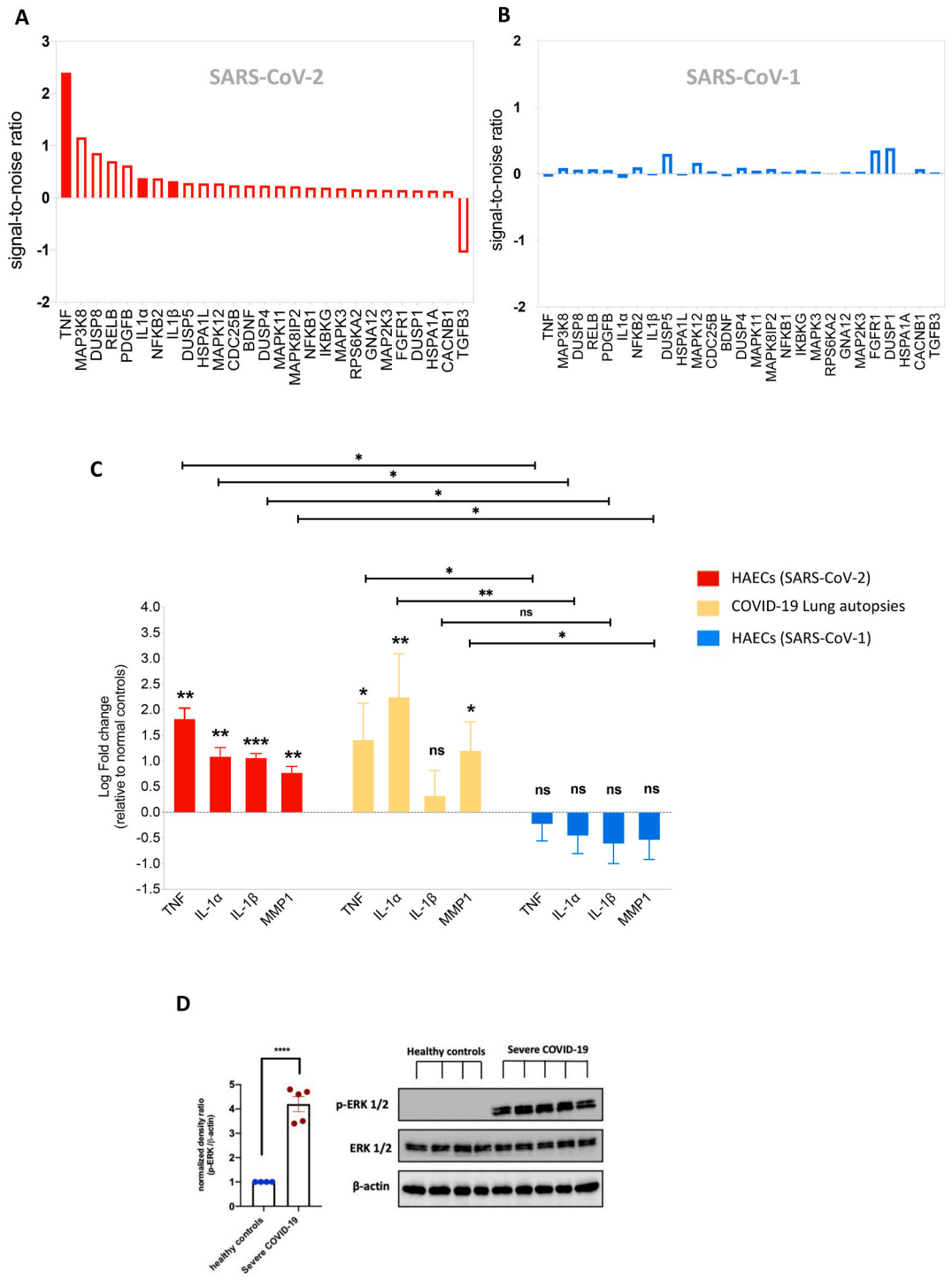

Fig. 2. Increased MAPK signaling and proinflammatory genes production following SARS-CoV-2 infection. **(A)** Enrichment of MAPK pathway in SARS-CoV-2 infected HAECs. (Enrichment Score (ES): 0.437, Normalized enrichment Score (NES): 1.57). **(B)** Enrichment of MAPK pathway in SARS-CoV-1 infected HAECs. **(C)** LogFC expression of proinflammatory genes in SARS-CoV-2 (n = 3 infected HAECs vs. n = 3 mock-treated HAECs; GSE147507) or SARS-CoV-1 (n = 11 infected HAECs vs. n = 9 mock-treated HAECs; GSE47960, GSE47961, and GSE47962) infected HAECs as well as lung autopsies of COVID-19 patients (n = 17 SARS-CoV-2 infected lung vs. n = 5 healthy lung biopsies; GSE150316). The data presented shows higher level of proinflammatory genes expression in SARS-CoV-2. **(E)** Protein expression of p-ERK 1/2 in nasopharyngeal swabs from severe COVID-19 patients (n = 5) and in healthy nasopharyngeal swabs (n = 4) that were randomly chosen from the 36 patient cohorts (Fig. 1D). The data presented shows increased expression of p-ERK 1/2 in COVID-19 nasopharyngeal swabs as compared to healthy nasopharyngeal swabs; indicative of increased MAPK signaling. Statistical test: LogFC was determined using adjusted *LIMMA* and two-way comparison was done using unpaired *t*-test or Mann-Whitney *U* test, depending on the skewness of the data. ns = non-significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

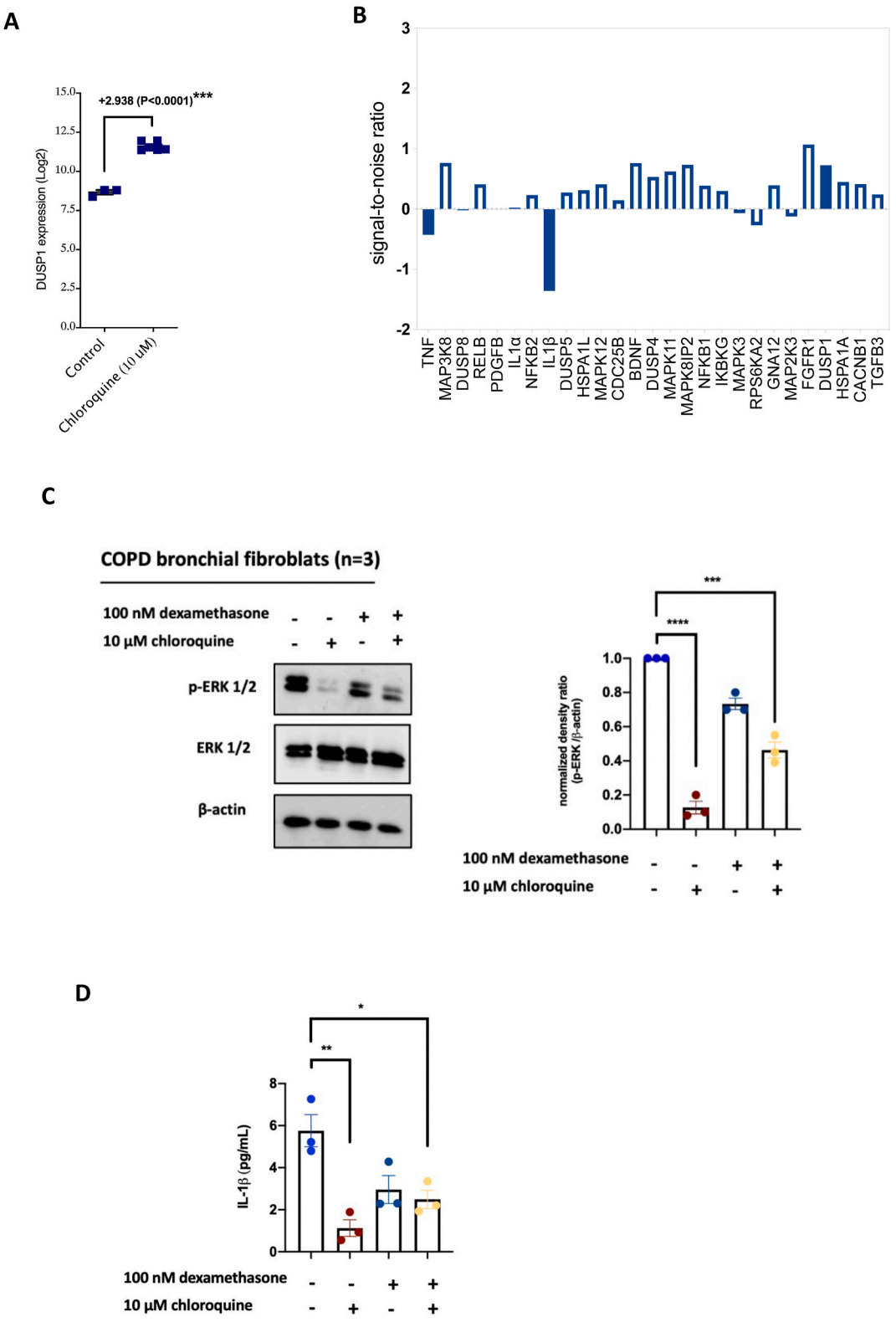

Fig. 3. Chloroquine attenuated MAPK signaling modulating steroid resistance. (A) The level of DUSP1 expression in chloroquine treated hepatocytes dataset (n = 6 treated hepatocytes vs. n = 3 untreated hepatocytes; GSE30351). The data presented shows elevated level of DUSP1 expression in chloroquine treated-compared to untreated hepatocytes. **(B)** Enrichment of MAPK pathway in chloroquine-treated hepatocytes. **(C)** Expression level of p-ERK 1/2 in chloroquine or/and dexamethasone treated bronchial fibroblasts of COPD. The data presented shows reduced level of p-ERK 1/2 expression in chloroquine treated as well as in chloroquine and dexamethasone treated compared to untreated bronchial fibroblasts of COPD; indicative of less MAPK activation. **(D)** Expression level of IL-1β in chloroquine or/and dexamethasone treated bronchial fibroblasts of COPD. The data presented shows reduced level of this cytokine in chloroquine treated as well as in chloroquine and dexamethasone treated fibroblasts compared to untreated fibroblasts. Two-way comparison was done using unpaired *t*-test or Mann-Whitney *U* test, depending on the skewness of the data. *P < 0.05, **P < 0.01.

3. Results

3.1. SARS-CoV-2 infection is associated with reduced lung DUSP1 expression

To understand the mechanism regulating response to steroid treatment following SARS-CoV-2 infections, we first assessed the level of expression of glucocorticoid-dependent receptor gene, NR3C1, in infected samples; since its upregulation is required for glucocorticoids repression of inflammatory genes (Miranda et al., 2013). Analysis of different gene expression data sets of *in vitro* model for SARS-CoV-2 and SARS-CoV-1 revealed that SARS-CoV-2, but not SARS-CoV-1 decreased the expression of NR3C1 in HAECs, at 24-h post infection, to level similar to those of COPD (Fig. 1A & Suppl. Fig. 1). The reduction in NR3C1 was even more pronounced in lung autopsies of COVID-19 patients (Fig. 1A). Decreased HDAC2 expression is one of the main mechanisms driving corticosteroid insensitivity (Barnes, 2013). However, the level of HDAC2 expression following SARS-CoV-2 infection was comparable to those of SARS-CoV-1 infection (Fig. 1A) and COPD (Suppl. Fig. 1).

Interestingly, the expression levels of anti-inflammatory effector genes, DUSP1 and TSC22D3 were significantly lower following infection of HAECs with SARS-CoV-2 compared to infection with SARS-CoV-1 (at least 2 log-fold decrease with DUSP1, $P = 0.0003$; and around a log-fold decrease with TSC22D3, $P = 0.032$, with SARS-CoV-2 vs SARS-CoV-1 infection, respectively), as well as in lung autopsies of COVID-19 patients (Fig. 1A). Reduced expression of DUSP1 and TSC22D3 during SARS-CoV-2 infection were apparent in gene expression data set of nasopharyngeal swabs obtained from COVID-19 patients (Fig. 1B for DUSP1 and Fig. 1C for TSC22D3). We have also noticed decreased expression of DUSP1, but not TSC22D3, to be associated with a higher viral load in nasopharyngeal swabs and lung autopsies of COVID-19 patients (Suppl. Fig. 2, for nasopharyngeal swabs; and Suppl. Fig. 3, for lung autopsies). Additionally, to confirm reduced expression of these genes during SARS-CoV-2 infection, we have evaluated the gene or/and protein expression levels of DUSP1 and TSC22D3 in nasopharyngeal swabs of our recruited patients with severe COVID-19 as well as in primary bronchial fibroblasts of COPD. Of note, the gene expression levels of DUSP1 and TSC22D3 were significantly reduced in nasopharyngeal swabs of severe COVID-19 patients compared to healthy controls (Fig. 1D) as well as in bronchial fibroblasts of COPD compared to healthy controls (Suppl. Fig. 4). A significant reduction in protein expression of DUSP1 was also noticed in the nasopharyngeal swabs of severe COVID-19 patients compared to healthy controls (Fig. 1E).

3.2. Reduced lung DUSP1 expression "switch on" proinflammatory p38 MAPK signaling

DUSP1, also known as MAPK phosphatase-1 (MKP-1), exert its anti-inflammatory effects through dephosphorylation of p38 MAPKs, and hence suppression of the pathway (Caunt and Keyse, 2013). In this regard, we have noticed that compared to SARS-CoV-1, infection with SARS-CoV-2 resulted in higher MAPK pathway activation, as apparent by the upregulation of the most common genes regulating this pathways (Fig. 2A for SARS-CoV-2 and Fig. 2B for SARS-CoV-1). In addition, significant increase in the levels of expression of MAPK-regulated pro-inflammatory genes such as TNF, IL-1α, and IL-1β was observed in both SARS-CoV-2 infected HAECs and lung autopsies of severe COVID-19 patients compared to those of SARS-CoV-1 infected HAECs (Fig. 2C). This suggest that MAPK pathway is activated to a higher level following SARS-CoV-2 compared to SARS-CoV-1 infection. Moreover, MMP1, a known marker of activator protein (AP)-1 activation (Chi et al., 2006), was significantly higher in SASR-CoV-2 infection further reflecting the upregulation of MAPK pathway during this infection (Fig. 2C). Additionally, increased p-ERK1/2 protein level in the nasopharyngeal swabs of our recruited COVID-19 patients indicate enhanced MAPK signaling during SARS-CoV-2 infection (Fig. 2D).

3.3. Chloroquine "switch off" proinflammatory MAPK signaling and attenuate steroid resistance

MAPK signaling has been shown to mediate glucocorticoid resistance in different clinical settings (Jones et al., 2015; Li et al., 2015). The upregulation of MAPK pathway during SARS-CoV-2 infection could mediate the steroid resistance observed in many patients with severe COVID-19 disease. Therefore, immunosuppressive mediators which regulate this pathway may be specifically effective in controlling inflammation during COVID-19. Early during COVID-19 pandemic, chloroquine has been widely used to treat severe COVID-19 patients due to its observed immunosuppressive effect. However, the mechanism behind this effect is not fully understood. Interestingly, analysis of a gene expression data set of chloroquine treated virus infected hepatocytes showed that this off-labeled COVID-19 drug (Wang et al., 2020), significantly upregulated the level of DUSP1 (Fig. 3A and B) in infected hepatocytes and reduced the expression of MAPK-related proinflammatory genes such as TNF, IL-1α, and IL-1β (Fig. 3B). Of note, Hepatocytes are known to express DUSP1 at a level comparable to that in the lung (Suppl. Fig. 5).

Next, to determine whether chloroquine mediated suppression of MAPK pathway enhance steroid responsiveness in lung, we treated primary bronchial fibroblasts isolated from COPD patients with chloroquine and/or dexamethasone. COPD is a prototype of poor response to steroid therapy, and increased activation of MAPK signaling has been shown in lung tissues of COPD patients (Renda et al., 2008). Our results showed that treating COPD bronchial fibroblasts with chloroquine, but not dexamethasone, significantly reduced p-ERK1/2 level (Fig. 3C), as well as IL-1β supernatant levels (Fig. 3D), indicative of less MAPK activation. Co-treatment of chloroquine with dexamethasone enhanced the suppressive effect of steroids on MAPK pathway (Fig. 3C and D).

4. Discussion

In this study, we have shown that SARS-CoV-2 infection is associated with a greater MAPK pathway activation compared to previous SARS-CoV-1 infection. This was apparent as the pathway inhibitor, DUSP1, was downregulated in lung autopsies of COVID-19 patients and nasopharyngeal swabs of our recruited severe COVID-19 patients. This could be the mechanism behind the observed enhanced activation of MAPK pathway in SARS-CoV-2 infection and the resulted steroid resistance. Chloroquine, an off-labeled drug for COVID-19 attenuated MAPK pathway modulating steroids effect.

The cytokine storm of increased TNF, IL-6, IL-1β, and another wide range of cytokines has been confirmed to characterize severe SARS-CoV-2 infections (Huang et al., 2020). The expression of these inflammatory cytokines is regulated in part by MAPK pathways (Chi et al., 2006). MAPK signaling occurs through phosphorylation of p38 MAPKs and activation of AP-1 (Chi et al., 2006). In this study, MMP1 gene expression level, an indicator of AP1 activation, is increased in HAEs infected with SARS-CoV-2 as well as in lung autopsies of COVID-19 patients (Fig. 2C). MMP1 was shown to be a predictor of lower airway obstruction and resistance to steroid-based treatment in patients with asthma (Huang et al., 2019; Lambrecht and Hammad, 2015). Therefore, the observed elevation of MMP1 gene expression in SARS-CoV-2 infected lung tissue and their association with immune pathway might be suggestive of steroid resistance in these patients.

Activated MAPK pathway is known to phosphorylate glucocorticoid receptors (GRs) *in vitro*, resulting in reduction of corticosteroid binding affinity and function (Irusen et al., 2002). This reduction in turn impairs the transcriptional activity of glucocorticoid-dependent genes regulated by corticosteroids, including DUSP1 (Barnes, 2006). Here, we have shown DUSP1 gene expression level in the nasopharyngeal swabs and lungs of COVID-19 decrease according to SARS-CoV-2 viral loads,

possibly reflecting the degree of COVID-19 severity as well as defect in the GRs within these patients. The level of DUSP1 might then be useful in distinguishing COVID-19 patients who may respond, or not, to steroid treatment. However, this should be addressed and confirmed in larger cohort studies.

Due to our limited access to an *in vitro* model that mimics SARS-CoV-2 infection in the lungs, we have discussed the context of COVID-19 steroid resistance through COPD lung cells. Similar to the lung tissues of COVID-19 patients, MAPK was also shown to be highly activated in the alveolar cells of COPD (Renda et al., 2008). This however appears to be involved in the pathogenesis of COPD mainly by activation of AP-1, increasing expression of proinflammatory genes such as IL-1β (Rahman and Adcock, 2006), and mediating steroid resistance (Irusen et al., 2002). Moreover, MAPK inhibitors were shown to improve corticosteroid sensitivity in severe asthma patients through induction of DUSP1 (Hoppstädter and Ammit, 2019). Here, we have shown that in both COVID-19 and COPD fibroblasts, MAPK signaling is activated resulting in production of IL-1β (Fig. 3C and D).

We have shown that immunosuppressive agent such as chloroquine, an off-labeled drug of COVID-19, may act as a MAPK inhibitor reducing IL-1β production and modulating steroid response (Jang et al., 2006). Our analysis of a gene expression data set of chloroquine treated virus infected cells confirmed that it significantly upregulated the level of DUSP1 and reduced the expression of IL-1β in these cells (Fig. 3A and B). Additionally, we were able to confirm the role of chloroquine as MAPK inhibitor in modulating steroids effect. Chloroquine co-treatment with dexamethasone enhanced the suppressive effect of steroids on MAPK pathway in the COPD lung fibroblasts (Fig. 3C). Therefore, upregulation of DUSP1, or the therapeutic inhibition of MAPK could hence constitute an effective approach for attenuating steroid resistance during COVID-19.

In general, the direct effect of viral infections on response to glucocorticoids is not well studied. Few exception include the reported decrease in NR3C1 transactivation following infection with respiratory syncytial virus (Hinzey et al., 2011), human rhinovirus (Rider et al., 2011), as well as treatment with the poly(I:C) double-stranded RNA (Rider et al., 2013). Moreover, since there are conflicting results for the use of glucocorticoid during the respiratory coronaviruses infections, here we were able to show that reduction of NR3C1 is specific to SARS-CoV-2 infection but not SARS-CoV-1 which could indicate greater level of glucocorticoid resistance following SARS-CoV-2 infection compared to SARS-CoV-1. Although, SARS-CoV-2 in silico modeling shows a structural sequence similarity of 74–79% with SARS-CoV-1; however, reports have shown SARS-CoV-2 to exhibit some differences (Baig et al., 2020). In support of our findings, SARS-CoV-2 has been shown to regulate transcription factors, such as AP-1 or NR3C1 (Quatrini and Ugolini, 2021; Zhu et al., 2021). Moreover, lower level of NR3C1 expression has been reported in neutrophils and macrophages of severe compared to the mild COVID-19 patients (Awasthi et al., 2021; Park and Lee, 2020).

Furthermore, reduction of anti-inflammatory effector genes, DUSP1 and TSC22D3 were more pronounced in HAECs with SARS-CoV-2 as compared to SARS-CoV-1 (2 log-fold decrease with DUSP1, $P = 0.0003$, and a log-fold decrease with TSC22D3, $P = 0.032$, with SARS-CoV-2 vs SARS-CoV-1 infection, respectively: Fig. 1A). Reduced DUSP1 and TSC22D3 gene expression levels were also clearly observed both in the nasopharyngeal swabs obtained from a large cohort of patients with COVID-19 ($n = 430$) and in our recruited patients ($n = 36$) as well as in the lung autopsies of COVID-19 patients. Although infection time-points and different treatment modalities might play a role in the level of these genes, lower expression of DUSP1 and TSC22D3 and consequently, higher expression of proinflammatory genes were still apparent in the lung autopsies of COVID-19 patients compared to those of healthy controls (Figs. 1A and 2C). This overall hints at a higher MAPK signaling during SARS-CoV-2 infection compared to SARS-CoV-1 as both DUSP1 and TSC22D3 attenuate the pathway through different mechanisms (Caunt and Keyse, 2013; Cheng et al., 2013). Lower DUSP1 and higher p-ERK1/2 protein levels in nasopharyngeal swabs of our recruited COVID-19 patients compared to healthy controls further support this notion (Fig. 1E). Therefore, induction of DUSP1 and TSC22D3 expression in SARS-CoV-2 infected lung tissues may represent a novel therapeutic modality with the potential to inhibit inflammatory cytokine recruitment.

In summary, we have observed that the expression level of DUSP1 and TSC22D3 is significantly reduced in both SARS-CoV-2 infected HAECs as well as in lung tissues of SARS-CoV-2 infected patients; which is expected to affect their response to corticosteroid treatment. Both gene expression and protein analysis confirmed that the level of DUSP1 was lower in nasopharyngeal swabs of severe COVID-19 patients compared to healthy controls. This SARS-CoV-2 induced suppression of DUSP1 could constitute the mechanism behind the observed enhanced activation of MAPK pathway, as well as steroid resistance reported during SARS-CoV-2 infection.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Fatemeh Saheb Sharif-Askari: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Narjes Saheb Sharif-Askari:** Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Swati Goel:** Data curation, Writing – original draft, Writing – review & editing. **Shirin Hafezi:** Data curation, Writing – original draft, Writing – review & editing. **Rasha Assiri:** Writing – review & editing. **Saleh Al-Muhsen:** Writing – review & editing. **Qutayba Hamid:** Funding acquisition, Project administration, Resources, Software, Writing – review & editing. **Rabih Halwani:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Appendix A. Supplementary data

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