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Short communication

Trypsin may induce cytokine storm in M1 macrophages, resulting in critical coronavirus disease

Hongyu Chen\textsuperscript{a}, Shirong Huang\textsuperscript{b}, Qingquan Chen\textsuperscript{b}, Qicai Liu\textsuperscript{c}, Xiaoting Lv\textsuperscript{d,}\textsuperscript{*}

\textsuperscript{a} Zhongwei Tech Innovation Medical Research Institute Co., Ltd, Beijing 100000, China
\textsuperscript{b} Department of Laboratory Medicine, Fujian Medical University, Fuzhou 350004, China
\textsuperscript{c} Center for Reproductive Medicine, 1st Affiliated Hospital, Fujian Medical University, Fuzhou 350004, China
\textsuperscript{d} Department of Respiratory and Critical Care Medicine, Research Laboratory of the Respiratory System Diseases, 1st Affiliated Hospital, Fujian Medical University, Fuzhou, Fujian 350004, China

\textbf{ABSTRACT}

Trypsin is a protease-activated receptor-2 (PAR2) activator that upregulates the interleukin (IL)-17 receptor signal in the airway epithelial cells and amplifies the inflammatory response, but does not modify the growth kinetics of the metapneumovirus. How does the coronavirus spread from cell to cell is yet an enigma. The present study analyzed the possible role of trypsin in the activation of coronavirus in \textit{in vitro} and \textit{in vivo}. We found that the overexpression of trypsin in A549 cells upregulated IL-17 and angiotensin-converting enzyme (ACE). In the humanized transgenic mice, trypsin activated M1 macrophages. Together, our results suggested that the upregulation of trypsin may support a new pathway for coronavirus transmission in patients.

1. Introduction

Autopsy shows that macrophage infiltration and high expression of interleukin (IL)-17 in SARS-CoV-2 patients’ tissues are the major causes of the cytokine storm (Xu et al., 2020; Wu et al., 2020b). Nonetheless, a comprehensive understanding of how coronavirus enters numerous host cell types is essential to further delineate the complex nature of the virus pathogenesis and develop targeted therapeutics. During the cell culture of porcine delta coronavirus (PDCoV), trypsin propagates the virus in the cells (Shirato et al., 2011; Wicht et al., 2014), although the underlying mechanism is yet to be clarified. It has been proposed that coronavirus spike protein can be cleaved by an endogenous or exogenous protease to induce the fusion between cells and the virus to enter the cell smoothly, which is crucial for the effective infection of the virus. In addition, the cleavage of spike protein on the CoV surface by trypsin enhances the infectivity of the virus to the cells (Wu et al., 2020a). Herein, we focused on the potential correlation between trypsin and virus infection \textit{in vitro} and \textit{in vivo}.

1.1. \textit{In vitro}

Transcriptome deep sequencing (RNA-seq) was performed using total RNA isolated from empty plasmid transfection (LV-NC) and overexpression (OE) in A549 cells. Genes with a strong positive correlation (Pearson’s $r > 0.9$) and a strong negative correlation (Pearson’s $r < -0.9$) with PRSS1 (trypsin) were screened, and the correlation coefficient matrix and heat map were constructed (Fig. 1a). IL-17RB, ACE, CD46, and other genes were positively correlated to PRSS1, which was beneficial to virus adhesion and immune escape. However, the expression of CD70, CD82, and other genes is strongly negatively correlated with PRSS1, and this deficiency is a probable molecular basis of susceptibility to the virus.

1.2. \textit{In vivo}

Macrophages are observed in the pancreas, lung, and small intestine of EIIA mice (overexpressing trypsin, systemic hPRSS1 transgenic mice produced by breeding the founder mice with EIIA-Cre heterozygotes). The M1 macrophages are significantly higher than that of Cre mice (Cre recombinase under the EIIA promoter), but did not differ significantly in the colon. Intriguingly, M1 macrophages accumulated around the bronchioles (Fig. 1b). In the lung tissue of EIIA, trypsin was highly expressed in the bronchial epithelial cell layer, while PAR2 was expressed in the alveolar epithelial cells (AECs) (Fig. 1c).
The putative mechanism of CoV infection is as follows: CoV enters the respiratory tract with granular floating substance and combines with angiotensin-converting enzyme (ACE) receptor of the bronchiole epithelial cells to promote trypsin synthesis. Then, trypsin activates the PAR2 on macrophages, alveolar endothelial cells, and neutrophils, leading to macrophage polarization, neutrophil activation, and increased capability of AECs. Trypsin is a protease-activated receptor-2 (PAR2) activator that mediates the upregulation of the IL-17 receptor signal in AECs and amplifies the inflammatory response (Fig. 1d).

2. Conclusions and outlook

IL-17 mediates the flow of neutrophils and macrophages in the inflammatory response of virus infection, increases the contraction of airway smooth muscle, and causes lung injury, but does not change the growth kinetics of metapneumovirus. Then, how does the virus spread from cell to cell? The spike protein of CoV is cleaved by trypsin, cathepsin, and other active proteases and stimulated for fusion (Glowacka et al., 2011), thereby releasing the virus genome into the cytoplasm. This vital phenomenon effectuates the fusion of cells and viruses, facilitating the viral spread rapidly throughout the body.

Therefore, the present study suggested that protease may play a major role in the process of virus aggregation, release, and replication, providing a novel method for the treatment of coronavirus infection by antagonizing the living enzymes.

**Ethics**

The protocols for the study and informed consent were approved by the ethics committee of First Affiliated Hospital of Fujian Medical University (Approval No. MRCTA, ECFAH of FMU [2020]153).

**CRediT authorship contribution statement**

Drs. Lv and Liu had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Conflict of Interest Disclosures**

None reported.

**Acknowledgements**

This work was supported by the National Nature Science Foundation of China (grant numbers 81800070).

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