

Single-cell transcriptional profile of ACE2 in healthy and failing human hearts

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Dear Editor,

The coronavirus disease 2019 (COVID-19) pandemic, which is caused by SARS-CoV-2, has gained serious attention from medical practitioners around the world in the past few months. Approximately 20% of critically ill COVID-19 patients were reported to have suffered myocardial injury. The specific mechanism of this pathology requires further investigation (Yang et al., 2020). It was reported that SARS-CoV-2 invades alveolar epithelial cells via the angiotensin converting enzyme (ACE2) cell receptor in the lungs, but whether cardiomyocytes can be directly infected with SARS-CoV-2 via ACE2 remains controversial (Zhou et al., 2020). The rapid development of single-cell RNA sequencing (scRNA-seq) in recent years has enabled us to analyze the transcriptome with a single-cell resolution. In this study, we analyzed the scRNA-seq data from human hearts in the Gene Expression Omnibus public database to investigate the expression profile of ACE2 and the expression profiles of other genes in different types of human heart cells.

Briefly, the scRNA-seq data from the heart samples of 14 healthy adults and four heart failure patients (GSE109816, GSE121893), as well as those from 20 fetal hearts (GSE106118), were included in the analysis. The Seurat R package (v.3.1.2) was used to perform data integration and clustering analysis to approximately 14,000 single cells (Stuart et al., 2019). The clusters were annotated based on canonical cell-type marker genes (Han et al., 2018; Volz et al., 2015). The main cell types of the heart, including cardiomyocytes and cardiac fibroblasts, were identified in both the hearts of adult humans and the hearts of human embryos (Figure 1A, B, and H). Consistent with the results of previous studies analyzing the expression levels of ACE2 in cardiomyocytes (Xu H et al., 2020), we detected that about 10% of cardiomyocytes had ACE2 expression (Figure 1C and D). The expression levels of ACE2 in the atria and ventricles were not significantly different (Figure 1F).

More interestingly, we found that nearly 30% of pericytes expressed ACE2 and that the expression level of ACE2 in pericytes was much higher than that in cardiomyocytes (Figure 1E). Our results are consistent with the results of a previous study that suggested ACE2 is highly expressed in pericytes in the heart tissue of humans (Chen et al., 2020). Furthermore, both the expression level and percentage of

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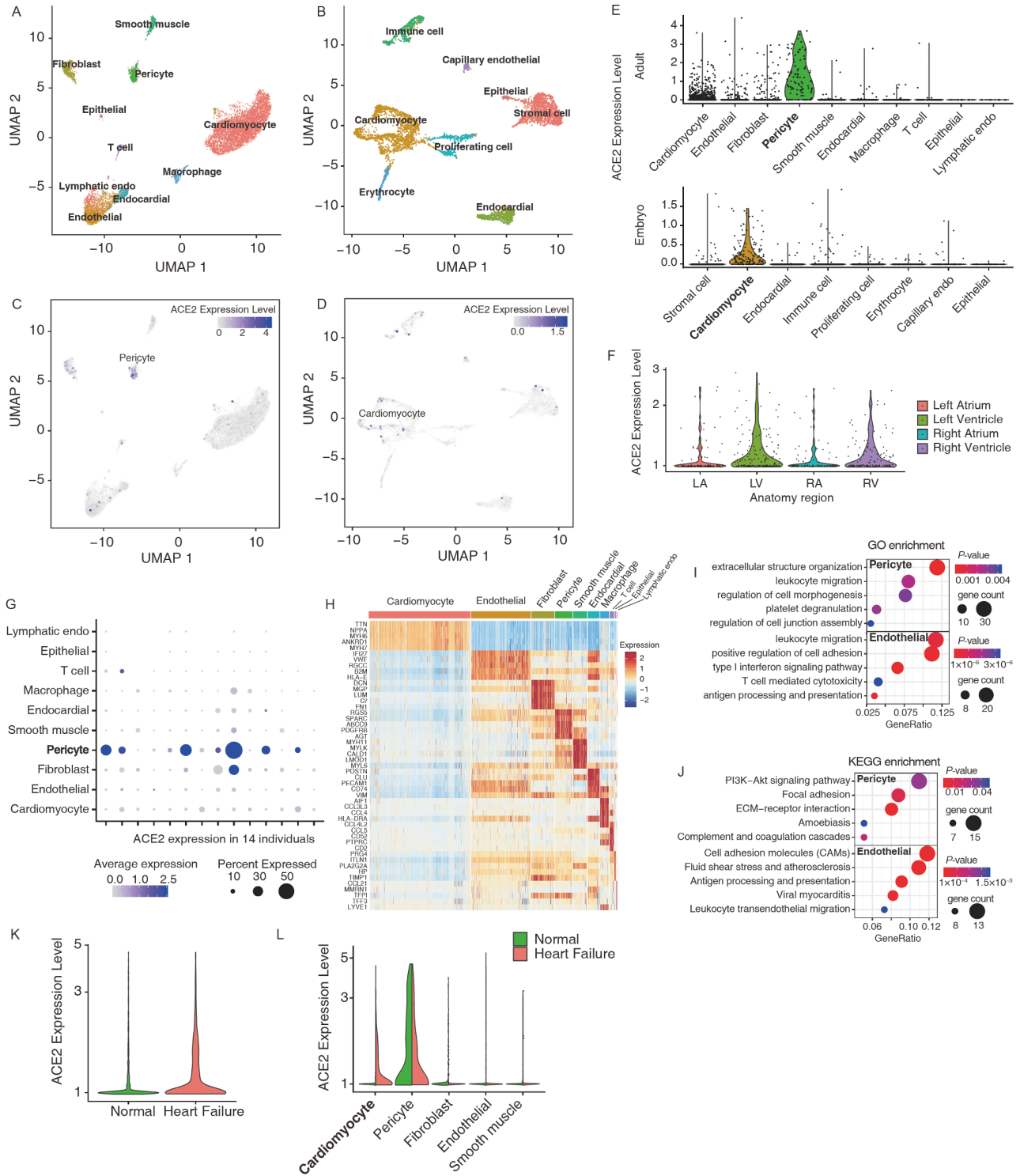


Figure 1 Characterization of the single-cell RNA expression transcriptional profile of ACE2 in the heart. A and B, Uniform manifold approximation and projection (UMAP) dimension reduction showing the results of unsupervised clustering for all filtered cells from the heart of an adult human (A), $n=10,314$, and from the heart of a human embryo (B), $n=4,617$. The cell types corresponding to each cluster are highlighted with their names labeled. C and D, Expression patterns of ACE2 in single cells from the heart of an adult human (C) and from the heart of a human embryo (D) under UMAP dimension reduction. Each dot represents a cell with a color indicating the normalized level of ACE2 expression. E, Violin plots showing the expression profiles of ACE2 in different cell types of the hearts of both human adults and embryos. F, Violin plot of normalized ACE2 expression levels in different anatomical regions of the heart in the human embryo. G, Expression profiles of ACE2 in different cell types of hearts from 14 adult human individuals. H, Heatmap showing the expression levels of the top 5 marker genes in each cluster from the hearts of adult humans. I and J, GO (I) and KEGG (J) enrichment using marker genes (log-fold change >0.5) of pericyte and endothelial cells from the heart of an adult human. For each group, five of the top 20 items are presented. K, Expression levels of ACE2 in normal individuals ($n=14$) and heart failure patients ($n=4$). L, ACE2 expression levels in different cell types of hearts for 14 adult human individuals and four heart failure patients.

pericytes were markedly higher in six of the adults than they were in the other eight adults (Figure 1G). However, whether ACE2 expression is heterogeneous in the general population requires further study. Since pericytes express ACE2 the highest among other cells and endothelial cells are closely related to pericytes in structure and function (Chiaverina et al., 2019; Stallcup, 2018), we next used markers of pericytes and endothelial cells to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis using the ClusterProfiler R package (v.3.15.2). As shown in Figure 1I and J, pericytes feature signaling pathways related to extracellular structure interactions and complement and coagulation cascades, while endothelial cells feature interferon and antigen presentation signaling pathways. These findings illustrate the importance of the roles that pericytes and endothelial cells play and their potential interactions, such as the joint regulation of leukocyte migration and cell adhesion.

Our results suggest that ACE2 transcripts in the heart are mainly concentrated in pericytes and not in cardiomyocytes. Moreover, the heart biopsy sample of a COVID-19 patient who died of cardiac arrest showed only a few interstitial mononuclear inflammatory infiltrates (Xu Z et al., 2020). Both of these findings suggest that the virus attacks mainly pericytes rather than cardiomyocytes in the healthy human heart and that it does so via ACE2. The classic paradigm of viral myocarditis cannot be directly applied to myocardial injury in the general population. Pericytes are contractile vascular mural cells that closely interact with endothelial cells, and together, these cells play an important role in regulating the vascular tone of coronary microcirculation. Similarly, disorders of pericytes alone can also lead to microvascular constriction, as it is in a no-reflow phenomenon (O'Farrell and Attwell, 2014). Appropriately, we propose that SARS-CoV-2 might attack pericytes via ACE2 and result in myocardial disorder in some patients.

A recent study suggested an incidence of thrombotic events including venous and arterial thrombosis of 31% in COVID-19 patients (Klok et al., 2020). Most studies indicate that thrombosis is closely related to endothelial dysfunction (Escher et al., 2020; Sardu et al., 2020), which is a finding that is in agreement with our finding that approximately 3% endothelial cells show expression of ACE2 (Figure 1E and G). Interestingly, our results show that the expression profile of pericytes features pathways related to coagulation cascades, indicating potential participation of pericytes in thrombotic events. Further studies should be performed to elucidate the roles of endothelial cells and pericytes in venous and arterial thrombosis in the pathogenesis of COVID-19.

In addition, we found that 10% of cardiac fibroblasts express ACE2 (Figure 1G). Cardiac fibroblasts secrete collagens and other extracellular matrix proteins to promote

fibrosis and diastolic dysfunction (Travers et al., 2016). Furthermore, activated fibroblasts may also regulate cardiomyocyte survival and hypertrophy by secreting growth factors or miRNA-containing exosomes, which contribute to myocardial injury (Humeres and Frangogiannis, 2019). We propose that dysregulation of cardiac fibroblasts might cause worsening of myocardial injury in heart disease patients with COVID-19.

Multiple mechanisms can lead to myocardial injury. The most suspected ones in COVID-19 are hypoxemia, hypotension, inflammatory and stress responses, and pre-existing heart diseases (Thygesen et al., 2019). Disseminated intravascular coagulation was observed in approximately 70% of COVID-19 non-survivors, and it is reasonable to suspect that thrombosis can occur within small tributaries of the coronary arteries and result in myocardial ischemia (Tang et al., 2020). The aforementioned pericyte-related microvascular disorder might also contribute to ischemia and result in myocardial injury. As the expression level of ACE2 is relatively low in cardiomyocytes, the presence of viral myocarditis in COVID-19 patients is highly suspected. In addition, only subclinical diastolic impairment without systolic involvement was observed in patients infected with SARS-CoV, which is a close relative to SARS-CoV-2. These results are inconsistent with the clinical characteristics of viral myocarditis (Li et al., 2003). Therefore, it is possible that the cardiac injury shown in COVID-19 is initiated from an attack on pericytes via ACE2.

Cardiovascular disease patients are more likely to be infected by SARS-CoV-2 than those without cardiovascular disease, which may aggravate the disease progression (Chen et al., 2020). Therefore, we further analyzed the expression of ACE2 in heart failure patients. Our results showed that ACE2 expression was significantly increased in patients with heart failure compared to healthy individuals (Figure 1K, $P < 0.001$, Wilcoxon test). To explore the potential mechanisms contributing to this finding, we examined the expression of ACE2 in the different cells of the heart for heart samples from healthy individuals and heart failure patients and compared the results between these two groups. We found that the expression of ACE2 in cardiomyocytes was significantly increased compared to the other cells in the heart failure patients (Figure 1L). These results suggest that the cardiomyocytes of heart failure patients may be directly attacked by the virus and that this may contribute to the development of myocardial injury and severe conditions.

A recent study showed that ACE2 is highly expressed in pericytes in the heart (Chen et al., 2020). Our results were produced from analyses that included even more heart samples, and our results further confirmed that ACE2 is highly expressed in pericytes in the heart. We speculate that SARS-CoV-2 mainly attacks pericytes via ACE2, which leads to coronary microvascular disorder and myocardial

injury in COVID-19 patients. Moreover, our results also indicated that the expression of ACE2 in pericytes for six adults was higher than that for the other eight adults, which implies that the heterogeneity of ACE2 expression in the hearts of those in the general population is worth further exploration. In addition, consistent with the results of a previous study, we found that ACE2 expression is increased in heart failure patients (Chen et al., 2020). More importantly, we further showed that the expression of ACE2 in the cardiomyocytes of heart failure patients is significantly higher than that in other heart cells of these patients. This finding suggests that heart failure patients infected by the SARS-CoV-2 virus may suffer viral myocarditis and severe myocardial injury by direct attack on their cardiomyocytes. These results imply that previous heart disease is closely related to whether people infected by the SARS-CoV-2 virus will develop myocardial damage.

In conclusion, the expression of ACE2 in cardiomyocytes is significantly increased in heart failure patients compared to healthy individuals. Heart failure patients may have a high risk for developing viral myocarditis and severe myocardial injury due to cardiomyocyte dysfunction.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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