The Physiological TMPRSS2 Inhibitor HAI-2 Alleviates SARS-CoV-2 Infection

Yuriko Tomita,* Shutoku Matsuyama,* Hideo Fukuhara, Katsumi Maenaka, Hiroaki Kataoka, Takao Hashiguchi, Makoto Takeda*

Department of Virology 3, National Institute of Infectious Diseases, Tokyo, Japan
Laboratory of Biomolecular Science and Center for Research and Education on Drug Discovery, Faculty of Pharmaceutical Sciences, Hokkaido University, Hokkaido, Japan
Section of Oncopathology and Regenerative Biology, Faculty of Medicine, Department of Pathology, University of Miyazaki, Miyazaki, Japan
Laboratory of Medical Virology, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

KEYWORDS SARS-CoV-2, HAI-2, TMPRSS2

The largest disease pandemic in modern human history, caused by severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), is still ongoing. The disease severity varies greatly (from asymptomatic to death) among individuals and by age (1, 2), but the mechanisms for the variation remain unclear. SARS-CoV-2 uses host proteases to activate its spike protein (3). The type II transmembrane serine protease TMPRSS2 plays an important role for spike protein activation (3–5). A recent study demonstrated that hepatocyte growth factor activator inhibitor 2 (HAI-2) is a cognate inhibitor of TMPRSS2 (6). Albeit less efficiently, HAI-1 also inhibits TMPRSS2 (6). In this study, the potential role of HAI-2 in inhibiting SARS-CoV-2 infection was analyzed. The His-tagged full-length ectodomain of HAI-2 was expressed in HEK293S cells lacking N-acetylglucosaminyltransferase I (293S GnTI- cells) and purified by Ni2+-NTA affinity column and Superdex 200 GL 10/300 gel filtration chromatography, as reported previously (7). Individual fractions were then tested for trypsin inhibition activity using a Pierce Fluorescent protease assay kit, and the fraction (fraction 18–20) that contained HAI-2 proteins with an expected size (27 kDa) and showing the highest activity was used for subsequent assays. SARS-CoV-2 may use TMPRSS2 at the plasma membrane (3–5) or lysosomal cathepsin L (8) for spike protein activation. Nafamostat and E-64d inhibit TMPRSS2- and cathepsin L-mediated coronavirus entry, respectively (9, 10). VeroE6 and VeroE6/TMPRSS2 cells (4) were infected with the SARS-CoV-2 Wk521 strain (4) in the presence or absence of inhibitors (10 μM nafamostat, 10 μM E-64d, or 20 μg/ml HAI-2), and the viral RNA levels at 6 h postinfection were quantified by real-time RT-qPCR as reported previously (4). In VeroE6/TMPRSS2 cells both TMPRSS2- and cathepsin L-mediated entry pathways are used, while in VeroE6 cells only the cathepsin L-mediated pathway is available. As expected, E-64d, but not nafamostat, blocked SARS-CoV-2 infection of VeroE6 cells, while neither nafamostat nor E-64d alone blocked SARS-CoV-2 infection of VeroE6/TMPRSS2 cells (Fig. 1A). The combined use of nafamostat and E-64d efficiently blocked SARS-CoV-2 infection of VeroE6/TMPRSS2 cells (viral RNA level reduced by 100-fold). In this experimental condition, HAI-2 showed comparable inhibitory ability to nafamostat (Fig. 1A). Dose-dependent inhibition of SARS-CoV-2 infection in VeroE6/TMPRSS2 cells by HAI-2 in the presence of 10 μM E-64d was also observed (Fig. 1B). HAI-2 is endogenously expressed in many cell types (11) and may thus inherently inhibit or alleviate SARS-CoV-2 infection. Expression of HAI-2 in human lung adenocarcinoma Calu-3 cells was knocked down (KD) by small interfering RNA (siRNA) transfection (Fig. 1C), and virus infection assays were performed. In a previous study using Middle East respiratory syndrome coronavirus, it was...
demonstrated that TMPRSS2 is mainly utilized during virus entry into Calu-3 cells and perhaps into the lung as well (12). The level of viral RNA in HAI-2 KD cells was 40 times greater than that in control siRNA-transfected cells (Fig. 1D). These data indicated that the endogenous level of HAI-2 in Calu-3 cells alleviated SARS-CoV-2 infection. The present study provides two key messages. First, the expression level of HAI-2 modulates the infection level of SARS-CoV-2. Because SARS-CoV-2 spreads systemically in vivo (3), even a small imbalance or change in TMPRSS2 and HAI-2 expression may modulate tissue tropism or disease severity caused by SARS-CoV-2. Second, studies on HAI-2 may open a way to develop protein-based therapeutics against SARS-CoV-2, as already suggested for the treatment of TMPRSS2-mediated malignant tumor invasions (6, 11).

ACKNOWLEDGMENTS
This study was supported by Grants-in-Aid from AMED (grant number 19fk0108111j [to T.H. and M.T.]) and JSPS (grant numbers 18H02665 [to M.T.] and 20H05773 [to T.H.]).

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