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

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The World Health Organization has recently declared the 2019-novel coronavirus (2019-nCoV) a global public health emergency. Huang et al\[1\] reported acute kidney injury (AKI) in 7% of the 41 patients infected with 2019-nCoV, this value was even higher (up to 31%) among intensive-care patients. Furthermore, Li et al\[2\] reported that plasma creatinine levels increased in 11 of 59 patients with 2019-nCoV infection, suggesting that kidney function was probably impaired when the disease progressed. Guan et al\[3\] reported that plasma creatinine level increased in 4.3% of severely diseased patients. 2019-nCoV is a highly contagious pathogen that predominantly causes pneumonia symptoms. Although respiratory failure has been associated with the highest mortality, the lung was not the only organ involved. Hoffmann et al\[4\] reported that 2019-nCoV and severe acute respiratory syndrome-associated coronavirus (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 2019-nCoV for membrane fusion. We investigated whether ACE2 and TMPRSS2 were expressed in kidney cells using precision-technology single-cell RNA sequencing.

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 (https://www.proteinatlas.org/ENSG00000130234-ACE2/tissue).

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.

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 to 18 weeks, and 7343 cells were retained for further analysis after quality control; the authorization to use the data from the KIT database was obtained through email). 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 fetal case [Supplemental Figure 1, http://links.lww.com/CM9/A212]. 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 1, http://links.lww.com/CM9/A212]. Single-cell RNA sequencing of fetal and adult kidney samples revealed that ACE2 was mainly expressed in tubule cells.

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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 2019-nCoV 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 2019-nCoV infection requires further investigation. Consistent with single-cell RNA sequencing data, ACE2 was predominantly expressed in the proximal tubules [Figure 1C].

Our results showed that ACE2 and TMPRSS2 were expressed in the human kidney, indicating that the kidney is a potential target organ of 2019-nCoV. 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 2019-nCoV 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.

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Conflicts of interest
None.

References
1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506. doi: 10.1016/s0140-6736(20)30183-5.
2. Li Z, Wu M, Guo J. Caution on kidney dysfunctions of 2019-nCoV patients. medRxiv 2020. doi: 10.1101/2020.02.08.20021212.
3. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020. doi: 10.1056/NEJMoa2002032. [Epub ahead of print].
4. Hoffmann M, Kleine-Weber H, Krüger N, Müller M, Drosten C, Pöhlmann S. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. BioRxiv 2020. doi: 10.1101/2020.01.31.929042.
5. Chu KH, Tsang WK, Tang CS, Lam MF, Lai FM, To KF, et al. Acute renal impairment in coronavirus-associated severe acute respiratory syndrome. Kidney Int 2005;67:698–705. doi: 10.1111/j.1523-1755.2005.67130.x.

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