

## Letter

### Angiotensin-converting Enzyme 2, a SARS-CoV-2 Receptor, Is Upregulated by Interleukin 6 through STAT3 Signaling in Synovial Tissues

To the Editor:

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has spread explosively worldwide and resulted in a pandemic of a new respiratory disease called coronavirus disease 2019 (COVID-19) in 2020. Symptoms of COVID-19 include fever, malaise, cough, and in severe cases, pneumonia and acute respiratory distress syndrome<sup>1,2</sup>. Coronaviruses possess envelope-anchored spike proteins that bind host cell surface receptors that then initiate viral entry into target cells. In the case of SARS-CoV-2, its spike protein mediates binding to the angiotensin-converting enzyme 2 (ACE2) receptor<sup>3</sup>. While ACE2 expression levels are relatively low in the airway mucosa and lungs, this protein is predominant in the stomach, intestines, gall bladder, kidney, and heart<sup>4</sup>. Therefore, in patients with COVID-19, SARS-CoV-2 can be detected not only in nasal and oral swabs but also in rectal samples; viral RNA has also been detected in blood samples<sup>5</sup>.

Rheumatoid arthritis (RA) is an autoimmune disease that is characterized by systemic synovitis and affects about 1% of the world population. The expression pattern of ACE2 in synovial tissues has not yet been reported, although patients with RA seem to have the same chance of contracting COVID-19 as anyone else<sup>6,7,8</sup>. In this study, we investigated ACE2 expression in the synovium and its regulatory expression mechanism.

Details of the study design are in the Methods section of the Supplementary Material (available with the online version of this letter). This study was approved by the clinical ethics committees of Hiroshima University Hospital, Dohgo Spa Hospital, and Ehime University Proteo-Science Center and Graduate School of Medicine and was conducted at these institutions (approval number: E-668; approval date: 01/02/2017). All experiments were performed in accordance with the approved guidelines. After obtaining informed and signed consent forms, synovial tissues were collected from 16 patients with RA who fulfilled the 1987 classification criteria of the American College of Rheumatology, and 3 patients with osteoarthritis (OA) who underwent total joint replacement.


Immunohistochemistry (IHC) analysis revealed that an active rheumatoid synovium, exhibiting substantial thickening of the synovial lining, mesenchymoid transformation of the synovial stroma, and palisading appearance of the synovial lining, had higher expression of ACE2 compared to inactive samples (Figure 1A). Data from reverse transcriptase-PCR (RT-PCR) for ACE2 mRNA extracted from the synovium supported these findings (Figure 1B). ACE2 expression was found to be increased in the synovial lining and sublining regions, suggesting that its expression was elevated in fibroblast-like synoviocytes (FLS).

Next, we investigated the stimulating agent and signaling pathway for ACE2 upregulation in inflamed FLS. Primary cultures of RA-derived FLS revealed that interleukin 6 (IL-6) stimulation increased ACE2 expression (Figure 1C). We showed slight upregulation of ACE2 by another inflammatory cytokine, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ; Supplementary Figure 1, available with the online version of this letter), whose alteration was not significant. IL-6 is known to regulate downstream target genes through activation of STAT3<sup>9</sup>. IL-6 stimulation led to tyrosine phosphorylation of STAT3 (Supplementary Figure 2). Indeed, the use of small interfering RNA against STAT3 reduced IL-6-dependent ACE2 expression in RA-FLS (Figure 1D and 1E). STAT3 is also known to be activated by cytokines of IL-6 family members, such as leukemia inhibitory factor, oncostatin M, and

IL-11, in the same manner as IL-6<sup>10</sup>. These other humoral factors might also be associated with ACE2 expression in FLS.

We also investigated the expression pattern in synovial specimens from severe OA, harvested during joint replacement surgery, using the IHC analysis. ACE2 expression was located in synovial lining, and both inflamed ACE2-positive lesions, characterized by the characteristics of multilayered lining (hyperplasia) and palisading appearance of the synovial lining, and non-inflamed ACE2-negative lesions were detectable in a same specimen heterogeneously (Figure 1F). Under severe joint destructive condition, IL-6 secreted from chondrocytes might affect ACE2 expression in synovial tissues from patients with OA.

We propose that ACE2 is upregulated in the active synovium of patients with RA, as well as in patients with severe OA, and is likely maintained by STAT3-mediated activation through the IL-6 pathway (Figure 1G). Therefore, RA activity and synovial fluid condition may be able to alter the efficiency of SARS-CoV-2 entry into synovial cells. These data imply that unplanned preventive withdrawal of disease-modifying antirheumatic drugs could lead to increased risk of COVID-19. Recently, phase II and III clinical trials for treatment of COVID-19 have been conducted using anti-IL-6 receptor antibodies, such as tocilizumab and sarilumab, and Janus kinase inhibitors, such as tofacitinib and baricitinib. Further analyses of IL-6-induced ACE2 transcription in other cells and tissues, besides FLS and synovial tissues, may help to elucidate the mechanisms of viral entry into target cells, because *in vivo* IL-6 levels are high in COVID-19 patients<sup>2</sup>.

Sho Mokuda<sup>1</sup> , MD, PhD  
Tadahi Tokunaga<sup>1</sup>, MD, PhD  
Junya Masumoto<sup>2</sup>, MD, PhD  
Eiji Sugiyama<sup>1</sup>, MD, PhD

<sup>1</sup>Department of Clinical Immunology and Rheumatology, Hiroshima University Hospital, Hiroshima;

<sup>2</sup>Department of Pathology, Ehime University Proteo-Science Center and Graduate School of Medicine, Toon, Japan.

This research was funded by JSPS KAKENHI (Grant Number 19K18499 to SM, 19K08908 to ES, 17H04656 and JP20H03719 to JM).

Address correspondence to Dr. S. Mokuda, Department of Clinical Immunology and Rheumatology, Hiroshima University Hospital 1-2-3, Kasumi, Minami-ku, Hiroshima, 734-8551, Japan.  
Email: sho-mokuda@hiroshima-u.ac.jp.

#### ACKNOWLEDGMENT

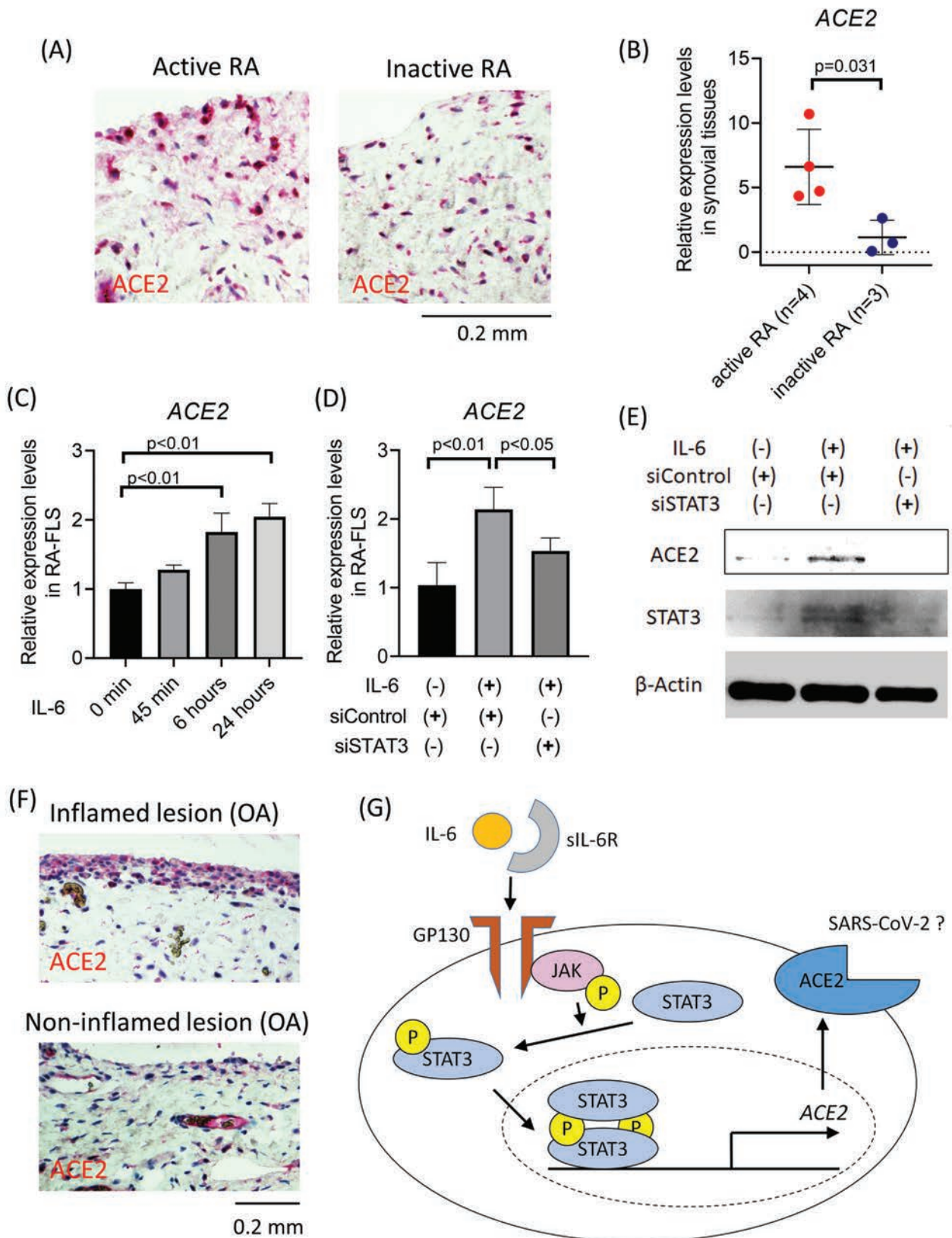
We thank the staff of the Department of Clinical Immunology and Rheumatology at Hiroshima University Hospital, Japan, and the staff of Dohgo Spa Hospital, Matsuyama, Ehime, Japan, for the preparation of patients' specimens.

#### ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this letter.

#### REFERENCES

1. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet* 2020;395:470-3.
2. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020;395:1033-4.
3. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;181:1-10.



**Figure 1.** ACE2 upregulation in inflamed synovial tissues through IL-6 signaling. (A) Immunohistochemistry (IHC) for ACE2 (red) in active and inactive rheumatoid synovial tissues. Images are representative from 3 specimens. Bar = 0.2 mm. (B) RT-PCR for ACE2 expression levels in rheumatoid tissues (n = 3, 4). Data are presented as mean ± SD and analyzed using a t test. Active rheumatoid synovitis was defined by the characteristics of multilayered lining (hyperplasia), mesenchymoid transformation, palisading appearance of synovial lining, and proliferation of blood vessels, all detected with H&E staining. C. RT-PCR for ACE2 expression levels in RA-FLS stimulated with rIL-6 (100 ng/ml) combined with rIL-6Rα (100 ng/ml; n = 3). Data are presented as mean ± SEM and analyzed using a Dunnett test. (D,E) RT-PCR (mean ± SEM, n = 3; D) and Western blot analyses (E) for ACE2 expression levels in RA-FLS stimulated with rIL-6 (100 ng/ml), rIL-6Rα (100 ng/ml), and small interfering RNA against STAT3. Data are presented as mean ± SEM and analyzed using a Dunnett test. (F) IHC for ACE2 (red) in synovial tissues from patients with OA. Images are representative from 3 specimens. Both inflamed and noninflamed lesions are from the same sample. Bar = 0.2 mm. (G) Schematic representation of IL-6-STAT3 signaling-mediated ACE2 expression. Expression levels detected with RT-PCR were normalized to GAPDH expression. ACE2: angiotensin-converting enzyme 2; IL: interleukin; RT-PCR: reverse transcriptase-PCR; RA: rheumatoid arthritis; RA-FLS: RA fibroblast-like synoviocytes; OA: osteoarthritis; sIL6-R: soluble IL-6 receptor; GP130: glycoprotein 130; JAK: Janus kinase; SARS-CoV-2: severe acute respiratory syndrome-coronavirus-2.

4. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci* 2020;12:8.
5. Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect* 2020;9:386-9.
6. Monti S, Balduzzi S, Delvino P, Bellis E, Quadrelli VS, Montecucco C. Clinical course of COVID-19 in a series of patients with chronic arthritis treated with immunosuppressive targeted therapies. *Ann Rheum Dis* 2020;17:667-8.
7. Favalli EG, Agape E, Caporali R. Incidence and clinical course of COVID-19 in patients with connective tissue diseases: a descriptive observational analysis. *J Rheumatol* 2020 Apr 25 (E-pub ahead of print).
8. Filocamo G, Minoia F, Carbogno S, Costi S, Romano M, Cimaz R, et al. Absence of severe complications from SARS-CoV-2 infection in children with rheumatic diseases treated with biologic drugs. *J Rheumatol* 2020 Apr 25 (E-pub ahead of print).
9. Wegenka UM, Buschmann J, Lütticken C, Heinrich PC, Horn F. Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. *Mol Cell Biol* 1993;13:276-88.
10. Kang S, Narazaki M, Metwally H, Kishimoto T. Historical overview of the interleukin-6 family cytokine. *J Exp Med* 2020;217:e20190347.