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Acetylated K676 TGFBIp as a severity diagnostic blood biomarker for SARS-CoV-2 pneumonia

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The outbreak of highly contagious and deadly SARS-CoV-2, also known as Coronavirus Disease 2019 (COVID-19) has posed a serious threat to public health across the globe, calling for the development of effective diagnostic markers and therapeutics. Here, we report a highly reliable severity diagnostic biomarker, acetylated 676th lysine transforming growth factor beta-induced protein (TGFBIp K676Ac). The TGFBIp K676Ac was consistently elevated in the blood of SARS-CoV-2 pneumonia patients (n=113); especially in patients of intensive care unit (ICU) than non-ICU. Patients' blood samples showed increased cytokines and lymphopenia, which are exemplary evidences of SARS-CoV-2 pneumonia. Treatment of TGFBIp neutralizing antibodies suppressed the cytokine storm. The increased level of TGFBIp K676 Ac in ICU patients implies that it can be used as a reliable severity diagnostic biomarker for SARS-CoV-2.

Introduction

In January 2020, SARS-CoV-2 was identified as the cause of the COVID-19 outbreak and spread around the globe, and was recently declared a pandemic by the World Health Organization (WHO) (1). This novel coronavirus is highly contagious and pathogenic, causing severe pneumonia symptoms in infected patients. Evidences show that the virus is a serious threat to people especially for elderly and/or with underlying diseases, such as cardiovascular disease (2), diabetes (3), chronic respiratory disease (4) and cancer (5). The SARS-CoV-2, although not as lethal than the other previously identified CoVs, has proved much more pervasive with a long incubation period of 14 days (6–8). SARS-CoV-2 is known to induce a life-threatening condition sepsis (9), which accompanies the degradation of the blood vessel (10). Because the leaky blood vessel causes subsequent damage of other organs, the diagnosis of SARS-CoV-2 infection before the severe organ damage is crucial.

SARS-CoV-2 has five major protein regions for virus structure assembly and viral replications (11), including replicase complex (ORF1ab), spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (12, 13). However, current diagnostic accuracy remains a problem (14), where occasional false-negative outcomes have been shown and due to lack of severity indication of the disease in patients.

In the current study, we sought to determine a biomarker

that can accurately discern the severity of the disease and be a potential therapeutic target for severely symptomatic patients. We analyzed transforming growth factor beta-induced protein (TGFBIp) and acetylated 676th lysine TGFBIp (TGFBIp K676Ac) levels in the blood of SARS-CoV-2 patients to diagnose the severity of SARS-CoV-2 infection. We showed that the TGFBIp K676Ac was consistently elevated in the blood of SARS-CoV-2 pneumonia patients (n=113) and the level was higher in patients of intensive care unit (ICU) than that of non-ICU cases. Currently, there is urgency to find and use a reliable biomarker that can accurately discern the severity of the disease, especially for those at imminent risk requiring urgent diagnostic care. Monitoring of TGFBIp K676Ac is a novel strategy in the diagnosis and treatment of patients with SARS-CoV-2 infection and may ultimately contribute to the development of therapeutic agents.

Results

TGFBIp and TGFBIp K676Ac levels are associated with SARS-CoV-2 infection and severity

From 113 blood samples of SARS-CoV-2 patients (85 of non-ICU and 10 of ICU cases, patient's information in the Table S1), the levels of TGFBIp were measured using the enzyme-linked immunosorbent assay (ELISA). The median serum TGFBIp value was 122.72 (77.2–195.65 ng/ml) in 20 healthy volunteers. The level of TGFBIp was increased to 428.65

(109.64-726.14 ng/ml) in the non-ICU case, and further increased to 757.69 (622.56-977.1 ng/ml) in ICU case (Fig. 1A). The recovered cases (designated as discharged) showed a reduced level of TGFBIp of 239.48 (128.34-393.09 ng/ml). When analyzed the acetylated 676th lysine (K676Ac) of TGFBIp, TGFBIp K676Ac, the difference was much clearer, 11.81 (5.22-23.02 ng/ml) for normal, 212.1 (22.06-459.89 ng/ml) for non-ICU, 724.63 (605.47-952.14 ng/ml) for ICU, and 66.62 (49.99-107.92 ng/ml) for discharged patients (Fig. 1B). The acetylation ratio, defined as the ratio of K676 TGFBIp Ac and total TGFBIp, was significantly higher in the SARS-CoV-2 ICU case than other cases and even two-fold higher than that of the non-ICU case (fig. S1A, fig. S1B). Using the molecular weight of TGFBIp, the concentration measurements of TGFBIp and TGFBIp K676Ac in patient blood (fig S1A) support that in Fig. 1A and Fig. 1B, as well as comparison with the control group from the immunoprecipitation and immunoblotting experiments (fig. S1B) are accurate. It is noted that the highest K676 TGFBIp level was observed in 10 deceased patients (fig. S2). Importantly, the TGFBIp K676Ac clearly discriminated at cut-off values which separate the degree of severity and recovery of symptoms; ~ 532.68 ng/ml for the determination of severity between ICU and non-ICU cases, and ~ 212.10 ng/ml for the determination of recovery. These results indicated that not only the TGFBIp Ac can serve as a biomarker for identifying the SARS-CoV-2-induced disease but also can diagnose the severity of diseases. For the diagnosis of pneumonia, lactate dehydrogenase (LDH), C-reactive protein (CRP), and procalcitonin (PCT) have been verified (15). LDH and CRP showed a sharp increase in the ICU cases of the blood samples of SARS-CoV-2 pneumonia patients, with no significant difference in PCT (Fig. 1, D to F). Although LDH and CRP showed statistically different values between non-ICU and ICU cases, the ranges of distribution in each case were overlapped, and thus determination of cut-off value for diagnosis was unavailable. The results indicate that the conventional biomarkers of pneumonia cannot be used for the determination of severity of SARS-Cov-2 pneumonia.

We analyzed the expression of pneumonia markers including TGFBIp K676Ac, TGFBIp, and LDH in plasma of nine SARS-CoV-2 ICU patients using western blot analysis. As shown in figure S1A, the pneumonia markers were detected in all patient plasmas. We also confirmed the detection of the pneumonia markers with immunoprecipitation (IP) technique (fig. S1B). TGFBIp K676Ac and TGFBIp were up-regulated in SARS-CoV-2 cases and such high level was rescued in the discharged cases. Considering the experimental results of ELISA, western blot and IP, the acetylated form of TGFBIp K676Ac was consistently expressed in SARS-CoV-2 patients, and thus proven to be used as a severity diagnostic biomarker for SARS-CoV-2 pneumonia.

Up-regulation of plasma inflammatory cytokine levels in SARS-CoV-2 patients

Acute respiratory distress syndrome (ARDS), sepsis and septic shock are the main death causes of SARS-Cov-2 (16, 17). Among them, ARDS and sepsis are the common immunopathological phenomena for SARS-CoV-2 infection. One of the main mechanisms for ARDS and sepsis in SARS-CoV-2 infection is the cytokine storm, a deadly uncontrollable systemic inflammatory response resulting from the release of large amounts of pro-inflammatory cytokines and chemokines by immune effector cells (16-18). According to the cytokine array analysis, the SARS-CoV-2 patients showed changes in the level of cytokines (Fig. 2A). The up-regulated cytokines include IL-6, IL-8, IL-1 β , IFN- γ , IL-4, TNF- α , CCL2, IL-10 and etc. The down-regulated cytokines include ENA-78, EGF, aggrecan, IL-33, CCL-20 and etc. Especially, the level of IL-1 β , IL-4, IL-6, IL-8, and IFN- γ was significantly increased in ten plasma samples obtained from SARS-CoV-2 ICU patients (Fig. 2, B to G). These increased cytokine level was consistent in all patient samples. Furthermore, the high level of cytokines observed in patient blood support the sudden rise in the systemic inflammatory response, 'cytokine storm' after the SARS-CoV-2 infection.

Lymphopenia symptoms in SARS-CoV-2 patients

It has been reported that the SARS-CoV-2 pneumonia patients lose their immune activity and thus aggravate their symptoms as a result of negative feedback. The number of each immune cell was counted to explain the loss of immune activity in ICU patients. In the ICU case of SARS-CoV-2 patients, the number of lymphocytes was decreased, indicating the lymphopenia symptom (Fig. 3D). However, the number of white blood cell, neutrophil, monocyte, and platelet did not show significant difference in non-ICU and ICU cases.

TGFBIp neutralizing antibody suppresses SARS-CoV-2-mediated cytokine storm

The peripheral blood mononuclear cells (PBMCs) are known to actively regulate the immune response upon invasion of pathogens, such as viruses by secreting various anti-inflammatory cytokines. Considering that cytokine storm is also observed in SARS-CoV-2 patients without underlying diseases, it is hypothesized that PBMCs are affected by the SARS-CoV-2 virus. In this regard, the expression of TGFBIp K676Ac as well as TGFBIp from the PBMCs of SARS-CoV-2 ICU patients was determined using immunofluorescence (Fig. 4A). The fluorescence for TGFBIp K676Ac was rescued in the PBMCs of the discharged patients. Furthermore, the acetylation of TGFBIp-related mRNAs was quantified (fig. S3). Although the mRNA level of CBP/p300 which acetylate the TGFBIp does not show significant difference, the mRNA levels of TGFBIp and IL-6 were significantly up-regulated in the

SARS-CoV-2 ICU patients.

Time-dependent reduction in cell viability of PBMCs isolated from SARS-CoV-2 ICU patients were observed, unlike the healthy group. However, the cell viability was significantly increased when PBMCs isolated from SARS-CoV-2 ICU patients were co-cultured with the TGFBIp neutralizing antibody (Fig. 4B).

To demonstrate the effects of neutralizing antibody on nuclear factor κ B (NF- κ B) activation and cytokine secretion, we treated TGFBIp monoclonal antibody (50 μ g/ml, 6 hours) to the PBMCs of SARS-CoV-2 patients. Data showed that the neutralizing TGFBIp antibody significantly reduced activation of NF- κ B (Fig. 4C) and secretion of cytokines (IL-1 β , IL-4, IL-6, IL-8, IFN- γ , and TNF- α) secretion (Fig. 4, D to H).

Discussion

TGFBIp is a 68 kDa sized protein in the extracellular matrix (ECM), usually found near the blood vessel (19). It contains an RGD domain after 676Lys, a binding motif that modulate cell adhesion and serves as a ligand recognition site for several integrins (20). In our recent study, we found that TGFBIp is acetylated after infection and undergo secretion into the circulation system (21). Previous reports have demonstrated that TGFBIp can induce signaling activation and up-regulation of NF- κ B (21, 22). NF- κ B is a family of inducible transcription factors that plays a vital role in different aspects of immune responses, and it is normally sequestered in the cytoplasm as inactive complexes via physical association with inhibitory proteins (23). In response to stress and/or immune stimuli, NF- κ B gets activated and moves to the nucleus to exert transcriptional functions (24). Although NF- κ B is vital for normal immune responses against infections, nuclear accumulation of activated NF- κ B can lead to increase in endothelial cell apoptosis, vascular permeability and secretion of endotoxins (25).

As inflammatory cytokines cannot be used as an infection biomarker due to very short half-life and are also increased in other diseases (26, 27), therefore, finding a good biomarker that can predict the symptoms from infectious disease is essential. We have observed that TGFBIp K676Ac is continuously secreted by SARS-CoV-2 and thus can be a suitable biomarker for diagnosis of the severity of infection. From the analysis of SARS-CoV-2 patient-derived blood samples, the level of K676 TGFBIp Ac was significantly higher indicating the possibility of increase in NF- κ B activation. We found that increase in the cytokine level was consistent in all patient samples, supporting the rise in the systemic inflammatory response is as a result of activation of the NF- κ B and secretion of endotoxins. In worst cases, subsequent up-regulation of the cytokines may elicit cytokine storm, leading to sepsis-induced organ failures and death. Because NF- κ B occupies a central role in signaling pathways important in sepsis,

modulation of NF- κ B activity via use of antibodies, such as acetylation shadow inhibitors may be an appropriate therapeutic target in SARS-CoV-2 patients.

In the majority of COVID-19 patients, primary cause of death is lung failure due to severe acute respiratory distress syndrome (16, 17). The syndrome is largely attributed to uncontrolled inflammatory responses characterized by cytokine storm, and edema and fibrosis in the lungs at the end stages. The fibrosis in the lung may be caused mainly by transforming growth factor-beta (TGF- β) activation (28). In addition, TGF- β is involved in the fluid homeostasis in the lung as well. This leads to the functional failure of the lungs and death of the patients. Thus, massive increase in active TGF- β in the lungs may be observed (29).

In our previous study, it was observed that under inflammatory conditions, the secreted TGFBIp becomes acetylated by CBP/p300, then the RGD domain of the secreted TGFBIp K676Ac binds with integrin α v β 5 and activates NF- κ B to induce inflammation (21, 30). When the secretion of TGFBIp is elevated by SARS-CoV-2, it was noticed that the p300 mRNA levels are increased by more than 1.5 fold, and we confirmed that TGFBIp K676Ac gets secreted (31, 32). Moreover, CBP/p300 gets activated and up-regulated upon inflammatory responses (33). In addition, the expression of TGFBIp, IL-6 is regulated by NF- κ B activation by infection (21). Here we found that CBP/p300 acetylates K676 of TGFBIp and gets secreted by SARS-CoV-2 infection, suggesting that it may be a diagnostic and therapeutic target for SARS-CoV-2. The data shows that CBP/p300 level in SARS-CoV-2 ICU was increased to a statistically significant level ($P < 0.05$) in response to the SARS-CoV-2 infection (fig. S3).

Previously, it was observed that the expression and extracellular secretion of TGFBIp is controlled by TGF- β and histone methylation (34). TGF- β increases recruitment of the H3K4 methyltransferase MLL1 and of SET7/9 and the binding of Smad3 to the promoters (34). SARS-CoV-2 virus infection and consequent strong immune and inflammatory responses induce massive activation of the TGF- β in the lungs (29). TGF- β can up-regulate TGFBIp expression through Smad transcription factors and E-box-dependent mechanisms (35, 36). Therefore, the possible mechanism in SARS-CoV-2 patients may be that, after the level of TGFBIp is increased by severe infectious responses, the TGFBIp becomes acetylated and accumulates in the granules and then the acetylated TGFBIp is secreted out of the cells (21, 30).

There lies a possibility that TGFBIp K676Ac may be a blood biomarker in common pneumonia and other diseases. Previously, we found that TGFBIp and TGFBIp K676Ac are associated with sepsis (21, 30). In this study, we verified that TGFBIp K676Ac could be used as a biomarker for severe disease caused by SARS-CoV-2 pneumonia. Although it may seem that TGFBIp K676Ac is SARS-CoV-2 specific, we should

not rule out the possibility of TGFBIp K676Ac in association with other pathogens. There are reports of SARS-CoV-2 mutants being found and also due to inaccurate diagnostics there are reports of reoccurrence of the infection as a result of poor antibody formation in the patient blood. Therefore, finding a good biomarker that can predict the severity of the SARS-CoV-2-mediated symptom is essential. Here, we suggest TGFBIp K676Ac protein in blood as an additional factor that can be used to diagnose the severity of SARS-CoV-2 infection. We believe that TGFBIp K676Ac could be used as a suitable biomarker as the level is increased only in SARS-CoV-2 patients and the level remains low in normal and discharged patients. In our cohort study, mild patients include the SARS-CoV-2 positive, but non-symptomatic patients (SARS-CoV-2 non-ICU patients). In the case of young patients, they showed no symptoms other than mild fever. As shown in Fig. 1D to Fig. 1E, some SARS-CoV-2 non-ICU patients displayed normal range of LDH and CRP which are the markers for COVID-19 diagnosis (dots in green part, normal range). An analysis of the association between co-founding factor such as age, sex, gender, comorbidities in SARS-CoV-2 patients and statistically significant increase of TGFBIp K676 secretion was confirmed in elderly patients and those with diabetes (table S1). Thus, the TGFBIp may not be an early diagnostic marker, but it can be a reliable severity diagnostic marker for SARS-CoV-2.

Herein, we report that the acetylated TGFBIp can serve as a more reliable severity diagnostic biomarker for SARS-CoV-2 pneumonia. This is the first evidence that show acetylation site K676 of TGFBIp as a novel biomarker for SARS-CoV-2. We have demonstrated that K676 TGFBIp Ac is the key player in up-regulation of cytokines via NF- κ B pathway. Recent reports have shown frequent cases of false negative diagnosis associated with the SARS-CoV-2, raising concerns over the reliability of commercially available test kits (37). There have been reports that SARS-like coronaviruses have a high rate of mutation (38). For patients requiring urgent diagnostic care, it is essential to use a reliable biomarker that can accurately discriminate the severity of the disease. Our experimental results from the patient blood sample support K676 TGFBIp Ac to be a more suitable diagnostic target for SARS-CoV-2 disease, and K676 TGFBIp Ac neutralizing antibody as potential therapeutic drug for severely symptomatic patients.

Materials and Methods

Experimental design

The purpose of this study was to identify and evaluate the reliability of acetylated 676th lysine (K676Ac) transforming growth factor beta-induced protein (TGFBIp) as a severity diagnostic biomarker for the SARS-CoV-2 pneumonia. A cohort study was designed for patients admitted to Yeungnam University Medical Center. First, the SARS-CoV-2-infected

patients were classified according to their symptoms (mild disease and sepsis). Blood was collected and analyzed from patients that were admitted to the hospital with mild disease and from those in the intensive care unit due to septic symptoms. The progression of sepsis was assessed by monitoring increases in the absolute number of neutrophils, lymphopenia and cytokine levels. In addition, TGFBIp and TGFBIp K676Ac were analyzed from patient blood samples were analyzed as indicators of severity of the disease caused by the viral infection. We treated TGFBIp neutralizing antibodies to the patient's peripheral blood mononuclear cells (PBMCs) to analyze suppression of SARS-CoV-2-mediated cytokine storm. Blood and immunohistochemical analyses were performed in a blinded manner. The study protocol (YUH 2020-03-057) was approved by the Institutional Review Board of Yeungnam University Hospital at Daegu in Korea.

Plasma sample

The patient was admitted to the department of internal medicine, Yeungnam University Medical Center, after SARS-CoV-2 infection was confirmed at a public health center in Daegu. SARS-CoV-2 ICU is a group that has progressed to acute respiratory distress syndrome (ARDS) or sepsis and receives respiratory intensive care in ICU. Healthy volunteers were used as controls and clinical data of all patients were collected. Plasma samples were generated by centrifuge at 2000 $\times$ g for 5 min within 48 hours after whole blood collection. The study protocol (YUH 2020-03-057) was approved by the Institutional Review Board of Yeungnam University Hospital at Daegu in Korea.

ELISA for TGFBIp/TGFBIp K676Ac

Rabbit anti-human TGFBIp K676 acetylation or non-acetylation antibody was produced by Abclon (Seoul, Korea). IP anti-TGFBIp antibody (10188-1-AP) was purchased from Proteintech (Rosemont, IL) and IB anti-TGFBIp antibody (ab89062) was purchased from Abcam (MA, USA). We performed competitive ELISA using antibodies that recognize the acetylated peptide RLAPVYQ(K-Ac)LLERMK or non-acetylated RLAPVYQKLLERMK (amino acid sequence 669-682 of hTGFBIp). TGFBIp peptide (RLAPVYQKLLERMK, 669-682 aa) or TGFBIp K676 Acetylation peptide (RLAPVYQ(K-Ac)LLERMK) were 2 μ g/100 μ l diluted and coated onto Nunc-Immuno MicroWell 96 well plates and incubated overnight at 4°C. Prior to use, the plates were washed 3 times with PBST and blocked with 3% BSA in PBS for 30 min 37°C. Primary antibody (1:1000 dilution) and plasma sample (20 μ g) was pre-incubated for 1 hour at 37°C and then the pre-incubated sample were transferred to peptide-coated plate and incubated for 1 hour at 37°C. The plate was washed 5 times with PBST 5 secondary antibody (1:3000 dilution) was incubated for 30 min at 37°C and then the plate was washed 5 times

with PBST. The washed plate was treated with TMB ELISA substrate 100 μ l/well for 10 min 37°C and then Stop Solution 100 μ l/well was added. The detection was performed at 450 nm by microplate reader (TECAN).

Laboratory tests

PCT was determined with an enzyme-linked fluorescent immunoassay (B.R.A.H.M.S. Diagnostica, Berlin, Germany). The test needs 20 min and 200 μ l of serum. CRP and LDH were determined using a Roche/Hitachi Modular DP Chemistry Analyzer. Blood cell count were performed using Sysmex XE-2100 Automated Hematology System.

MPO ELISA

To quantify the release of granule matrix proteins upon degranulation in peripheral blood mononuclear cells (PBMCs) of SARS-CoV-2-infected patients and mice, plasma were analyzed using a human MPO ELISA kit (BMS2038INST, Invitrogen) and mouse myeloperoxidase ELISA kit (MBS700747, MyBioSource), respectively.

Protein profiling

Plasma pools of patients with normal or SARS-CoV-2 patients were processed as indicated in the Human XL Cytokine Array Kit (R&D Systems). Developed films were scanned, the obtained images were analyzed using ImageJ version 1.43.

ELISA for IL-1 β , IL-4, IL-6, IL-10, IFN- γ , and TNF- α

The concentrations of cytokines in SARS-CoV-2 patients' plasma was quantified according to the manufacturer's instructions using a commercially available ELISA kit. Values were measured using an ELISA plate reader (Tecan, Austria GmbH, Austria). Human IL-1 β Quantikine ELISA Kit (DLB50, R&D Systems, Minneapolis, MN, USA), Human IL-4 Quantikine ELISA Kit (D4050, R&D Systems, Minneapolis, MN, USA), Human IL-6 Quantikine ELISA Kit (D6050, R&D Systems, Minneapolis, MN, USA), Human IL-10 Quantikine ELISA Kit (D1000B, R&D Systems, Minneapolis, MN, USA), Human IFN- γ Quantikine ELISA Kit (DIF50, R&D Systems, Minneapolis, MN, USA), and Human TNF- α Quantikine ELISA Kit (DTA00D, R&D Systems, Minneapolis, MN, USA)

PBMC isolation and culture

Samples from healthy, SARS-CoV-2 pneumonia patients, or discharged patients were obtained from Yeungnam University Medical Center. The relevant local Institutional Review Boards and Ethics Committees approved the study. Heparinized blood samples were used fresh within 4 hours, and peripheral blood mononuclear cells (PBMCs) were separated from blood using Ficoll-Hypaque or Nycoprep according to the manufacturer's recommendations. Following this, more refined PBMCs were obtained via MACSprep PBMC

Isolation Kit and cultured in RPMI-1640 with 1 mM Sodium pyruvate, 2 mM L-glutamine, 4.5 mg/L glucose, 10mM HEPES and 2 mg/L sodium bicarbonate.

Polyclonal rabbit anti-TGFBIp neutralizing antibody is obtained and purified as described previously (39). To verify the effect of TGFBIp neutralizing antibodies on suppressing cytokines secretion, PBMCs isolated from SARS-CoV-2 patients and the TGFBIp neutralizing antibodies (50 μ g/ml) were cultured for 6 hours. The supernatant was used for analysis of cytokines ELISA, and lysate was used for NF- κ B activity analysis.

Immunofluorescence staining

For TGFBIp/TGFBIp K676Ac staining, PBMCs were fixed in 4% formaldehyde in PBS (v/v) for 15 min at room temperature. After fixation, cells were blocked in blocking buffer (5% BSA in PBS) overnight at 4°C. Then, cells were then incubated with primary mouse monoclonal TGFBIp antibody, anti-mouse alexa 488 (green), primary rabbit polyclonal TGFBIp K676AC antibody, and anti-mouse alexa 594 (red). And PBMCs were visualized by fluorescence microscopy at a 200 $\times$ magnification (Leica microsystem, Germany).

WST-1 cell proliferation assay

Following exposure of PBMCs to TGFBIp neutralizing antibody, 10 μ L of WST-1 reagent were added per well and incubated at 37°C with 5% CO₂. At indicated time points, measurements of absorbance were taken at 480 nm and 600 nm (background) on Tecan Spark microplate reader.

NF- κ B activity kit

Nuclear extracts preparation and TransAM assay were performed as previously described³³. The activity of individual NF- κ B subunits was detected by an ELISA-based NF- κ B family transcription factor assay kit (43296; Active Motif, Carlsbad, CA, USA). Briefly, nuclear extracts (2 μ g) were incubated in a 96-well plate, which were immobilized NF- κ B consensus oligonucleotides. The captured complexes were incubated with specific NF- κ B primary Abs and subsequently detected with HRP-conjugated secondary Abs (included with the kit). Finally, the optical density (OD) value at 450 nm was measured by Tecan Spark microplate reader.

Western blotting and immunoprecipitation

TGFBIp, acetylated lysine (K), and acetylated K676 TGFBIp in plasma of healthy volunteers, SARS-CoV-2 patients, and discharged were detected by immunoblotting. After SDS-PAGE, we performed an immunoblotting assay with each antibody. Plasmas were incubated with the anti-TGFBIp antibody at 4°C overnight. Immunoprecipitates were recovered using protein A/G-S magnetic beads, washed four times with IP buffer, and resuspended in the sample buffer of SDS-

PAGE, followed by boiling for 10 min. Bound proteins were then analyzed by immunoblotting using anti-TGFBIP or anti-acetylated K676 TGFBIP antibodies.

Real-time PCR of TGFBIP, CBP/p300, IL-6

To generate cDNA from PBMCs incubation with/without TGFBIP neutralizing antibodies samples, 1 µg of total RNA was reverse transcribed with random hexamers using expand reverse transcription polymerase (Roche). Real-time PCR was performed using the LightCycler FastStart DNA Master SYBR Green I from Roche Diagnostics GmbH according to the manufacturer's protocol. The following LightCycler conditions were used: Initial denaturation at 95°C for 10 min, followed by 45 cycles with denaturation at 95°C for 10 min, annealing at 60°C for 5 min, and elongation at 72°C for 15 min. Quantities of specific mRNA in the sample were measured according to the corresponding gene-specific standard curves.

Statistical analysis

All experiments were performed independently at least three times. Statistically significant differences were determined using unpaired *t* test. Prism software was used for statistical analyses. Data are reported as mean ± SEM with significance set at *P* < 0.05. *p*-values for each experiment is provided in the figure legends

REFERENCES AND NOTES

1. J. T. Wu, K. Leung, M. Bushman, N. Kishore, R. Niehus, P. M. de Salazar, B. J. Cowling, M. Lipsitch, G. M. Leung, Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China. *Nat. Med.* **26**, 506–510 (2020). [doi:10.1038/s41591-020-0822-7](https://doi.org/10.1038/s41591-020-0822-7) [Medline](#)
2. Y.-Y. Zheng, Y.-T. Ma, J.-Y. Zhang, X. Xie, COVID-19 and the cardiovascular system. *Nat. Rev. Cardiol.* **17**, 259–260 (2020). [doi:10.1038/s41569-020-0360-5](https://doi.org/10.1038/s41569-020-0360-5) [Medline](#)
3. L. Fang, G. Karakiulakis, M. Roth, Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir. Med.* **8**, e21 (2020). [doi:10.1016/S2213-2600\(20\)30116-8](https://doi.org/10.1016/S2213-2600(20)30116-8) [Medline](#)
4. H. Shi, X. Han, N. Jiang, Y. Cao, O. Alwalid, J. Gu, Y. Fan, C. Zheng, Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: A descriptive study. *Lancet Infect. Dis.* **20**, 425–434 (2020). [doi:10.1016/S1473-3099\(20\)30086-4](https://doi.org/10.1016/S1473-3099(20)30086-4) [Medline](#)
5. H. Wang, L. Zhang, Risk of COVID-19 for patients with cancer. *Lancet Oncol.* **21**, e181 (2020). [doi:10.1016/S1470-2045\(20\)30149-2](https://doi.org/10.1016/S1470-2045(20)30149-2) [Medline](#)
6. D. Baud, X. Qi, K. Nielsen-Saines, D. Musso, L. Pomar, G. Favre, Real estimates of mortality following COVID-19 infection. *Lancet Infect. Dis.* **20**, 773 (2020). [doi:10.1016/S1473-3099\(20\)30195-X](https://doi.org/10.1016/S1473-3099(20)30195-X) [Medline](#)
7. L. Du, Y. He, Y. Zhou, S. Liu, B.-J. Zheng, S. Jiang, The spike protein of SARS-CoV—A target for vaccine and therapeutic development. *Nat. Rev. Microbiol.* **7**, 226–236 (2009). [doi:10.1038/nrmicro2090](https://doi.org/10.1038/nrmicro2090) [Medline](#)
8. W. Tai, L. He, X. Zhang, J. Pu, D. Voronin, S. Jiang, Y. Zhou, L. Du, Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: Implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cell. Mol. Immunol.* **17**, 613–620 (2020). [doi:10.1038/s41423-020-0400-4](https://doi.org/10.1038/s41423-020-0400-4) [Medline](#)
9. P. Mehta, D. F. McAuley, M. Brown, E. Sanchez, R. S. Tattersall, J. J. Manson; HLH Across Speciality Collaboration, UK, COVID-19: Consider cytokine storm syndromes and immunosuppression. *Lancet* **395**, 1033–1034 (2020). [doi:10.1016/S0140-6736\(20\)30628-0](https://doi.org/10.1016/S0140-6736(20)30628-0) [Medline](#)
10. T. van der Poll, F. L. van de Veerdonk, B. P. Scicluna, M. G. Netea, The immunopathology of sepsis and potential therapeutic targets. *Nat. Rev. Immunol.* **17**, 407–420 (2017). [doi:10.1038/nri.2017.36](https://doi.org/10.1038/nri.2017.36) [Medline](#)
11. D. Forni, R. Cagliani, M. Clerici, M. Sironi, Molecular evolution of human coronavirus genomes. *Trends Microbiol.* **25**, 35–48 (2017). [doi:10.1016/j.tim.2016.09.001](https://doi.org/10.1016/j.tim.2016.09.001) [Medline](#)
12. J. Won, S. Lee, M. Park, T. Y. Kim, M. G. Park, B. Y. Choi, D. Kim, H. Chang, V. N. Kim, C. J. Lee, Development of a Laboratory-safe and Low-cost Detection Protocol for SARS-CoV-2 of the Coronavirus Disease 2019 (COVID-19). *Exp. Neurobiol.* **29**, 107–119 (2020). [doi:10.5607/en20009](https://doi.org/10.5607/en20009) [Medline](#)
13. Y. Zhou, Y. Hou, J. Shen, Y. Huang, W. Martin, F. Cheng, Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. *Cell Discov.* **6**, 14 (2020). [doi:10.1038/s41421-020-0153-3](https://doi.org/10.1038/s41421-020-0153-3) [Medline](#)
14. S. Kim, D.-M. Kim, B. Lee, Insufficient sensitivity of RNA dependent RNA polymerase gene of SARS-CoV-2 viral genome as confirmatory test using Korean COVID-19 cases. *Preprints*, DOI: [10.20944/preprints202002.0424.v](https://doi.org/10.20944/preprints202002.0424.v)
15. S. Lu, T. Li, X. Xi, Q. Chen, X. Liu, B. Zhang, J. Ou, J. Liu, Q. Wang, B. Zhu, X. Liu, C. Bai, J. Qu, H. Lu, Z. Zhang, Y. Song, Prognosis of 18 H7N9 avian influenza patients in Shanghai. *PLOS ONE* **9**, e88728 (2014). [doi:10.1371/journal.pone.0088728](https://doi.org/10.1371/journal.pone.0088728) [Medline](#)
16. C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497–506 (2020). [doi:10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5) [Medline](#)
17. F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, J. Xiang, Y. Wang, B. Song, X. Gu, L. Guan, Y. Wei, H. Li, X. Wu, J. Xu, S. Tu, Y. Zhang, H. Chen, B. Cao, Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet* **395**, 1054–1062 (2020). [doi:10.1016/S0140-6736\(20\)30566-3](https://doi.org/10.1016/S0140-6736(20)30566-3) [Medline](#)
18. R. Channappanavar, S. Perlman, in *Seminars in immunopathology*. (Springer, 2017), vol. 39, pp. 529–539.
19. A. Venkatraman, E. Murugan, S. J. Lin, G. S. L. Peh, L. Rajamani, J. S. Mehta, Effect of osmolytes on in-vitro aggregation properties of peptides derived from TGFBIP. *Sci. Rep.* **10**, 4011 (2020). [doi:10.1038/s41598-020-60944-0](https://doi.org/10.1038/s41598-020-60944-0) [Medline](#)
20. M. P. Ween, M. K. Oehler, C. Ricciardelli, Transforming growth Factor-Beta-Induced Protein (TGFBIP)/βig-H3: A matrix protein with dual functions in ovarian cancer. *Int. J. Mol. Sci.* **13**, 10461–10477 (2012). [doi:10.3390/ijms130810461](https://doi.org/10.3390/ijms130810461) [Medline](#)
21. W. Lee, E. J. Park, O. K. Kwon, H. Kim, Y. Yoo, S. W. Kim, Y. K. Seo, I. S. Kim, D. H. Na, J. S. Bae, Dual peptide-dendrimer conjugate inhibits acetylation of transforming growth factor β-induced protein and improves survival in sepsis. *Biomaterials* **246**, 120000 (2020). [doi:10.1016/j.biomaterials.2020.120000](https://doi.org/10.1016/j.biomaterials.2020.120000) [Medline](#)
22. G. Min, S.-K. Ku, T. Lee, J.-S. Bae, Suppressive effects of zingerone on TGFBIP-mediated septic responses. *Arch. Pharm. Res.* **41**, 276–287 (2018). [doi:10.1007/s12272-017-0919-9](https://doi.org/10.1007/s12272-017-0919-9) [Medline](#)
23. S. K. Ramakrishnan, H. Zhang, X. Ma, I. Jung, A. J. Schwartz, D. Triner, S. N. Devenport, N. K. Das, X. Xue, M. Y. Zeng, Y. Hu, R. M. Mortensen, J. K. Greenon, M. Cascalho, C. E. Wobus, J. A. Colacino, G. Nunez, L. Rui, Y. M. Shah, Intestinal non-canonical NF-κB signaling shapes the local and systemic immune response. *Nat. Commun.* **10**, 660 (2019). [doi:10.1038/s41467-019-08581-8](https://doi.org/10.1038/s41467-019-08581-8) [Medline](#)
24. M. Czerkies, Z. Korwek, W. Prus, M. Kocharczyk, J. Jaruszewicz-Błńska, K. Tudelska, S. Błński, M. Kimmel, A. R. Brasier, T. Lipniacki, Cell fate in antiviral response arises in the crosstalk of IRF, NF-κB and JAK/STAT pathways. *Nat. Commun.* **9**, 493 (2018). [doi:10.1038/s41467-017-02640-8](https://doi.org/10.1038/s41467-017-02640-8) [Medline](#)
25. I. S. Afonina, Z. Zhong, M. Karin, R. Beyaert, Limiting inflammation—the negative regulation of NF-κB and the NLRP3 inflammasome. *Nat. Immunol.* **18**, 861–869 (2017). [doi:10.1038/ni.3772](https://doi.org/10.1038/ni.3772) [Medline](#)
26. N. Aziz, R. Detels, J. J. Quint, Q. Li, D. Gjertson, A. W. Butch, Stability of cytokines, chemokines and soluble activation markers in unprocessed blood stored under different conditions. *Cytokine* **84**, 17–24 (2016). [doi:10.1016/j.cyto.2016.05.010](https://doi.org/10.1016/j.cyto.2016.05.010) [Medline](#)
27. X. Zhou, M. S. Fragala, J. E. McElhaney, G. A. Kuchel, Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. *Curr Opin Clin Nutr Metab Care.* **13**, 541–547 (2010). [doi:10.1097/MCO.0b013e32833cf3bc](https://doi.org/10.1097/MCO.0b013e32833cf3bc) [Medline](#)

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28. A. L. Tatler, G. Jenkins, TGF- β activation and lung fibrosis. *Proc. Am. Thorac. Soc.* **9**, 130–136 (2012). [doi:10.1513/pats.201201-003AW](https://doi.org/10.1513/pats.201201-003AW) [Medline](#)
29. W. Chen, A potential treatment of COVID-19 with TGF- β blockade. *Int. J. Biol. Sci.* **16**, 1954–1955 (2020). [doi:10.7150/ijbs.46891](https://doi.org/10.7150/ijbs.46891) [Medline](#)
30. J.-S. Bae, W. Lee, J.-O. Nam, J.-E. Kim, S.-W. Kim, I.-S. Kim, Transforming growth factor β -induced protein promotes severe vascular inflammatory responses. *Am. J. Respir. Crit. Care Med.* **189**, 779–786 (2014). [doi:10.1164/rccm.201311-2033OC](https://doi.org/10.1164/rccm.201311-2033OC) [Medline](#)
31. A. R. Brasier, The nuclear factor- κ B–interleukin-6 signalling pathway mediating vascular inflammation. *Cardiovasc. Res.* **86**, 211–218 (2010). [doi:10.1093/cvr/cvq076](https://doi.org/10.1093/cvr/cvq076) [Medline](#)
32. Y. S. Maeng, Y. J. Choi, E. K. Kim, TGF β 1 regulates differentiation of EPC (CD133(+)-C-kit(+) Lin(-)) cells to EC through activation of the Notch signaling pathway. *Stem Cells* **33**, 2052–2062 (2015). [doi:10.1002/stem.2003](https://doi.org/10.1002/stem.2003) [Medline](#)
33. Y.-Z. Ge, M.-T. Pu, H. Gowher, H.-P. Wu, J.-P. Ding, A. Jeltsch, G.-L. Xu, Chromatin targeting of de novo DNA methyltransferases by the PWWP domain. *J. Biol. Chem.* **279**, 25447–25454 (2004). [doi:10.1074/jbc.M312296200](https://doi.org/10.1074/jbc.M312296200) [Medline](#)
34. Y.-S. Maeng, G.-H. Lee, S.-I. Choi, K. S. Kim, E. K. Kim, Histone methylation levels correlate with TGF β 1 and extracellular matrix gene expression in normal and granular corneal dystrophy type 2 corneal fibroblasts. *BMC Med. Genomics* **8**, 74 (2015). [doi:10.1186/s12920-015-0151-8](https://doi.org/10.1186/s12920-015-0151-8) [Medline](#)
35. M. Kato, J. Zhang, M. Wang, L. Lanting, H. Yuan, J. J. Rossi, R. Natarajan, MicroRNA-192 in diabetic kidney glomeruli and its function in TGF- β -induced collagen expression via inhibition of E-box repressors. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 3432–3437 (2007). [doi:10.1073/pnas.0611192104](https://doi.org/10.1073/pnas.0611192104) [Medline](#)
36. Y. Shi, J. Massagué, Mechanisms of TGF- β signaling from cell membrane to the nucleus. *Cell* **113**, 685–700 (2003). [doi:10.1016/S0092-8674\(03\)00432-X](https://doi.org/10.1016/S0092-8674(03)00432-X) [Medline](#)
37. E. Y. P. Lee, M.-Y. Ng, P.-L. Khong, COVID-19 pneumonia: What has CT taught us? *Lancet Infect. Dis.* **20**, 384–385 (2020). [doi:10.1016/S1473-3099\(20\)30134-1](https://doi.org/10.1016/S1473-3099(20)30134-1) [Medline](#)
38. V. D. Menachery, B. L. Yount Jr., K. Debbink, S. Agnihothram, L. E. Gralinski, J. A. Plante, R. L. Graham, T. Scobey, X.-Y. Ge, E. F. Donaldson, S. H. Randell, A. Lanzavecchia, W. A. Marasco, Z.-L. Shi, R. S. Baric, A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat. Med.* **21**, 1508–1513 (2015). [doi:10.1038/nm.3985](https://doi.org/10.1038/nm.3985) [Medline](#)
39. J. E. Kim, E. H. Kim, E. H. Han, R. W. Park, I. H. Park, S. H. Jun, J. C. Kim, M. F. Young, I. S. Kim, A TGF-beta-inducible cell adhesion molecule, betaig-h3, is downregulated in melorheostosis and involved in osteogenesis. *J. Cell. Biochem.* **77**, 169–178 (2000). [doi:10.1002/\(SICI\)1097-4644\(20000501\)77:2<169::AID-JCB1>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1097-4644(20000501)77:2<169::AID-JCB1>3.0.CO;2-L) [Medline](#)

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SUPPLEMENTARY MATERIALS

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Fig. 1. Circulating TGFBIp and TGFBIp K676Ac levels are associated with SARS-CoV-2 infection and severity. (A, B) Plasma was obtained from 20 healthy subjects, 113 SARS-CoV-2 patients, and 25 discharged patients. TGFBIp and TGFBIp K676Ac levels were measured by ELISA. (C) In SARS-CoV-2 patients, the ratio of TGFBIp K676Ac/TGFBIp maybe a diagnostic and prognostic factor. LDH (D) and CRP (E) in SARS-CoV-2 ICU patients were particularly elevated and PCT (F) was not altered. *** $P < 0.001$ vs. Normal; ### $P < 0.001$ vs. SARS-CoV-2

Fig. 2. Plasma cytokines levels in SARS-CoV-2 patients. (A) Proteome analysis of inflammatory factors in plasma from healthy volunteers (n=2) and SARS-CoV-2 patients (n=2). (B-G) Series of comparisons of plasma cytokines levels in SARS-CoV-2 Non-ICU, SARS-CoV-2 ICU, and discharged patients. **P<0.01 vs. Normal; ##P<0.01 vs. SARS-CoV-2 patients.

Fig. 3. Number of blood cell subsets in SARS-CoV-2 patients. A series of comparisons of WBCs (A), Neutrophils (B), ANC (C), Lymphocytes (D), ALC (E), Monocytes (F), Hemoglobin (G), and Platelets (H) between in SARS-CoV-2 Non-ICU and SARS-CoV-2 ICU. *P<0.05 vs. SARS-CoV-2 Non-ICU.

Fig. 4. Imaging of TGFB1p K676 Ac and anti-inflammatory effect of TGFB1p neutralizing antibody in PBMC of SARS-CoV-2 patients. (A) Expression and acetylation of TGFB1p was shown in isolated PBMC from SARS-CoV-2 patients by immunofluorescence staining (x 200) (green: TGFB1p and red: TGFB1p K676Ac). (B) Relative cell viability of PBMCs after the treatment of TGFB1p neutralizing antibody. (C) Binding activity of NF- κ B p65 in PBMC cultured with PBS, mouse IgG antibodies (50 μ g/ml, 6 hours), or TGFB1p neutralizing antibodies (50 μ g/ml, 6 hours) was measured using the Trans AM NF- κ B p65 assay kit. (D-I) Series of comparisons of plasma cytokines levels in PBS-, mouse IgG-, or TGFB1p neutralizing antibodies-treated PBMC of SARS-CoV-2 patients. **P<0.01 vs. IgG treat group; ***P<0.001 vs. ICU patients PBMC + IgG treat group.