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Rapid report

Single-cell RNA sequencing data suggest a role for angiotensin-converting enzyme 2 in kidney impairment in patients infected with 2019-nCoV

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Conflict of interest

None.

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Introduction

The World Health Organization has recently declared severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) a global public health emergency. Chaolin Huang et al. reported acute kidney injury (AKI) in 7% of the 41 patients infected with SARS-CoV-2, this value was even higher (up to 31%) among intensive-care patients[1]. Furthermore, Zhen Li et al. reported that plasma creatinine levels increased in 11 of 59 patients with SARS-CoV-2 infection, suggesting that kidney function was probably impaired when the disease progressed[2]. Weijie Guan et al. reported that plasma creatinine level increased in 4.3% of severely diseased patients[3]. SARS-CoV-2 is a highly contagious pathogen that predominantly causes pneumonic symptoms. To date, infection by this virus has caused tens of thousands of fatalities, and hundreds of thousands of people have been isolated as a preventive measure. Although respiratory failure has been associated with the highest mortality, the lungs were not the only organs involved. Hoffmann et al. reported that SARS-CoV-2 and SARS-CoV share a common receptor angiotensin-converting enzyme 2 (ACE2) that is required to enter target cells, and cellular protease transmembrane protease serine 2 (TMPRSS2) can cleave and activate the spike protein of SARS-CoV-2 for membrane fusion[4]. We investigated whether ACE2 and TMPRSS2 were expressed in kidney cells using precision-technology single-cell RNA sequencing.

Methods

Data sources

Single-cell RNA sequencing data were acquired from the Gene Expression Omnibus (GEO) database and from the Kidney Interactive Transcriptomics (KIT) database (http://humphreyslab.com/SingleCell/). Original sequence data were downloaded from the GEO database for further analyses (accession numbers GSE131685, GSE112570, GSE109564, and GSE114156), and immunohistochemical staining results were acquired from the Human Protein Atlas (http://www.proteinatlas.org).

Data processing

R software (version 3.6.1, https://www.r-project.org/) and the Seurat package (version 3.1, https://satijalab. org/seurat/) were used for the single-cell RNA sequencing data processing.

Results

Single-cell RNA sequencing data confirmed ACE2 expression in the human kidney

In order to investigate whether ACE2 was expressed in a specific cell type in human kidneys, published single-cell RNA sequencing data were downloaded from the GEO and KIT databases. Kidney samples assigned the GEO accession numbers GSE109564 and GSE114156 originated from a healthy donor, and 4487 cells were retained for further analysis after quality control. Kidney samples under accession number GSE131685 originated from para-carcinoma tissue of three patients with tumors, and 23,366 cells were retained for further analysis after quality control; data from four samples were combined for further analysis. Fetal kidney samples originated from embryos of 8–18 weeks, and 7343 cells were retained for further analysis after quality control. ACE2 was mainly expressed in proximal tubule cells in cases under the accession numbers GSE109564 and GSE114156 (Figure 1A). Accordingly, ACE2 was found to be expressed predominantly in tubular precursors of the kidney of the fatal case (Supplemental figure). Similarly, in GSE131685, ACE2 was also expressed mainly in proximal tubule cells (Figure 1B). TMPRSS2 was predominantly expressed in the loop of Henle and in the collecting duct in GSE109564 and GSE114156 (Supplemental figure). Single-cell RNA sequencing of fetal and adult kidney samples revealed that ACE2 was mainly expressed in tubule cells.

Immunohistochemical staining confirmed ACE2 protein expression in human organs

After verifying ACE2 expression in specific kidney cell types at RNA level, we investigated whether this was consistent at a protein level using the Human Protein Atlas. Interestingly, ACE2 was found to be expressed in several human organs such as the intestines, adrenal gland, gallbladder, and in the kidneys, and it was highly expressed in the urogenital and digestive

systems. ACE2 was highly expressed in the glandular cells of the intestine and gallbladder (Figure 1C). As SARS-CoV-2 preferably occurs in the lungs, we tested whether ACE2 was also expressed in lung tissue; however, we found that ACE2 showed only low expression levels in normal lungs, and only some positive staining was observed in lung macrophages (Figure 1C). Therefore, whether ACE2 levels would increase due to SARS-CoV-2 infection requires further investigation. Consistent with single-cell RNA sequencing data, ACE2 was predominantly expressed in the proximal tubules (Figure 1C).

Discussion

Our results showed that ACE2 and TMPRSS2 were expressed in the human kidney, indicating that the kidney is a potential target organ of SARS-CoV-2. These findings may suggest that antibodies or biological inhibitors targeting virus proteins such as spike protein, the ACE2 receptor, or protease TMPRSS2 could potentially be part of therapeutic strategies.

Among patients infected with SARS-CoV, 6.7% (36/536) exhibited AKI with a median duration of 20 days (from 5 to 48 days) despite normal plasma creatinine levels at the first clinical presentation, and those who experienced AKI eventually suffered extremely high mortality of up to 91.7% (33/36)[5]. Middle East respiratory syndrome-related coronavirus (MERS-CoV) has also been found in 26.7% (8/30) of the patients with AKI, and the mean and median durations until occurrence of AKI from symptom onset were 18 and 16 days, respectively. The receptor of MERS-CoV, DPP4, is also expressed in kidney cells such as tubule cells and podocytes. Furthermore, tubules are often found to be severely damaged during AKI caused by various reasons. High expression of the coronavirus receptors ACE2 and DPP4 in kidney tubule cells suggests that the kidney is at high risk of coronavirus infection.

Thus, there is an urgent need to develop specific drugs that target coronavirus receptors so as to prevent kidney damage. Moreover, kidney functions in patients infected with SARS-CoV-2 should be monitored frequently, particularly in patients with increased levels of plasma creatinine. Early interventions, including continuous renal replacement therapies, should be applied as early as possible to preserve kidney function in patients who show signs of kidney

failure such as increased concentrations of urine protein, blood urea nitrogen, or plasma creatinine.

Acknowledgement

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Figure legend

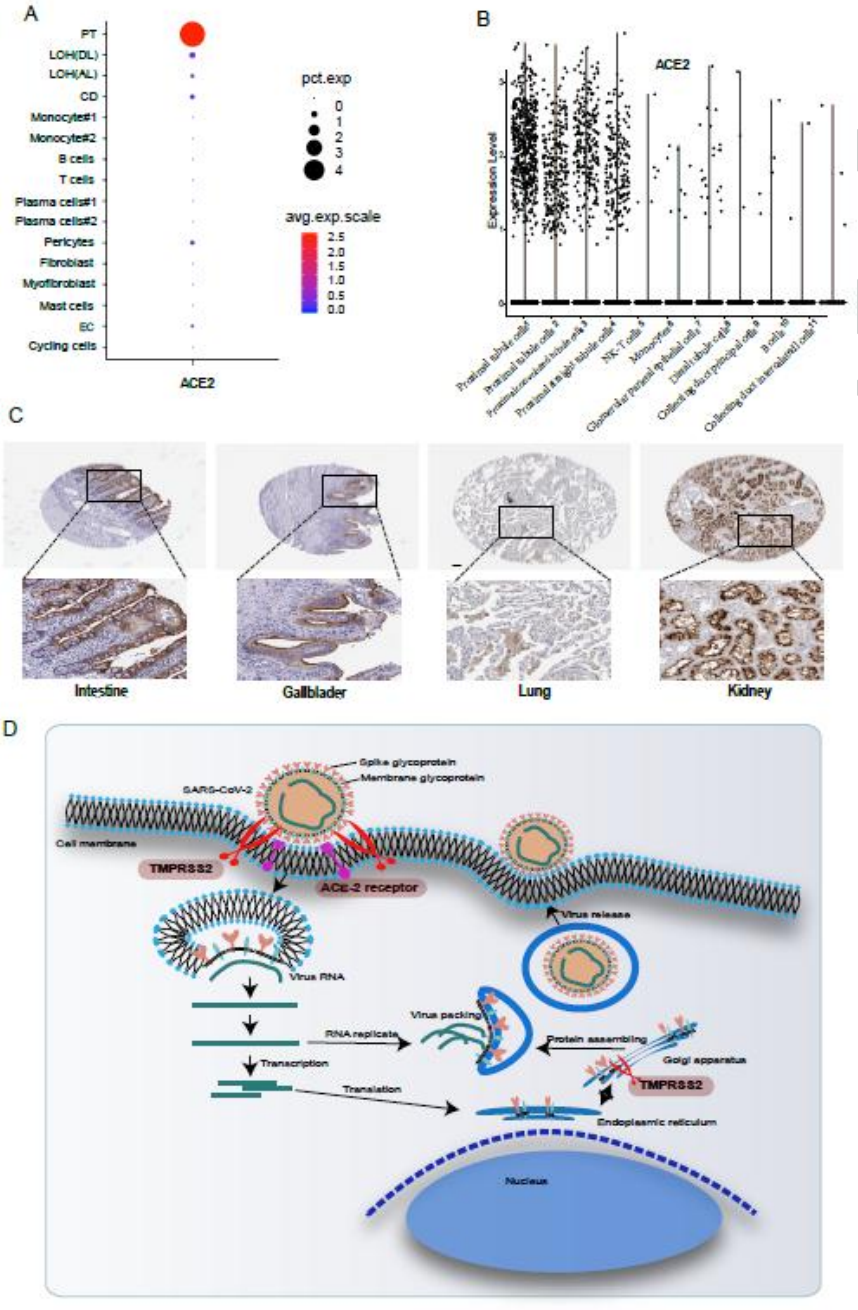

Figure 1. (A) Expression of ACE2 in different cell clusters of cases with accession numbers GSE109564 and GSE114156. (B) Expression of ACE2 in different cell clusters of accession number GSE131685. (C) Immunohistochemical staining of ACE2 in human organs. (D) Illustration of SARS-CoV-2 entering the target cell.