Angiotsensin-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 syndrome1,2. 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) receptor3. 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 heart4. 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 samples5.

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 else6,7,8. 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 piling or 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-α (TNF-α; 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 STAT39. 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-610. 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 piling or 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.

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ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this letter.

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