Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis

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Abstract

Purpose: Acute kidney injury (AKI) is a severe symptom of the 2019 novel coronavirus disease (COVID-19), especially for patients in a critical condition. This study explored the potential mechanism of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on AKI at the single-cell level.

Methods: 15 normal human kidney samples were collected and analyzed using single-cell RNA sequencing (scRNA-seq). Subsequently, we analyzed the components and proportions of kidney cells expressing the host cellular receptor ACE2 and the key protease TMPRSSs family, and analyzed the expression differences in Occidental and Asian populations.

Results: We drafted the currently available world's largest human kidney cell atlas with 42,589 cells and identified 19 clusters through unsupervised hierarchical clustering analysis. ACE2 and TMPRSSs genes were significantly co-expressed in podocytes and proximal convoluted tubules as potential host cells targeted by SARS-CoV-2. Comparative analysis showed that ACE2 expression in kidney cells was no less than that in the lung, esophagus, small intestine and colon, suggesting that the kidney may be an important target organ for SARS-CoV-2. In addition, given the high expression of ACE2 and kidney disease-related genes in Occidental donors relative to Asian donors, Occidental populations with SARS-CoV-2 infection might be a higher risk of of kidney injury.

Conclusions: AKI may be infringed by synergistic assaults from the virus-induced cytopathic effect and systemic inflammatory response, especially in severe patients. Therefore, we need to closely monitor renal function in patients with COVID-19 and take early clinical interventions.

Key words: SARS-CoV-2, COVID-19, acute kidney injury, angiotensin converting enzyme II (ACE2), transmembrane serine protease (TMPRSSs)

Introduction

In December 2019, a nightmare of unknown pneumonia enveloped Wuhan, the capital city of Hubei Province and soon spread to other parts of China. The source of this fatal pneumonia is a novel coronavirus named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1], which was isolated from human airway epithelial cells[2]. As of February 21, 2019, 75,571 cases of SARS-CoV-2 infection had been diagnosed by laboratory testing, including 11,633 critical cases, and 2,239 deaths. In addition, patients with the 2019 novel coronavirus disease (COVID-19) have also been found in 24 other countries around the world, becoming a Public Health Emergency of International Concren (PHEIC). The genomic characterization of SARS-CoV-2 was found to be closely related to severe acute respiratory syndrome (SARS)-like coronaviruses detected in two bat-derived coronaviruses (bat-SL-CoVZC45, sequence identity 87·99%; bat-SL-CoVZXC21, sequence identity 87·23%)[3], as such it was officially designated as SARS-CoV-2 [1].

The clinical symptoms of COVID-19 are very similar to those in patients with SARS coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), including fever, cough and diarrhea [4]. Although most patients had mild symptoms, nearly one-third of the patients developed severe pneumonia with serious comorbid conditions, such as acute respiratory failure, multiple organ failure or even death[4, 5]. Acute kidney injury (AKI) is one of the most frequent organ damage of coronavirus, with a sharp rise in serum creatinine (SCr) level and a sharp decrease in urine output [6]. Systematic analysis showed that 75% (9/12) of MERS-CoV infected patients developed acute renal failure (ARF) with a median interval of 11 days from symptom onset, and 6.7% (36/536) SARS-CoV infected patients with ARF were diagnosed after a median of 20 days from symptom onset [6, 7]. The latest epidemiological and clinical characteristics study showed that 3-19%

patients with COVID-19 had symptoms of AKI, and about 9% patients with renal failure received continuous blood purification [5, 8]. But whether the AKI of COVID-19 is caused by a coronavirus-induced cytopathic effect or cytokine storm-induced systemic inflammatory response remains unclear.

Like SARS-CoV infection, the spike (S) protein of SARS-CoV-2 was found to engage angiotensin converting enzyme 2 (ACE2) as host cellular receptor and employ cellular transmembrane serine proteases (TMPRSSs) family (especially TMPRSS2 and TMPRS11D) for priming [9-11]. After the virus S protein binding to the receptor ACE2 on host cells, the S protein was activated and cleaved by the TMPRSSs, allowing the virus to release fusion peptide for membrane fusion [12]. Therefore, co-expression of ACE2 and TMPRSSs is a key determinant for entry of SARS-CoV-2 into host cells. Previous studies reported that the expression of ACE2 and TMPRSSs was distributed in the epithelia of the gastrointestinal, urogenital, and respiratory tracts, which provide superior host conditions for coronavirus [9, 13-15]. Due to the lack of signal specificity in detection of ACE2 and TMPRSSs expression level by immunohistochemical or Real-Time PCR assays, it is difficult to accurately and quantitatively distinguish which cellular component plays a leading role in virus infection.

To investigate possible causes of AKI in humans after SARS-CoV-2 infection, we used single-cell RNA sequencing (scRNA-seq) analysis to identify ACE2 and TMPRSSs expression in all cellular components of the human kidney and also analyzed differences in the expression of kidney disease-related genes in Occidental and Asian populations. In addition, we used bioinformatics analysis data combined with clinical characteristics to deduce the underlying mechanism of AKI during the SARS-CoV-2 outbreak.

Methods

Patients and specimens

Fifteen human single-cell expression matrices of normal kidneys were collected for scRNA-seq analyses, including 5 samples from Occidental donors and 10 samples from Asian donors. Among the 10 Asian donors, 7 single-cell expression matrices of scRNA-seq data were derived from the Gongli Hospital of Second Military Medical University (Shanghai, China). The remaining 8 single-cell expression matrices were obtained from the published researches.

We received approval from the Institution Review Board (IRB) of the Gongli Hospital of Second Military Medical University, and signed informed consent was obtained from seven patients.

Statistical analysis

Statistical analysis of all scRNA-seq data was performed in R (version 3.6.1). Statistical significance was accepted for $P < 0.05$.

Detailed experimental and analytical methods and materials

Detailed experimental methods and analytical procedures are presented in Supplementary experimental and analytical procedures.

Results

Identification of human kidney cell populations

In this study, we included 3 datasets of human normal kidney tissuses for scRNA-seq analysis: one Occidental (dataset 1, 5 samples) and two Asian (dataset 2, 7 samples; dataset 3, 3 samples). We used the gene expression count matrices to present cell distribution of kidney. As the UMAP plot showed, we identified 6 clusters using unsupervised clustering analysis among 42,589 cells (Fig.1A). The 6 clusters were annotated with expression of known cell type signal genes, including Epithelial cluster (28,801 epithelial cells), Endothelial cluster (7,366 endothelial cells), Vascular smooth muscle cells

cluster (225 VMSC), T cluster (3,625 T cells), B cluster (1,033 B cells) and Myeloid cluster (1,539 Myeloid cells) (Fig.1A).

Cells of each cluster were subdivided into some subclusters, which were identified by known cell type signal genes (Fig.1A). The epithelial cluster as the largest cluster was subdivided into 7 subclusters, including proximal convoluted tubule cells (PT convoluted), Loop of henle, Distal tubules (DT), Podocyte, Collecting duct (CD), proximal straight tubule cells (PT straight), and proximal tubule cells (PT) (Fig.1A) . Kidney epithelial cells, as the main source of kidney cells, were composed of 86.9% proximal tubules, 5.2% collecting duct, 2.9% Loop of henle, 2.9% distal tubules and 2.0% podocyte (Fig.1B). In addition, Endothelial cluster, T cluster, B cluster and Myeloid cluster were respectively subdivided into 3 endothelial subclusters (Assending wasa recta, Clomerular endothelium and Descending wasa recta), 3 T subclusters ($CD4^+$T, $CD8^+$T and NKT), 2 B subclusters (B cell and Plasma) and 3 Myeloid subclusters (monocyte, M1 and M2) (Fig.1A).

Significant differences in transcriptional activity and signature gene expression were clearly presented among 19 subclusters, suggesting that identification of human kidney cell populations was successful (Fig. S1A, Supplementary Table 1). The dot-plot model also perfectly showed differences in signature gene expression between all these subclusters, which could clearly distinguish each subcluster (Fig. S1B-F).

Cell population-specific ACE2 and TMPRSSs expression

To investigate which cell type was the candidate host cell for SARS-CoV-2 in the kidney, we explored the expression level of ACE2 and TMPRSSs genes in all kidney cell populations, and found that the expression of ACE2 gene was relatively high in podocytes, proximal convoluted tubule cells and proximal straight tubule cells, which were main subclusters of epithelial cell clusters (Fig. 2A,B). TMPRSSs genes were widely distributed in all epithelial cell subclusters,

especially the collecting duct and distal tubule cells (Fig. 2A,B). Colocalization analysis of ACE2 and TMPRSSs genes showed relatively high co-expression in podocytes and proximal straight tubule cells, which were candidate host cells (Fig. 2A-C). In addition, the endocytosis-related signature genes, which affected virus entry into host cells, were highly and extensively expressed in all the 7 epithelial cell subclusters, especially podocytes (Fig. 2B). TMPRSS2 gene, as an important helper for SARS-CoV-2 to enter host cells[9, 10], was found to be co-expressed with ACE2 in podocytes (Fig. 2B).

Ethnic differences of ACE2 and TMPRSSs expression

In this study, we collected 5 normal kidney samples from Occidental donors and 10 normal kidney samples from Asian donors. The cellular composition of kidney epithelial cells was similar between Occidental and Asian donors, except for some minor differences due to deviations in the samples (Fig. 3A). Compared with the kidney tissues from the Asian donors, the podocytes and proximal straight tubule cells from the Occidental donors were highly expressed in kidney disease-related genes FABP1, PLIN2 and GNB2L1 (Fig. 3B, Supplementary Table 2), which could increase the risk of kidney disease[16, 17]. Simultaneously, the expression of ACE2 gene in the podocytes and proximal straight tubule subclusters from the Occidental donors was significantly higher than that from the Asian donors (Fig. 3C). However, an insignificant difference in TMPRSSs expression in the proximal straight tubule subclusters was observed between the different races (Fig. 3D). In addition, genesets related to kidney injury showed ethnic differences between the Occidental and Asian donors (Fig. S2). It is noteworthy that the expressions of diabetic kidney disease-related biomarkers Cystatin C (CST3), DPP4 and RBP4 [18-20] in podocytes of the Occidental donors were significantly higher than those in the Asian donors (Fig. 3E). These findings indicated that Occidental populations might be a high risk for developing AKI during the

COVID-19.

Inter-organ differences in ACE2 and TMPRSSs expression

To evaluate the possibility of the kidney as a target organ for SARS2, we comparatively analyzed the co-expression levels of ACE2 and TMPRSSs genes in the human gastrointestinal, urogenital, and respiratory tracts, including the kidney, lung, esophagus, ileum and colon. The data of cell types by scRNA-seq analysis were based on our previously published research [9]. It was found that ACE2 and TMPRSSs genes were co-expressed extensively in host cells of the human organs, including podocytes and proximal straight tubule cells (kidney), type II alveolar cells (lung), upper and stratified epithelial cells (esophagus), absorptive enterocytes and progenitor absorptive cells (ileum) and enterocytes and immature enterocytes cells (colon) (Fig. 4A, Fig. S3). The expression of endocytosis- and exocytosis-related signature genes in podocytes was relatively high compared with host cells of other human organs, second only to host cells of the colon (Fig. 4B). The RNA-seq data of the kidney, lung, esophagus, stomach, small intestine, colon-transverse and colon-sigmoid were obtained from GTEx database. The expression of ACE2 gene in the kidney tissue was higher than that in the other human organs, except for the small intestine (Fig. 4C).

A potential mechanism of virus-induced AKI

Histologically and embryologically, the mesoderm-derived metanephros differentiates into the metanephrogenic blastema and sequentially differentiates into proximal tubules, loop of henle, and distal tubules[21]. In this study, we clearly identified all kidney cell components (Figure 5A). Through Differentiation trajectory and RNA Velocity analyses, we found that podocytes and proximal straight tubule cells were located close to each other on the same differentiation trajectory and had similar differentiation trends,

suggesting intercellular homology (Fig. S4A, B). We found that a population of kidney epithelial cells positioned as a starting point of the differentiation process with a high expression of stem cell-associated genes ACAT2, PDGFRB and NDUFA4L2, might be relatively primitive epithelial cells with an active differentiation ability (Fig. S4A-C). Furthermore, Gene Set Variation Analysis (GSVA) showed that kidney epithelial cells were a highly enriched signature in kidney disease-related signaling pathways, especially in podocytes (Fig. S4D, Supplementary Table 3). These findings further validate that the assault of candidate host cells (podocytes and proximal straight tubule cells) could lead to AKI (Fig. 5B). At the same time, virus replication and immune regulation-related gene [22, 23] analysis showed that podocytes and proximal straight tubule cells could provide superior conditions for unbridled viral replication (Fig. 5C, D).

Discussion

Acute kidney injury is a severe symptom after coronavirus infection, the incidence of which is second only to respiratory system injuries [24]. It was reported that 8.3-23% patients with ICU care were infringed by AKI during COVID-19 [5, 25]. Previous research demonstrated that MERS-CoV could disseminate infection in the lungs and kidneys, inducing direct damage through a nonhuman primate (common marmosets) model [26]. In addition, positive SARS-CoV RNA polymerase gene fragments were detected in kidney specimens of autopsy patients who died of SARS by immunohistochemistry and in situ hybridization [27]. Intriguingly, kidney epithelial cells were found to be more infectious MERS-CoV progeny than bronchial epithelial cells, suggesting a viral kidney tropism [6]. The mechanism of kidney damage may be that MERS-CoV induces kidney cell apoptosis through mediating the increase of FGF2 and Smad7 expression [26]. Conversely, silencing SMAD7 could effectively inhibit MERS-CoV replication, thus protecting kidney cells

against the virus-induced cytopathic effect [26]. These findings suggest that renal damage following coronavirus infection may be closely related to virus-targeted assault on the renal tissue.

ACE2, known as an entry receptor and TMPRSSs activating coronavirus S protein, is the determining factor of SARS-CoV-2 entry into host cells [9, 10]. It was found in our study that ACE2 and TMPRSSs genes were significantly co-expressed in podocytes and proximal straight tubule cells, playing critical roles in urine filtration, reabsorption and excretion. Podocytes (co-expression of ACE2 and TMPRSS2 genes), as a major component of the glomerular filtration barrier (GFB), is particular vulnerable to viral and bacterial attacks [28]. Studies demonstrated that podocyte injury easily induced heavy proteinuria [28], which is consistent with the previous report of proteinuria as a typical clinical symptom in SARS-CoV patients with AKI [7]. Recently published data showed that about 63% SARS-CoV-2 infected patients had clinical symptoms of proteinuria [8], suggesting that SARS-CoV-2 may invade human podocytes cells to cause the virus-induced cytopathic effect. Furthermore, a positive expression of MERS-CoV nucleocapsid protein was detected in renal tubular cells and podocytes in an ex vivo human kidney culture model [26].

Some recent scRNA-seq analysis studies [9, 29, 30] showed that ACE2 gene was highly expressed in lung type II alveolar cells, esophageal upper epithelial cells and absorptive enterocytes from the ileum and colon. In addition, a study [8] reported that the expression of ACE2 gene in the kidney tissue was almost 100-fold higher than that in the lung tissue. In fact, as precisely targeted host cells, podocytes in the kidney and type II alveolar cells in the lung have similar co-expression levels of ACE2 and TMPRSSs genes (**Fig. 5A**). We also found extensive co-expression of ACE2 and TMPRSSs genes in host cells of other human organs, including the esophagus, ileum and colon. Obviously, host cells of SARS-CoV-2 are widely distributed in various human organs, which

may be a potential fatal hazard for severe patients. In addition, Occidental populations with COVID-19 had a higher risk of AKI compared with Asian populations

Once the coronavirus spillover from the infected lung gains access into the systemic circulation, it implies a very unfavorable outcome for patients with subsequent severe comorbidities and clinical features [31-33]. At 2-3 weeks after symptom onset, positive SARS-CoV RNA was detected in human blood and urine samples [34]. Meanwhile, SARS-CoV patients developed ARF at a median of 20 days from symptom onset [7], suggesting that renal damage is closely related to kidney transmission of coronavirus [35]. According to the current published data for SARS-CoV-2, the interval from symptom onset to the detection of viral RNA in the blood is about 6-7 days for patients with COVID-19 [5, 33]. At the same time, one week from symptom onset is also the time when patients with SARS-CoV-2 infection develop AKI [4, 5]. Furthermore, a recent study [36] reported the detection of positive viral RNA in urine samples of patients with severe SARS-CoV-2 infection. It is reasonable to suppose that the source of AKI for patients with positive viral RNA in blood samples and heavy proteinuria may be caused by the SARS-CoV-2-induced cytopathic effect. But in critical cases of SARS-CoV-2 infection, the role of the cytokine storm-induced systemic inflammatory response in causing multiple organ damage is unlikely to be trivial [37]. Studies reported that the level of plasma proinflammatory factors (IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A and TNFα) in severe cases was significantly higher than that in mild cases, suggesting that cytokine storm occurred in severe cases [5]. Once a cytokine storm is formed, the immune system may not be able to kill the SARS-CoV-2, instead it may kill large numbers of normal human cells of the lung, kidney, heart and digestive tract. Therefore, the synergistic effect of cytokine-induced systemic inflammatory response and virus-induced cytotoxic effect may be the cause of AKI during COVID-19.

Conclusions

In the present study, we used scRNA-seq analysis to have accurately located the expression and distribution of ACE2 and TMPRSSs genes in kidney cell components, and found that podocytes and proximal straight tubule cells were potential host cells targeted by SARS-CoV-2, resulting in AKI caused by the virus-induced cytopathic effect. During the course of fatal pneumonia, AKI patients may be infringed by synergistic assaults from the virus-induced cytopathic effect and systemic inflammatory response, especially in severe and critical cases with positive viral RNA in blood samples and heavy proteinuria. The findings of the present study may have important guiding significance for planning the clinical treatment strategies of AKI and early protection of the renal function of patients with COVID-19.

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Author contributions

Xin-gang Cui had full access to all the data in the study and takes

responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Cui, Wang, Zhou, Pan. Acquisition of data: Xu, Gong, Chen, Meng. Analysis and interpretation of data: Pan, Xu, Zhang, Gong, Chen. Drafting of the manuscript: Pan, Xu, Zhang, Ye. Critical revision of the manuscript for important intellectual content: Cui, Wang, Zhou, Ye. Statistical analysis: Xu, Zhang, Gong, Chen, Meng. Obtaining funding: Cui, Pan. Administrative, technical, or material support: Ye, Gan, Qu, Chu. Supervision: Cui, Wang, Zhou, Pan.

Compliance with ethical standards

Conflicts of interest

The authors declare no potential conflicts of interest.

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Figure Legend

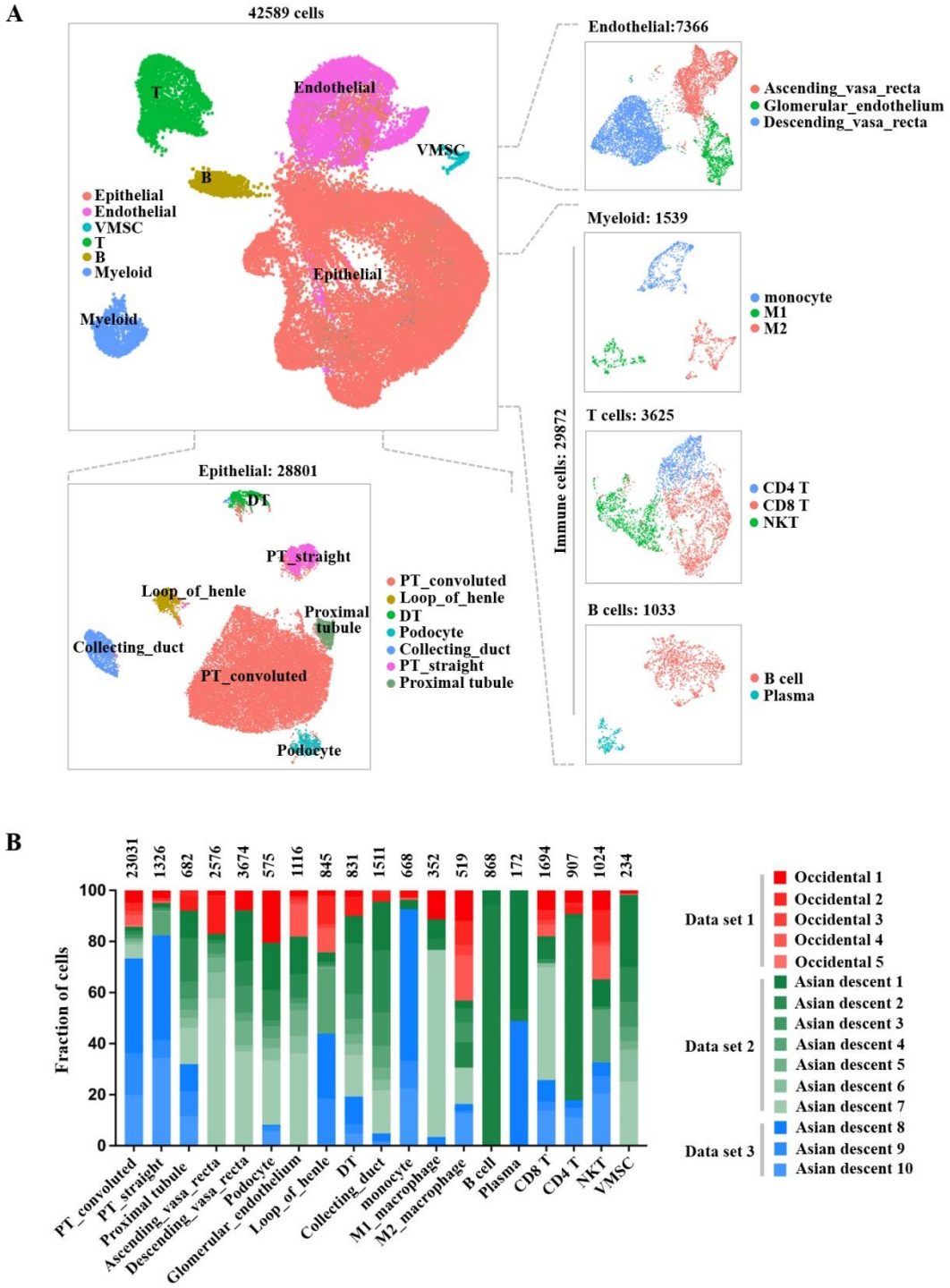


Figure1. Single cell atlas of normal kidney cells. (A). UMAP plots showing 42589 kidney cells to visualize 6 cell clusters (epithelial, endothelial, myeloid, T and B cells) and the corresponding subclusters; each cluster is labeled with different colors and the cell numbers. (B). Barplot showing the fraction of cells for the 19 cell clusters of 10 patients. The number at the top of the barplot

represents the cell number of the corresponding cell clusters. Data set1 including data from 5 patients is labeled red, data set2 including data from 7 patients is labeled green, and data set3 including data from 3 patients is labeled blue.

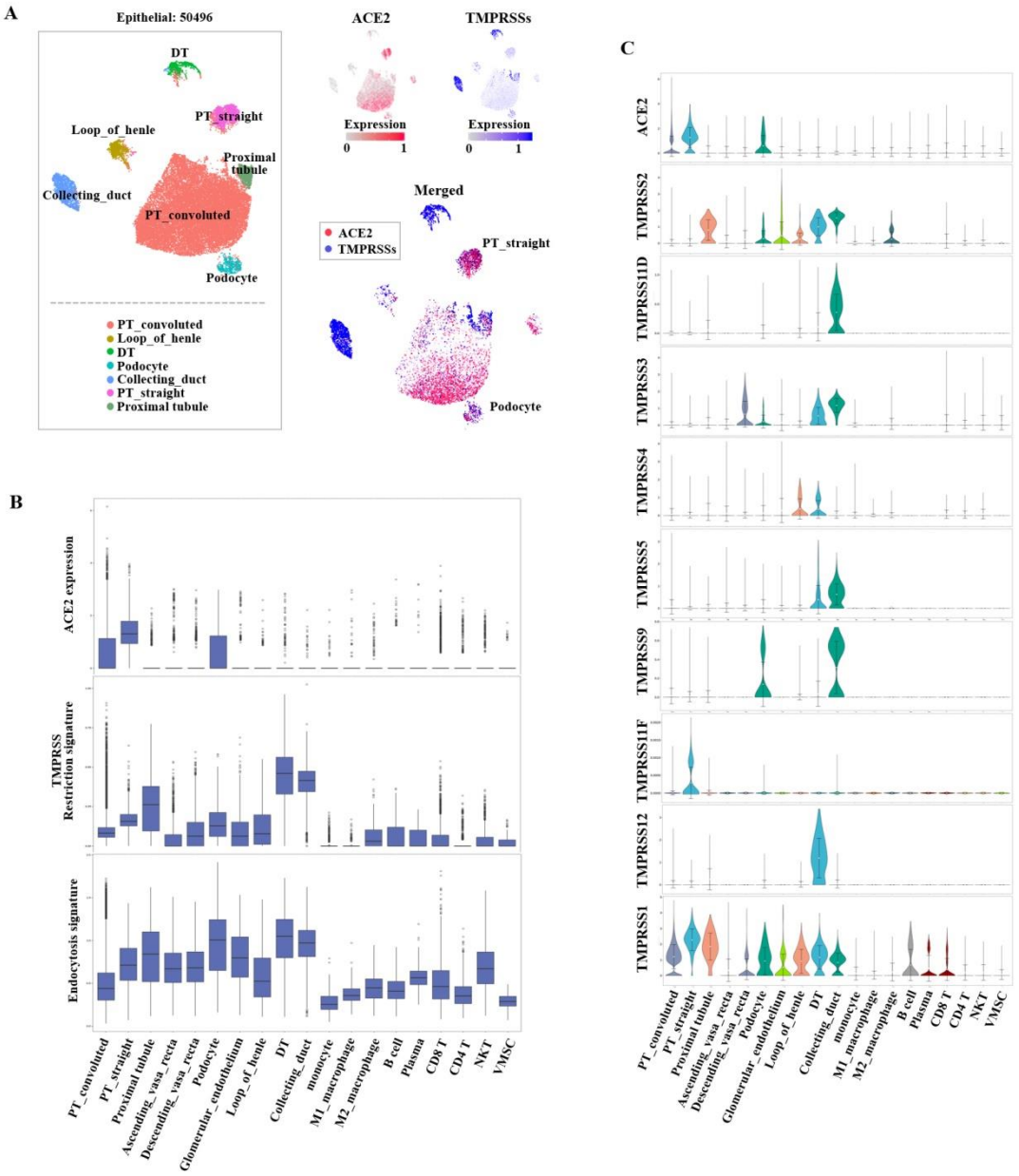


Figure2. Single cell atlas of renal epithelial cells and the expression of ACE2 and functional genesets. (A). UMAP plot showing 7 subclusters of renal epithelial cells and the expression of ACE2(red) and TMPRSSs genes(blue), the merging image showing the co-expression of ACE2 and TMPRSSs genes especially in proximal tubules and podocytes. (B). Boxplot showing the expression of ACE2, and the mean expression of TMPRSSs family genes and endocytosis related genes in 19 clusters of renal cells. (C). Violin plot showing the expression of ACE2 and each gene of TMPRSSs family genes in 19 clusters of renal cells, and the expression is measured as the log2

(TP10K+1) value.

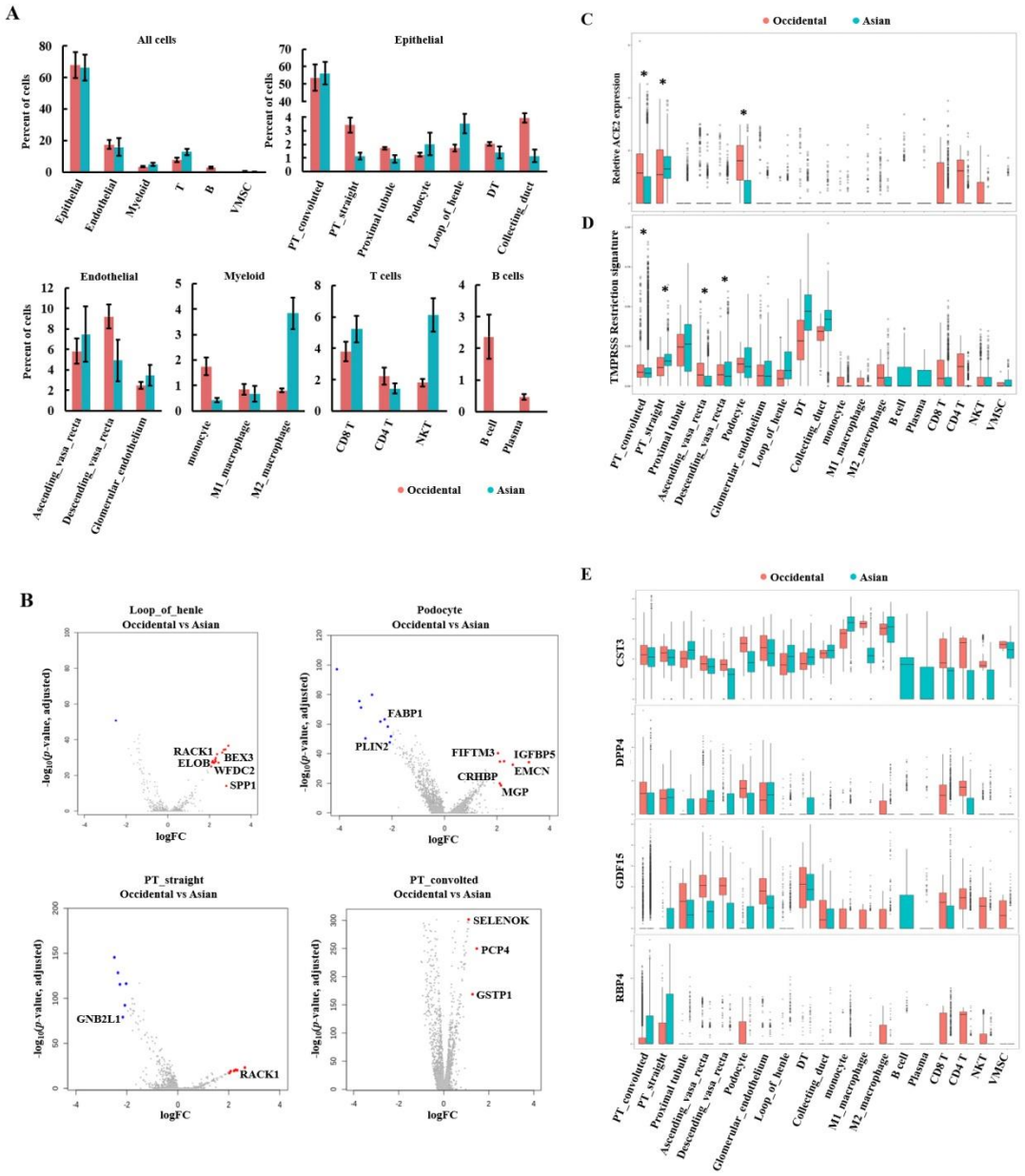


Figure3. The different expression genes between Asian and Occidental populations. (A). Boxplot showing the proportion of cells in different clusters of Accidental and Asian donors. (B). Volcano plot showing the different genes between Asian and Occidental donors in 4 clusters (loop of henle, podocyte, PT_straight and PT_convoluted). The red dots represent the genes expressed in Occidental donors higher than Asian donors and the blue dots represent the genes expressed in occidential donors lower than Asian donors (P< 0.01). (C). Boxplot plot showing the expression difference of ACE2 between Asian and Occidental donors (* representing P<0.01). (D). Boxplot showing the

expression difference of TMPRSSs family genes between Asian and Occidental donors. (E). Boxplot showing the expression difference of kidney injury related genes between Asian and Occidental donors.

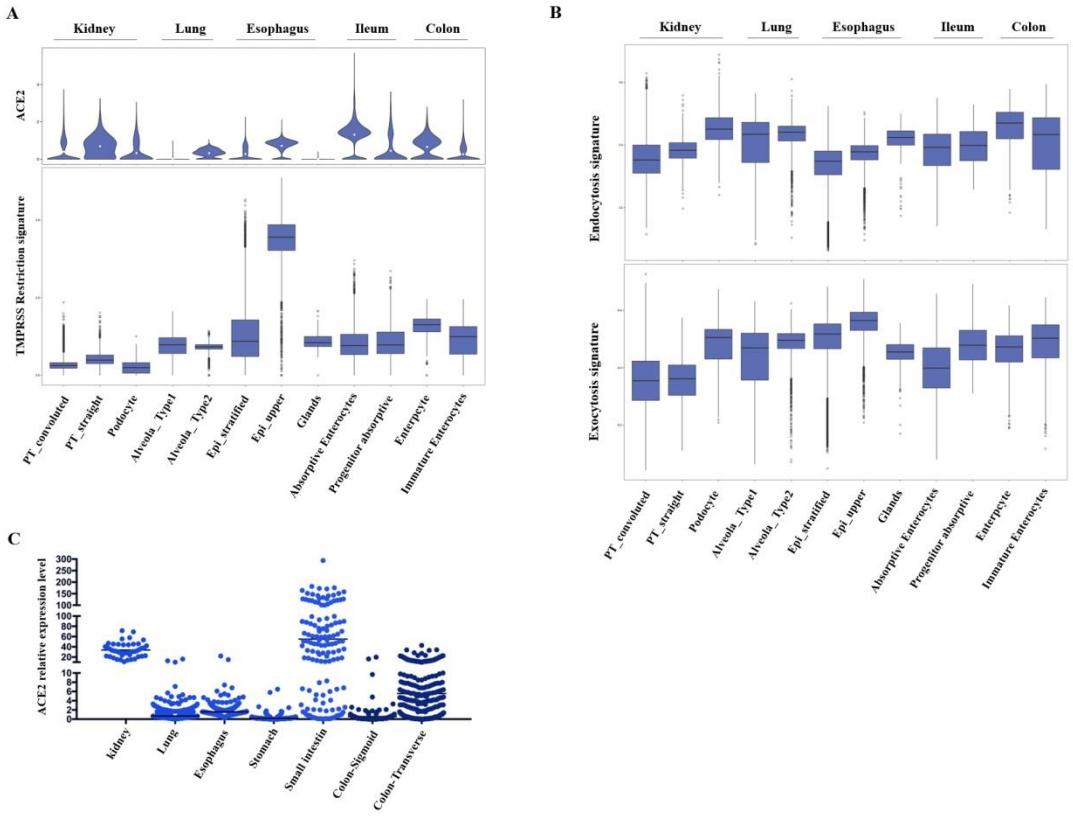


Figure4. The expression level of ACE2 and functional genesets in different tissues. (A).Violin plot showing the expression of ACE2 and mean expression of TMPRSSs family genes in different tissues (kidney, lung, esophagus, small intestine and colon); the expression is measured as the log2 (TP10K+1) value. (B).Boxplot showing the expression of endocytosis and exocytosis related functional genes in different tissues. (C).Scattered dotplots showing the RNA relative expression from GTEx database in different tissues. The Horizontal line represents the mean expression level of ACE2.

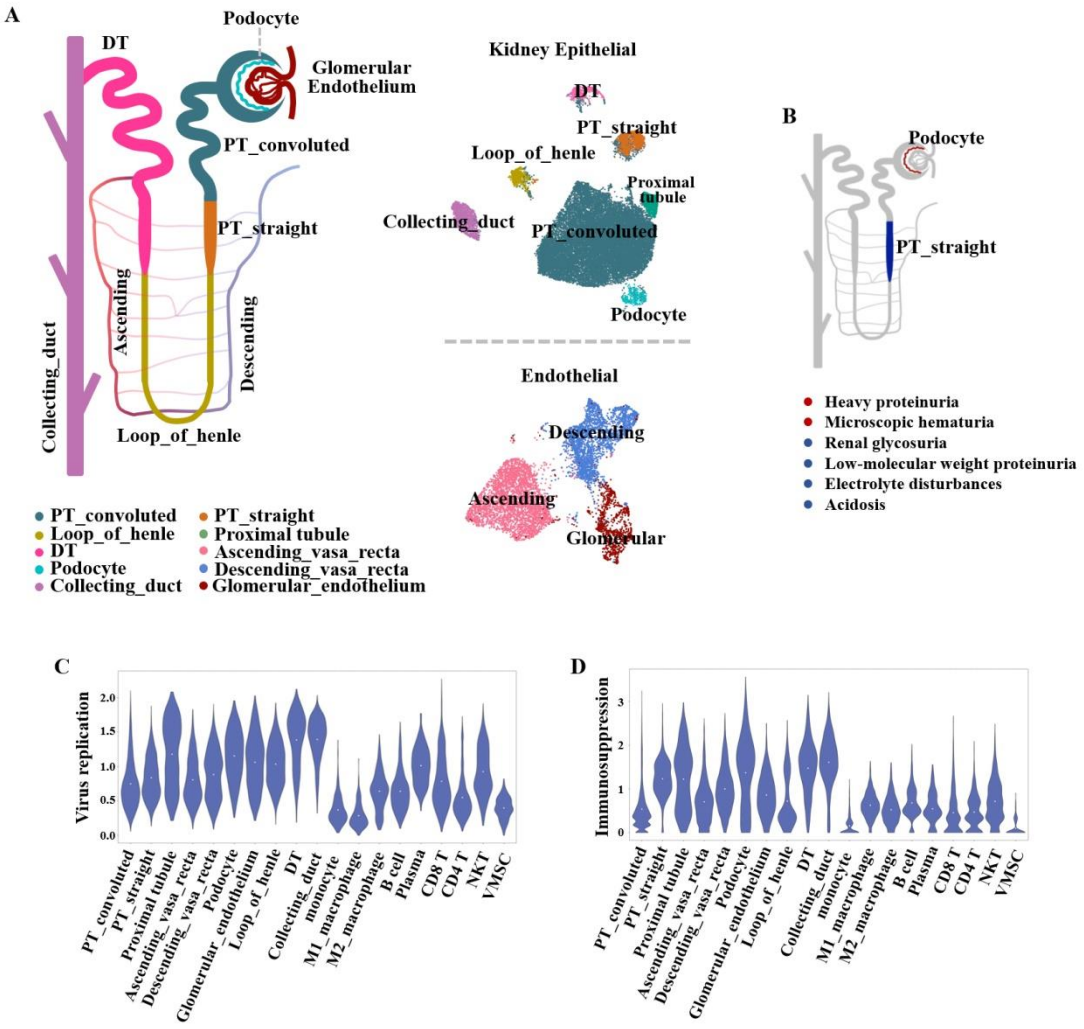
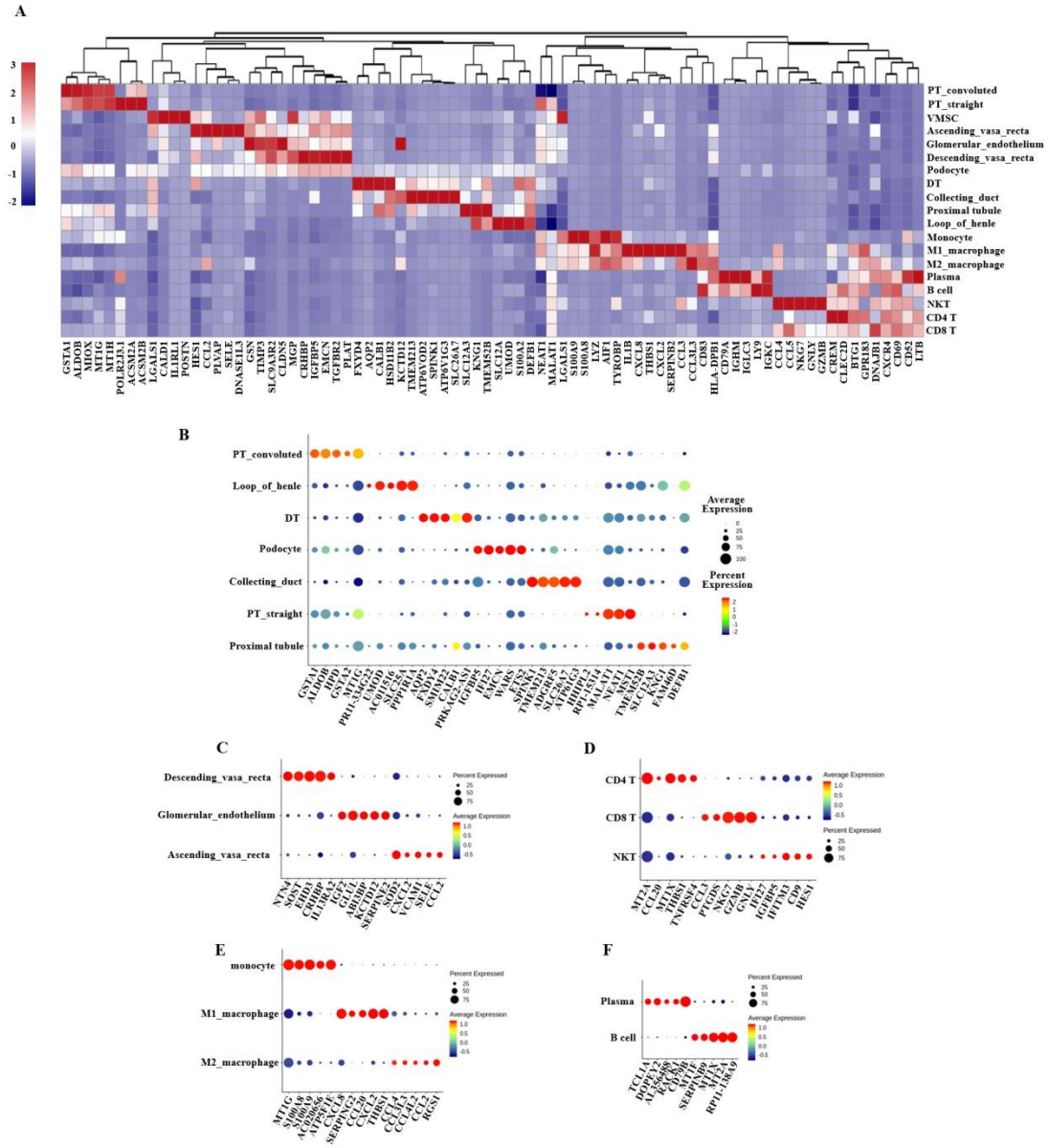
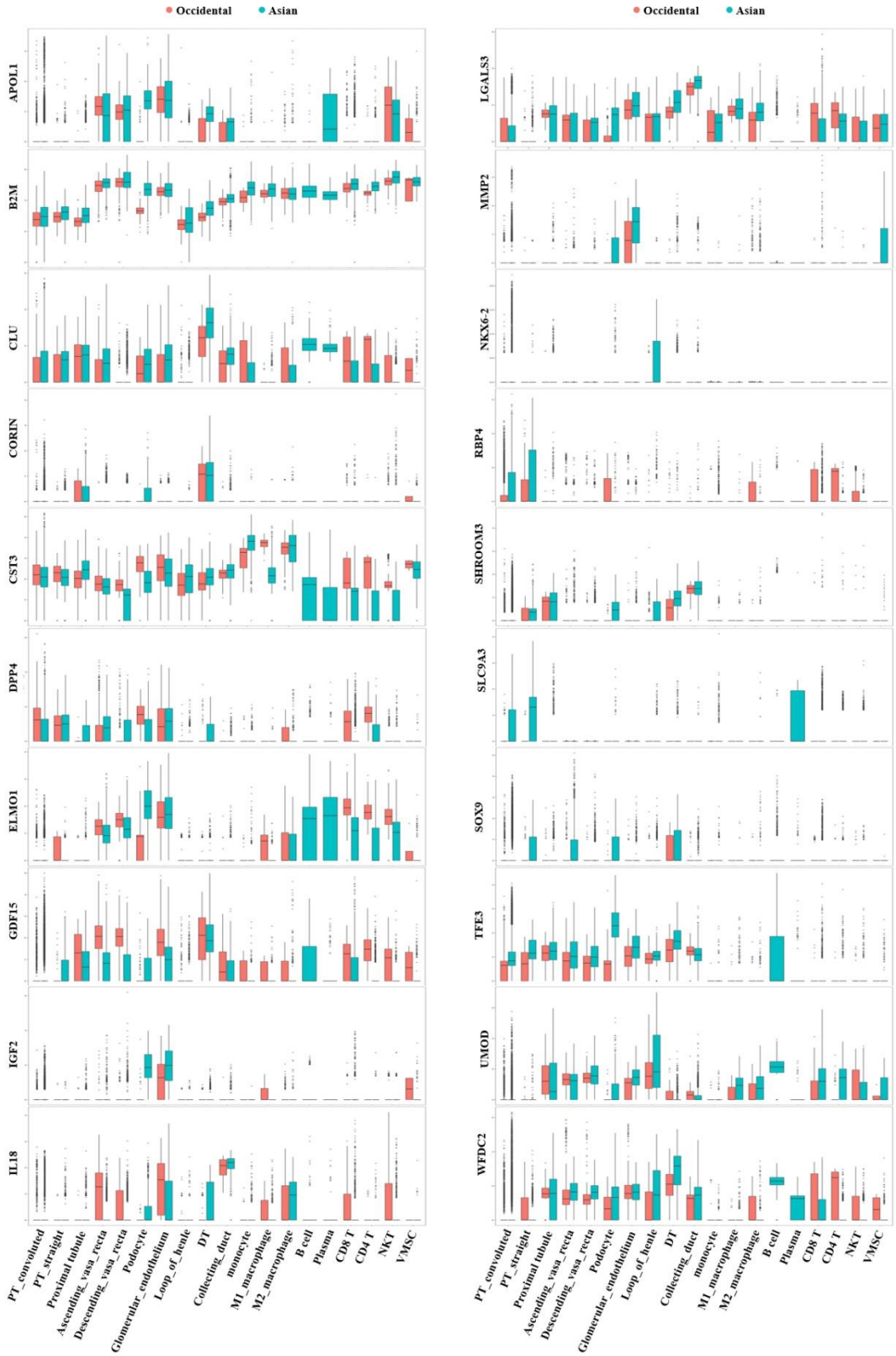


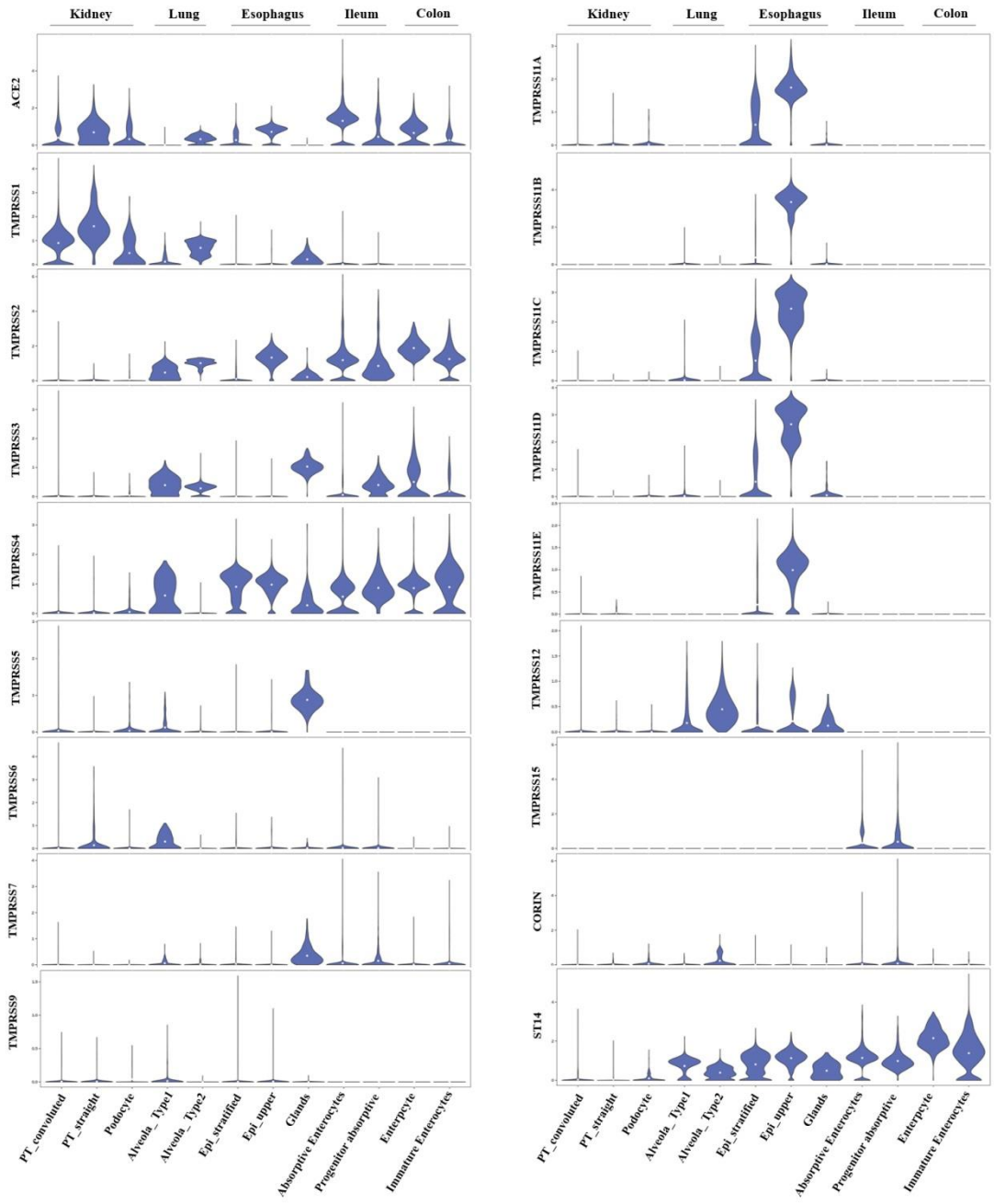
Figure5. The outline of nephron and symptoms of kidney injury at different sites. (A). Sketch map showing the position of subclusters of renal epithelial and endothelial cells. (B). Sketch map showing the different clinical symptoms caused by different cell cluster injury. (C). Violin plot showing the mean expression of functional genesets related to virus replication. (D). Violin plot showing the mean expression of functional genesets related to innate immunity of host cells. The genes are the targets of the virus to suppress the host cell immunity.



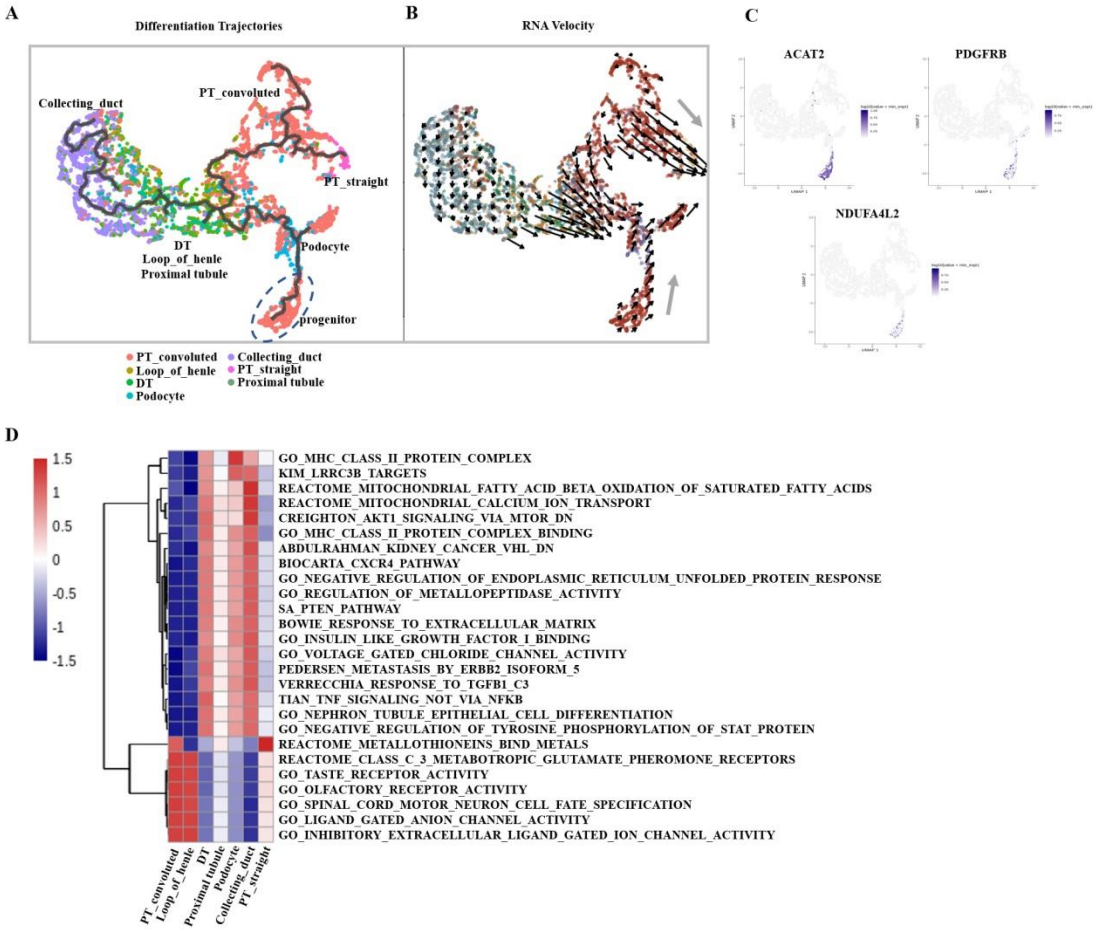
Supplementary Figure 1. Cell markers of the subclusters. (A). Heatmap showing the marker genes of 19 cell clusters which are highlighted . (B). Dot plot showing the marker genes of the subclusters of renal epithelial cells. The size of each dot represents the proportion of cells expressing the corresponding genes and the color of dot represents the expression degree of the marker genes. (C). Dot plot showing the marker genes of the subclusters of renal endothelial cells. (D). Dot plot showing the marker genes of the subclusters of renal T cells. (E). Dot plot showing the marker genes of the subclusters of renal myeloid cells. (F). Dot plot showing the marker genes of the subclusters of renal B cells.



Supplementary Figure 2. The expression difference of genesets related to kidney injury. Boxplot showing the expression difference of kidney injury related genes between Asian and occidential donors, the expression is measured as the log2 (TP10K+1) value.



Supplementary Figure 3. The expression level of ACE2 and TMPRSSs family genes in different tissues. Violin plot showing the expression of ACE2 and TMPRSSs family genes in different tissues(kidney, lung, esophagus, small intestine and colon) , the expression is measured as the log2 (TP10K+1) value.



Supplementary Figure 4. The evolution and signaling pathways of renal epithelial cells. (A). Monocle pseudeotime trajectory predicting the potential evolution of renal epithelial cells. (B). Velocity field showing in the UMAP plot among epithelial cells, the direction of arrows representing the future sate of cells. (C). Feature plots showing the expression level of ACAT2, PDGFRB and NDUFA4L2. (D). The heatmap showing the enrichment of different cell pathways in each cell cluster of renal epithelial cells.