Antihypertensive drug treatment and susceptibility to SARS-CoV-2 infection in human PSC-derived cardiomyocytes and primary endothelial cells

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SUMMARY

The pathogenicity of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been attributed to its ability to enter through the membrane-bound angiotensin-converting enzyme 2 (ACE2) receptor. Therefore, it has been heavily speculated that angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) therapy may modulate SARS-CoV-2 infection. In this study, exposure of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) and human endothelial cells (hECs) to SARS-CoV-2 identified significant differences in protein coding genes involved in immunity, viral response, and cardiomyocyte/endothelial structure. Specifically, transcriptome changes were identified in the tumor necrosis factor (TNF), interferon α/β, and mitogen-activated protein kinase (MAPK) (hPSC-CMs) as well as nuclear factor kappa-B (NF-κB) (hECs) signaling pathways. However, pre-treatment of hPSC-CMs or hECs with two widely prescribed antihypertensive medications, losartan and lisinopril, did not affect the susceptibility of either cell type to SARS-CoV-2 infection. These findings demonstrate the toxic effects of SARS-CoV-2 in hPSC-CMs/hECs and, taken together with newly emerging multicenter trials, suggest that antihypertensive drug treatment alone does not alter SARS-CoV-2 infection.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for coronavirus disease 2019 (COVID-19), has been the source of a widespread pandemic of respiratory illness. In its most virulent form, SARS-CoV-2 can lead to severe pneumonia, pulmonary edema, and acute respiratory distress syndrome, thereby being predominantly characterized as a pulmonary disease (Chen et al., 2020). However, growing clinical evidence has suggested the multi-systemic spread of COVID-19 into various organs, including the lungs, nervous system, and most notably the heart (Puntnmann et al., 2020; Rajpal et al., 2020; Shi et al., 2020). Newly emerging studies have described malignant arrhythmias (ventricular tachycardia/ventricular fibrillation) (Guo et al., 2020), cardiac arrest (Thapa et al., 2020), and cardiac injury (Lala et al., 2020; Shi et al., 2020) as complications and contributing factors to in-hospital mortality of COVID-19 patients, suggesting a strong association between viral infection and potential cardiac damage. Hospitalized patients with COVID-19 who sustain cardiac injury have been shown to require mechanical ventilation, have increased rates of complications (acute respiratory distress syndrome, acute kidney injury, and coagulation disorders), and have a significantly higher mortality compared with patients without cardiac injury (Shi et al., 2020). Furthermore, it has been reported that individuals with underlying comorbidities, including cardiovascular disease, are particularly susceptible to SARS-CoV-2 infection, cardiac injury-related events, and increased mortalities (Wang et al., 2020; Yang et al., 2020). Cardiovascular disease is associated with endothelial dysfunction (Perticone et al., 2001), and severe SARS-CoV-2 infections can result in diffuse endothelial inflammation and vascular disease (see reviews by Pons et al., 2020; Siddiqi et al., 2021), which, in turn, could propagate the multi-systemic effects, thromboembolic complications, and hidden cardiac consequences of SARS-CoV-2. Therefore, growing concern has been expressed about the effects of SARS-CoV-2 on the heart and the susceptibility of developing COVID-19 in certain patient populations.

SARS-CoV-2 infects the host through membrane-bound angiotensin-converting enzyme 2 (ACE2) receptors. Interaction between the viral spike glycoprotein and the aminopeptidase ACE2 receptor facilitates SARS-CoV-2 entry into human cells (Xu et al., 2020); therefore, organs such as the lungs, kidneys, intestine, and heart may be at high risk for potential infection due to their expression of the
ACE2 receptor (Zou et al., 2020). Physiologically, ACE2 is a counter-regulatory protease to the renin-angiotensin-aldosterone system (RAAS) and functions to metabolize angiotensin II into angiotensin 1,7, a vasodilator peptide (Figure 1) (Oudit et al., 2003). Angiotensin II receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs) are first-line medical treatments offered to patients with hypertension, heart failure, and chronic kidney disease, allowing neurohormonal regulation of the RAAS system. Previously, it was demonstrated that ACE2 mRNA expression increased with ACEI and ARB therapy in rodent hearts (Ferrario et al., 2005). Therefore, it was heavily suspected that the use of ACEIs or ARBs may affect the susceptibility of certain patients to SARS-CoV-2 infection. However, more recent data show conflicting evidence regarding the correlation between SARS-CoV-2 disease severity and antihypertensive drug therapy.

Two population-based studies from Lombardy, Italy, and New York City, United States, confirmed there was no evidence that ACEIs or ARBs affected the susceptibility (Mancia et al., 2020) or disease severity (Reynolds et al., 2020), respectively, of COVID-19 patients. However, recent single nucleic RNA sequencing (RNA-seq) of cardiac tissue from patients with aortic stenosis and heart failure showed that patients treated with ACEIs had significantly higher ACE2 expression compared with patients treated with ARBs (Ncin et al., 2020), suggesting that ACEIs may aggravate cardiovascular pathology in patients with SARS-CoV-2 infection, through the upregulation of ACE2 receptors. Conversely, two retrospective studies from China concluded that hospitalized COVID-19 patients with hypertension had a lower risk of all-cause mortality with the use of ACEI/ARB therapy compared with non-users (Zhang et al., 2020) and that patients receiving ACEI/ARB therapy had a lower rate of severe disease and decreased peak viral load compared with non-ARB/ACEI-treated patients (Meng et al., 2020). Therefore, there is conflicting evidence regarding the susceptibility of certain individuals to SARS-CoV-2 and the risk factors that predispose patients to increased cardiac damage. Current published data are limited by human sample size, sample availability, and confounding effects from patient comorbidities and prescription use. Furthermore, the results from these studies only account for late-stage disease findings instead of...
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early-stage or disease-onset mechanisms. As with most cardiac disease modeling, it is difficult to obtain human primary cardiomyocytes; therefore, to circumvent these drawbacks and to directly examine the primary effects of SARS-CoV-2, in this study we used both human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) and primary hECs to (1) identify the expression levels of genes implicated in ACEI/ARB therapy and SARS-CoV-2 infection during hPSC-CM differentiation, (2) identify differences in the transcriptomic signatures of SARS-CoV-2-infected hPSC-CMs and hECs, and (3) determine potential changes in SARS-CoV-2 viral load with two widely prescribed antihypertensive medications, losartan and lisinopril.

RESULTS

hPSC-CMs express genes involved in antihypertensive drug treatment and SARS-CoV-2 viral entry

Recently, growing evidence has suggested that SARS-CoV-2 has a cardiac-specific tropism; however, the degree of tropism for specific cardiac cell types and the consequences of the viral load on the heart are still largely unknown. To determine the role cardiomyocytes play in SARS-CoV-2 infection and to characterize human induced pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) as a model for SARS-CoV-2 cell entry, we assessed the expression levels of the SARS-CoV-2 entry receptor (ACE2), entry co-receptor neuropilin 1 (NRP1), viral entry proteases (transmembrane serine protease [TMPRSS2], furin [FURIN], cathepsin L [CTSL] and cathepsin B [CTSB]), endosomal membrane trafficking protein coding gene FYVE-type zinc finger-containing phosphoinositide kinase (PIKFYVE), and genes targeted by ACEI/ARB therapy within the RAAS pathway (ACE and angiotensin II receptor type 1 [AGTR1]). Transcriptome analysis from bulk RNA-seq of hPSC-CMs during cardiomyocyte differentiation showed that ACE2 expression increased over time, peaking around day 9 in culture, while the expression pattern of ACE remained relatively low and constant (Figure 2A). The cell surface receptors AGTR1 and NRP1, as well as CTSL, showed increased expression at day 9, while TMPRSS2 expression decreased over time (Figure 2A). To further validate the relevant SARS-CoV-2 entry markers in TNNT2-positive cardiomyocytes, uniform manifold approximation and projection (UMAP) plots from single-cell RNA-seq analysis of hPSC-CMs were analyzed and demonstrated that ACE2 expression was detected early in cardiomyocyte development (day 14 versus day 45), whereas ACE and AGTR1 expression remained low over time (Figure 2B). Although TMPRSS2 was not detected in hPSC-CMs at the single-cell level, endosomal CTSL, CTSB, FURIN, and PIKFYVE, all involved in the endocytosis of viral fusion proteins (Kang et al., 2020; Riva et al., 2020), were identified on days 14 and 45 of hPSC-CM differentiation. These results suggest that human cardiomyocytes are susceptible to SARS-CoV-2 infection; however, viral entry may not involve the cell surface serine protease TMPRSS2. This is in contrast to other human beta-coronaviruses (e.g., SARS-CoV and Middle East respiratory syndrome [MERS]-CoV), which have limited reports of cardiovascular involvement (Xiong et al., 2020).

SARS-CoV-2 infection results in significant transcriptional changes in hPSC-CMs and hECs

Given that SARS-CoV-2 has been detected in human cardiomyocytes (Pesaresi et al., 2020), it is unclear whether injury to the myocardium is a result of direct viral infection or secondary to systemic inflammation and hypoxia. To begin addressing this question, we first examined the transcriptional changes associated with direct SARS-CoV-2 exposure in hPSC-CMs. Since these cells are devoid of any systemic or neurohormonal response, they provide a suitable model for testing the primary effects (as opposed to secondary or compensatory effects) of viral infection. Therefore, hPSC-CMs were infected with SARS-CoV-2 at 0.1 MOI for 24 h (Figure S1A), at days 9–14 of hPSC-CM differentiation in culture (as this was the time interval when ACE2 expression was highest; p = 0.008, one-way ANOVA, day 5 versus day 9; Figure 2A), and subsequently subjected to RNA-seq. Transcriptome analysis showed significant differentially expressed genes (DEGs) (1,160 genes, q < 0.05) between SARS-CoV-2-infected hPSC-CMs and uninfected controls (Figure 3A). STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) network analysis identified significant enrichment of protein coding genes involved in three key proinflammatory and antiviral signaling pathways in infected hPSC-CMs compared with controls: tumor necrosis factor (TNF), interferon alpha/ beta (IFN-α/β), and mitogen-activated protein kinase (MAPK) (Figure S1B). When differentially regulated

Figure 2. Expression profiles of select ACEI/ARB and SARS-CoV-2 targeted genes in human iPSC-CMs

(A) Retrospective analysis of published bulk RNA-seq (Churko et al., 2018) in hPSC-CMs during differentiation demonstrates that the expression of ACE2, AGTR1, CTSL, and NRP1 increases over time, whereas human ACE and TMPRSS2 expression remains low during cardiac differentiation. Data are expressed as mean ± SEM (N = 3 independent cardiac differentiations).

(B) UMAP plots from previously reported single-cell RNA-seq (Churko et al., 2018) of TNNT2-positive hPSC-CMs, at days 14 and 45, display expression profiles for ACE, ACE2, AGTR1, TMPRSS2, FURIN, CTSL, CTSL, NRP1, and PIKFYVE.
Figure 3. Acute transcriptional effects of SARS-CoV-2-infected hPSC-CMs and hECs

(A) Heatmap analysis generated from bulk RNA-seq showing DEGs (1,160 genes, q < 0.05) between SARS-CoV-2-infected and -uninfected hPSC-CMs at 24 h post infection (N = 3 independent cardiac differentiations per condition).

(B) Transcriptional expression profiles from bulk RNA-seq of control SARS-CoV-2-uninfected (red) and -infected (orange) hPSC-CMs depicting upregulated and downregulated genes involved in immunity and viral response, SARS-CoV-2 entry, and cardiomyocyte structure and function (empirical Bayes moderated t test, *p < 0.05, **p < 0.005, ***p < 0.0005, N = 3 independent differentiations).

(C) Heatmap analysis generated from bulk RNA-seq of hECs demonstrating DEGs (845 genes, p < 0.05) between SARS-CoV-2-infected and -uninfected cells (N = 3 independent endothelial cell expansions).

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genes from SARS-CoV-2-exposed hPSC-CMs were further subdivided (Figure 3B), genes associated with innate immunity, viral response, and infection were found to be significantly upregulated (REL, TNAIP3, NFKIBZ, and BCL6). Genes involved in the SARS-CoV-2 entry pathway were predominantly unaltered, with the exception of CTSL. Upregulation of genes associated with cellular stress response (ATF3) and apoptotic cell death (NR4A1 and FOS) were also observed in infected hPSC-CMs. Interestingly, significant transcriptional changes were identified in genes encoding cardiomyocyte structure (MYZAP) and sarcomeric proteins (TMOD1, TNNT2, and TNNT1) as well as genes associated with cardiomyocyte function (HBEGF, KCNJ8), metabolism (AKT), and cardiac development (TBX5, HRG1, and NOTCH1). Overall, pathway analysis identified genes associated with cellular respiration as significantly enriched, while antioxidant and muscle contraction pathways were significantly downregulated in infected hPSC-CMs (Figure S1C). Gene Ontology (GO) term enrichment analysis further showed upregulation of genes linked to cell death with concurrent downregulation of genes involved in sarcomere structure and the cardiomyocyte contractile machinery.

Given that SARS-CoV-2 infection can result in severe endothelial dysfunction with subsequent inflammation and thrombosis in various organ systems, including the kidneys, heart, lung, and liver (Varga et al., 2020), we next examined the transcriptional changes associated with SARS-CoV-2 infection in primary hECs. Following SARS-CoV-2 infection, transcriptome analysis showed significant DEGs (845 genes, p < 0.05) between infected and uninfected hECs (Figure 3C). Similar to SARS-CoV-2-infected hPSC-CMs, infected hECs also showed significant differences in genes associated with immunity and viral response; however, they did not display significant changes in genes involved with SARS-CoV-2 viral entry (Figure 3D). Interestingly, SARS-CoV-2-infected hECs had downregulated genes involved in the NF-κB pathway (TNFRSF4), which regulates cell apoptosis, cell-cycle progression (ZBTB16), and cell-cell adhesions (PCDH12 and PCDH1). Genes involved in cellular stress response (SGK1) and endothelial disease (ESM1) were found to be significantly elevated. When DEGs in SARS-CoV-2-infected hPSC-CMs were compared with infected hECs, 78 genes common to both cell types were identified (Figure 3E). Within these genes, four enriched gene target groups were identified: viral translation, MORC2 (transcriptional repression in response to DNA damage), SETD1A (histone methylation), and NFRKB (transcriptional regulation and DNA repair) (Figure 3F). Taken together, these results indicate a robust inflammatory response and significant transcriptional changes in SARS-CoV-2-infected hPSC-CMs and hECs.

**Antihypertensive drug treatment in SARS-CoV-2 exposed cells did not alter the susceptibility of hPSC-CMs or hECs to viral infection**

Particular attention has been placed on a specific population of patients who are prescribed antihypertensive medications, such as lisinopril (an ACEI) and losartan (an angiotensin II receptor blocker) because not only do these patients present with underlying cardiovascular disease but there is ongoing debate surrounding the protective and/or harmful use of these therapies due to the possible upregulation of ACE2. Since ACE2 is a key component in SARS-CoV-2 viral entry and an important downstream effector within ACEI/ARB therapy, we first aimed to confirm the importance of ACE2 in SARS-CoV-2 entry in hPSC-CMs. Therefore, we generated an ACE2 knockout (KO) hPSC-CM line (Figure 4A), which showed significantly reduced ACE2 mRNA levels (Figure 4B) and significantly decreased SARS-CoV-2 viral load (Figure 4C) with the use of two US Food and Drug Administration (FDA)-approved primers for SARS-CoV-2 (N1 and N3).

The abolition of SARS-CoV-2 viral transcripts in ACE2 KO hPSC-CMs confirmed the necessity of ACE2 in hPSC-CM SARS-CoV-2 infection. Next, to capture the potential primary effect(s) of antihypertensive therapy on the susceptibility of SARS-CoV-2 infection and viral load, we treated both hPSC-CMs and hECs with either 5 μM lisinopril, 10 μM losartan carboxylic acid (a potent active metabolite of losartan), or DMSO for 36–72 h. To ensure maximal effect of each drug, concentrations above published half maximal inhibitory concentration (IC50) values and treatment time intervals (Burnier, 2001; Desideri et al., 2008; Patchett et al., 1980; Sachinidis et al., 1993) (with no observed cellular toxicity) were used. Comparison between hPSC-CMs treated with lisinopril or losartan revealed negligible differences in gene expression (51–93 genes using an empirical Bayes moderated t test, p < 0.05) and pathways specific to each treatment (Figure 4D). Enriched pathways...
Figure 4. Antihypertensive treatment and susceptibility of hPSC-CMs and hECs to SARS-CoV-2 infection

(A) A schematic of the generation of the ACE2 KO hPSC-CM line.

(B and C) qPCR quantification of (B) ACE2 and (C) SARS-CoV-2 transcript levels in control hPSC-CMs and ACE2 KO hPSC-CMs (N = 3 independent cardiac differentiations, ****p < 0.0001).

(D) Heat map generated from bulk RNA-seq demonstrating DEGs (MarkerFinder algorithm) and enrichment terms for hPSC-CMs (N = 4 independent cardiac differentiations) treated with lisinopril or losartan. Brackets indicate the FDR value for each pathway.

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in lisinopril-treated versus -untreated hPSC-CMs were observed in cholesterol biosynthesis and transcriptional regulation. Accordingly, hECs treated with lisinopril revealed genes involved in estrogen signaling and lipid metabolism, while hECs treated with losartan showed enrichment in genes encoding cholesterol homeostasis and immune function (Figure 4E). To examine the acute effect(s) of SARS-CoV-2 infection in hPSC-CMs and hECs, cells were infected with SARS-CoV-2 at 0.1 MOI for 24 h. Overlapping DEGs in lisinopril- or losartan-treated hPSC-CMs with DEGs in SARS-CoV-2-infected hPSC-CMs revealed nine gene differences to overlap in lisinopril-treated and 22 gene differences in losartan-treated cells, compared with 3,053 genes identified in infected SARS-CoV-2 hPSC-CMs. ACE2 was not identified as a DEG (Figure 4F). To further validate this result, we examined ACE2 expression in drug-treated versus -untreated hPSC-CMs and found no significant difference in ACE2 transcript levels with either lisinopril or losartan (Figure 4G). Overall, this indicates that antihypertensive drug treatment may have a marginal effect on the transcriptional regulation of hPSC-CMs and hECs in vitro.

To investigate the potential effects of antihypertensive therapy with SARS-CoV-2 infection, we utilized both hPSC-CMs and hECs to determine if treatment with antihypertensive drugs and subsequent infection with SARS-CoV-2 alters (1) expression of genes/proteins associated with viral entry, and (2) susceptibility of hPSC-CMs and hECs to SARS-CoV-2 infection. When hPSC-CMs and hECs were infected with SARS-CoV-2 for 24 h, viral load was significantly higher in hPSC-CMs compared with hECs (Figure 4H). This was demonstrated by coverage map visualizations, which identified reads that aligned to the SARS-CoV-2 viral genome in both SARS-CoV-2-infected hPSC-CMs and hECs, albeit at far lower levels in infected hECs (Figure S1D). Next, hPSC-CMs and hECs were treated with either lisinopril or losartan and subsequently infected with SARS-CoV-2. Viral load remained unchanged in both hPSC-CM and hEC treated cells after 24 h of SARS-CoV-2 exposure (Figures 4I and 4J). Furthermore, to confirm whether SARS-CoV-2 infection and concomitant use of antihypertensive therapy effects downstream proteins associated with viral entry, immunoblot analysis was performed in hPSC-CMs (Figure 4K). Protein expression of ACE2 and CTSL was not significantly altered in hPSC-CMs infected with SARS-CoV-2 and/or treated with lisinopril or losartan. Given that the hPSC-CM in vitro system may have decreased levels of natural components (e.g., angiotensin I or II) of the renin-angiotensin axis, we interrogated our latter findings in the presence of angiotensin II, a key substrate for AT1R and ACE2. Therefore, hPSC-CMs were treated in isolation or in combination with angiotensin II/losartan. Following infection with SARS-CoV-2, no significant changes in viral load (Figure S1E) or ACE2 transcript levels were detected (Figure S1F). However, immunoblot analysis showed a reduced, but non-significant (p = 0.059), change in expression of ACE2 in the presence of angiotensin II (Figure S1G). Altogether, these results indicate that hPSC-CMs are more susceptible to SARS-CoV-2 infection compared with hECs and that antihypertensive therapy does not alter the susceptibility of either cell type to SARS-CoV-2 infection.

**DISCUSSION**

Emerging reports have highlighted the potential magnitude of cardiac damage that ensues following SARS-CoV-2 infection, both in vivo (Guo et al., 2020; Lala et al., 2020; Puntmann et al., 2020; Shi et al., 2020) and in vitro (Marchiano et al., 2021; Pérez-Bermejo et al., 2020). Currently, there is a diverse spectrum of cardiac manifestations that have been reported in COVID-19 patients: cell necrosis (Fox et al., 2020a), myocarditis (Rajpal et al., 2020; Siripanthong et al., 2020), and right ventricular dilatation (Fox et al., 2020b). These studies suggest that SARS-CoV-2 infection can cause acute cardiac injury, as well as chronic effects on cardiac function. Furthermore, antihypertensive therapy has been shown to reduce the risk of cardiovascular events in COVID-19 patients. However, these findings remain preliminary and require further investigation. Overall, these results highlight the importance of understanding the interaction between SARS-CoV-2 infection and antihypertensive therapy, and the potential role of antihypertensive therapy in preventing or mitigating COVID-19-related cardiac damage.
all of which have culminated in the observed secondary increases in cardiogenic shock (Kim et al., 2020), malignant arrhythmias (Guo et al., 2020), myopericarditis (Purohit et al., 2020), in-hospital arrest (Thapa et al., 2020), and overall mortality (Shi et al., 2020) in this patient population. Furthermore, patients who have recovered from COVID-19 have been reported to have ongoing myocardial inflammation (Puntmann et al., 2020). Collectively, these studies have identified the heart as a target of SARS-CoV-2 infection; however, how this virus infects the myocardium and utilizes the cardiac environment to cause subsequent damage is largely still unknown. Many reports to date have focused on late-stage disease onset or post mortem cardiac injury; therefore, identifying primary effects of SARS-CoV-2 infection on the heart has been limited. The use of hiPSC-CMs has become beneficial in attempting to identify the primary effects of SARS-CoV-2 on the heart in an environment that is free of secondary neurohormonal compensatory mechanisms. In this study, we utilized both hiPSC-CMs and primary hECs to recapitulate the primary effects of SARS-CoV-2.

Consistent with previous reports (Pérez-Bermejo et al., 2020; Sharma et al., 2020), we identified hiPSC-CMs to express ACE2, indicating that ACE2 is present early in cardiac differentiation. Interestingly, TMPRSS2 was not detected in hiPSC-CMs; however, gene members of the endolysosomal pathway CTSL, CTSF, FURIN, and PLKfyve were identified. This suggests that viral entry of SARS-CoV-2 in hiPSC-CMs is independent of TMPRSS2-mediated cleavage at the membrane and utilizes cathepsin-mediated fusion as opposed to direct plasma membrane fusion. Furthermore, we observed that removal of ACE2 from hiPSC-CMs abolished SARS-CoV-2 transcript levels. The absence of SARS-CoV-2 viral entry in ACE2 KO cells was also previously validated in hiPSC-CMs, which showed undetectable fluorescence of a SARS-CoV-2-mNG (mNeonGreen) reporter with the loss of ACE2 expression (Marchiano et al., 2021). Taken together, these findings indicate that not only is the heart susceptible to SARS-CoV-2 viral infection and that ACE2 is a key component of infectivity but that future drug therapies could target PIKfyve or cathepsins as therapeutic targets for viruses that utilize the endolysosomal entry pathway.

Interestingly, we observed significant transcriptome changes in SARS-CoV-2-infected hPSC-CMs and hECs after only a 24-h incubation period with a low viral MOI (0.1 MOI). Three inflammatory and antiviral pathways were discovered to be significantly upregulated in hPSC-CMs (TNF, IFN-α/β, and MAPK signaling), suggesting a robust cardiac inflammatory response to SARS-CoV-2 infection. Previously, TNFα has been described as a proinflammatory cytokine involved in the innate immune response and regulation of tissue injury and inflammation (see review by Cicha and Urschel, 2015). Specifically, it has been reported that TNFα has cardiodepressant effects, leading to reduced contractility (Dörge et al., 2002), increased cardiomyocyte apoptotic activity (Krown et al., 1996), downregulation of contractile proteins (Patten et al., 2001), and overall myocardial remodeling and structural alterations (Jobe et al., 2009). Furthermore, adverse effects of interferon on the cardiovascular system have been reported to contribute to the pathogenesis of atherosclerotic lesions (Boshuizen and de Winther, 2015), as well as increased arrhythmias and myocardial disease (Teragawa et al., 1996). The upregulation of these pathways, in the setting of SARS-CoV-2 infection, could potentially explain the increased incidence of out-of-hospital cardiac arrest and sudden cardiac death observed in geographic locations that had a high cumulative incidence of COVID-19 (Baldi et al., 2020). Similarly, NF-κB signaling (a master regulator of the inflammatory response in endothelial cells) was upregulated in SARS-CoV-2-infected hECs. Upregulation of this pathway could provide a potential explanation for the enhanced activation of endothelial cells observed in severe COVID-19 disease, emphasizing the possible importance of the NF-κB pathway in endothelial dysfunction.

COVID-19 pathogenesis has been strongly associated with a robust production of cytokines, referred to as the "cytokine storm," and there has been much speculation surrounding the question of whether this cytokine storm induces direct or indirect cardiotoxic effects. While systemic overproduction of cytokines in a cytokine storm is seen only in a subset of severe COVID-19 patients, dysregulated and prolonged localized production of cytokines could by itself propagate tissue damage in different organs. In this study, we observed significant changes in genes involved with cytokine-mediated immune and inflammatory pathways in hPSC-CMs (TNFAIP3, NFKBIZ, BCL6, RELB) and in hECs (F2RL3, SPNS2, TNFRSF4), which could provide insights into the potential immune response involved in cardiac injury/endothelial dysfunction and warrants further research to determine potential therapeutic strategies. Additionally, genes involved in cellular stress response (ATF3, SGK1), apoptosis (NR4A1, TNFRSF4), and platelet aggregation (THBS1) were upregulated in infected hPSC-CMs and hECs, respectively, further advocating for the detrimental cardiac/endothelial damage that occurs with SARS-CoV-2 infection.

At the structural level of the cardiomyocyte, we observed transcriptional downregulation in genes encoding proteins abundant at the intercalated disc (MYZAP), involved in sarcomeric thinfilament assembly (TMOD1, TNNT2, and TNNT1) and cardiac electrical activity (KCNJ8) in SARS-CoV-2-infected hPSC-CMs. These transcriptional alterations...
suggest a compromise in cardiomyocyte integrity and structural assembly, as was observed in our immunostaining of infected hPSC-CMs, and could explain the myofibrillar fragmentation (Pérez-Bermejo et al., 2020), cessation of cardiomyocyte contraction (Bailey et al., 2021; Marchiano et al., 2021; Sharma et al., 2020), and impaired electrical signaling (Marchiano et al., 2021) observed by others in infected hPSC-CMs. Overall, SARS-CoV-2 appears to demonstrate a robust ability to initiate detrimental cytopathic effects in cardiomyocytes, most likely resulting in severe cardiac dysfunction.

Since ACE2 has been identified as the host receptor for SARS-CoV-2, it has been proposed that viral infection can potentially lead to downregulation of the angiotensin 1–7 (Ang1-7)/Mas axis (see review by Brojakowska et al., 2020). Due to the protective effects of this pathway, dysregulation of Ang1-7/Mas could confer decreased disease protection and increased organ damage in COVID-19 patients. However, with the use of antihypertensive medications such as lisinopril and losartan, which selectively inhibit angiotensin-converting enzyme (ACE) and the angiotensin II receptor (AT1R) respectively, it has been suggested that ACE2 expression and activity may be upregulated (Bai et al., 2016; Igase et al., 2005; Ishiyama et al., 2004). Although much of this has been the result of murine studies, it is difficult to disregard that the upregulation of ACE2 could pose either a therapeutic benefit (increased Ang1-7 production) or disadvantage (increased ACE2 transcript levels in the presence of losartan) (Marchiano et al., 2021) observed. This suggests that antihypertensive therapy intake and other comorbidities that predispose vulnerable populations to infection. These results, taken together with newly emerging multicenter trials, propose that antihypertensive therapy most likely does not alter susceptibility to SARS-CoV-2 infection at the cellular level and is presently not associated with an increased risk for severe COVID-19 (Hakeam et al., 2020; Lopes et al., 2021).

With much conflicting evidence surrounding the use of antihypertensive therapy during the SARS-CoV-2 pandemic, this study provides insight into the cross-talk between ACEI/ARB therapy in the presence of SARS-CoV-2 and addresses the correlation hypothesis between antihypertensive medications and susceptibility of SARS-CoV-2 infection at the cellular level. However, it is important to note that these therapeutics do not function alone in vivo, and their response is heavily influenced by neurohormonal feedback as well as a diseased state (Yakubova et al., 2018). In our cultured system, some of the necessary substrates for the renin-angiotensin axis may be present at lower levels and therefore we were only able to examine the downstream effects of direct ACE and AT1R inhibition. To circumvent this, we added angiotensin II to hPSC-CMs and found no significant difference in SARS-CoV-2 viral load or ACE2 transcript levels in the presence of losartan treatment. Since the renin-angiotensin axis is a complex hormonal system involving multiple organs, it is critical to corroborate the relationships discovered in this study at the gross level and further investigate confounding factors (e.g., age, gender, comorbidities), other antihypertensive therapies (e.g., calcium channel blockers), as well as chronic effects of antihypertensive therapy intake and COVID-19 clinical outcomes. Understanding these connections can provide more personalized treatment options for individuals with COVID-19.

**EXPERIMENTAL PROCEDURES**

For further details, see supplemental experimental procedures.

**Primary human endothelial and human pluripotent stem cell-derived cardiomyocyte drug treatments**

hECs and hPSC-CMs (H7s) were treated with 5 μM lisinopril (Cayman Chemical Co.), 10 μM losartan carboxylic acid (a potent active metabolite of losartan) (Cayman Chemical Co.), or DMSO (Sigma-Aldrich) for 36–72 h. To ensure maximal effect of each drug, drug concentrations above published IC50 values and treatment time intervals (Burnier, 2001; Desideri et al., 2008; Patchett et al., 1980; Sachinidis et al., 1993) (with no observed cellular toxicity) were used. Endothelial cells and hPSC-CMs were then infected with SARS-CoV-2 at 0.1 MOI for 24 h, lysed with TRIzol...
reagent (Zymo Research), and total RNA was extracted using the Direct-zol RNA Miniprep Plus kit (Zymo Research). cDNA was synthesized from 100 ng of total RNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Template cDNA was then used for real-time quantitative PCR (qPCR) using the PowerUp SYBR Green Master Mix kit (Thermo Fisher Scientific) to determine relative gene expression on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific). Primers included 18S (forward, 5'-ACCCGTTGAACCCATTCGTA; and reverse, 5'-GCCTCATAAAACCATCCAATCGG); 2019-nCoV_N1 (forward, 5'-GACCCCAAAAATCAGCGAAAT; and reverse, 5'-TCTGGTTAATCAACGTGAT); 2019-nCoV_N3 (forward, 5'-GGGAGGCCCCTGATTACACCAA; and reverse, 5'-AGACCATTCCACCTCATCTC).

**hPSC-cardiomyocyte angiotensin II treatment**

After metabolic selection, hPSC-CMs (H7s) were reseeded at a density of 500,000 to 1 million cells per well of a standard six-well cell culture plate. At 48 h post reseeding, hPSC-CMs were treated with either 1 μM angiotensin II (Sigma-Aldrich), a combination of 1 μM angiotensin II and 10 μM losartan carboxylic acid (a potent active metabolite of losartan) (Cayman Chemical Co.), or DMSO (Sigma-Aldrich) for 36 h. hPSC-CMs were then infected with SARS-CoV-2 at 0.1 MOI for 24 h. The cells were lysed with Trizol reagent (Zymo Research) and total RNA was extracted using the Direct-zol RNA Miniprep Plus kit (Zymo Research). cDNA was synthesized from 200 ng of total RNA using the High-Capacity cDNA Reverse Transcriptation Kit (Thermo Fisher Scientific). Template cDNA was then used for real-time qPCR using the iQ SYBR Green Supermix (Bio-Rad) to determine relative gene expression on a Quant Studio 5 (Thermo Fisher Scientific).

**RNA-seq and analysis**

Single-cell RNA-seq (10X Chromium system: 10X Genomics, Pleasanton, CA) data, obtained from a previously published dataset (Churko et al., 2018), were analyzed for ACEI/ARB and SARS-CoV-2 marker expression on days 14 and 45 of hPSC-CM differentiation from healthy donors). Matrices were filtered for hPSC-CMs using >20 TNNT2 counts per cell in R. Clustering, UMAP plots, and count visualization were performed using Seurat 3.2.3. For bulk RNA-seq, hECs and hPSC-CMs were lysed using TRizol reagent (Zymo Research) and total RNA was extracted using the Direct-zol RNA Miniprep Plus kit (Zymo Research). During RNA isolation, on-column DNAse digestion was performed for 15 min at room temperature. RNA-seq libraries were performed at Novogene using the Zymo-Seq Ribofree Total RNA Library Kit for SARS-CoV-2-infected cells or NEB Next Ultra II non-directional kit for lisinopril- and losartan-treated cells. Sequencing was performed using a 2 × 150-bp output on the NovaSeq 6000 (Illumina). Transcriptomes of hPSC-CMs throughout differentiation were previously performed (Churko et al., 2018) and assessed for expression of ACE2, ACE, AGTR1, CTS1, NRPI, and TMRPSS2. Fastq files were aligned using STAR to the Ensembl GRCch38 annotation. DEGs were calculated using AltAnalyze (Emig et al., 2010) using an empirical Bayes moderated t test (p < 0.05). The heatmap.2 R package was used to generate the heatmap of SARS-Cov-2-infected hPSC-CMs versus control hPSC-CMs. The MarkerFinder algorithm and heatmap generation tool integrated within AltAnalyze was used to generate heatmaps specific for ACEI/ARB-treated primary endothelial cells and hPSC-CMs. Gene set enrichment analysis (GSEA) was used to acquire enrichment terms specifically for ACEI/ARB treatment or for SARS-CoV-2-infected hPSC-CMs (Subramanian et al., 2005). Network analysis was performed using STRING analysis (Szklarczyk et al., 2019). To assess relative SARS-CoV-2 levels per sample, FASTQ files were aligned to the SARS-CoV-2 genome (Ensembl ASM985889v3) using BWA-MEM (Li and Durbin, 2009). Read counts for 12 annotated genes were quantified using featureCounts (Liao et al., 2019) and normalized by total number of reads per sample. Coverage maps were generated using IGV with reads counts per million as scaling.

**Quantification and statistical analysis**

All data are represented as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism version 9.1.0 (GraphPad Software Inc., San Diego, CA). Two-tailed Student’s t test, one-way ANOVA, or two-way ANOVA with Tukey’s post hoc test was used depending on the number of groups or variables per experiment. A P value <0.05 was considered significant, unless otherwise indicated. qPCR and immunoblot data were normalized to a housekeeping marker (18S or GAPDH, respectively) and presented as fold change over the control group.

**Data and code availability**

All relevant data are available from the authors. RNA-seq of iPSC-CM differentiation data is deposited under the Gene Expression Omnibus (GEO) accession number: GSE81585. Single-cell RNA-seq of iPSC-CMs (10x Genomics) is deposited under synapse ID: syn7818379. RNA-seq of lisinopril- and losartan-treated hPSC-CMs, as well as SARS-CoV-2-infected hPSC-CMs, is deposited under GEO accession number: GSE164784.

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.stemcr.2021.08.018.

**AUTHOR CONTRIBUTIONS**

Conceptualization, J.M.C. and J.I.; methodology, J.M.C., S.G.K., J.L., J.N.-Z., T.S., B.S., and S.K.C.; validation, J.M.C., S.G.K., and S.K.C.; formal analysis, J.M.C., T.L., and S.G.K.; investigation, J.M.C., S.G.K., T.K., S.L., J.L.U., L.J., and S.P.M.; resources, J.M.C., S.K.C., T.K., C.C.G., S.L., J.N., J.P.K., and M.K.; writing – original draft, J.I.; writing – review & editing, J.M.C., J.I., S.G.K., S.K.C., J.P.K., J.N.-Z., and C.C.G.; visualization, J.M.C., J.L., S.G.K., and S.P.M.; supervision, J.M.C.; funding acquisition, J.M.C.

**CONFLICT OF INTERESTS**

The authors declare no competing interests.

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REFERENCES

Bai, F., Pang, X.F., Zhang, L.H., Wang, N.P., McKallip, R.J., Garner, R.E., and Zhao, Z.Q. (2016). Angiotensin II AT1 receptor alters ACE2 activity, eNOS expression and CD44-hyaluronan interaction in rats with hypertension and myocardial fibrosis. Life Sci. 153, 141–152.

Bailey, A.L., Dmytrenko, O., Greenberg, L., Bredemeyer, A.L., Ma, P., Liu, J., Penna, V., Winkler, E.S., Sviben, S., Brooks, E., et al. (2021). SARS-CoV-2 infects human engineered heart tissues and models COVID-19 myocarditis. JACC Basic Transl. Sci. 6, 331–345.

Baldí, E., Sechi, G.M., Mare, C., Canevari, F., Brancaglione, A., Primi, R., Klersy, C., Palò, A., Contri, E., Ronchi, V., et al. (2020). Out-of-hospital cardiac arrest during the COVID-19 outbreak in Italy. N. Engl. J. Med. 383, 496–498.

Bojkova, D., Wagner, J.U., Shumliakivska, M., Aslan, G.S., Saleem, U., Hansen, A., Luxán, G., Günther, S., Pham, M.D., Krishnan, J., et al. (2020). SARS-CoV-2 infects and induces cytoxic effects in human cardiomyocytes. Cardiovasc. Res. 116, 2207–2215.

Bosshuizen, M.C.S., and de Winther, M.P.J. (2015). Interferons as essential modulators of atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 35, 1579–1588.

Brojakowska, A., Narula, J., Shimony, R., and Bander, J. (2020). Clinical implications of SARS-CoV-2 interaction with renin angiotensin system: JACC review topic of the week. J. Am. Coll. Cardiol. 75, 3085–3095.

Burnier, M. (2001). Angiotensin II type 1 receptor blockers. Circulation 103, 904–912.

Cantuti-Castelvetri, L., Ojha, R., Pedro, L.D., Djammatian, M., Franz, J., Kuivanan, S., van der Meer, F., Kallio, K., Kaya, T., Anastasina, M., et al. (2020). Neuporin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science 370, 856–860.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395, 507.

Churko, J.M., Garg, P., Treutlein, B., Venkatasubramaniam, M., Wu, H., Lee, J., Wessells, Q.N., Chen, S.Y., Chen, W.Y., Chetel, K., et al. (2018). Defining human cardiac transcription factor hierarchies using integrated single-cell heterogeneity analysis. Nat. Commun. 9, 1–14.

Cicha, I., and Urschel, K. (2015). TNF-alpha in the cardiovascular system: from physiology to therapy. Int. J. Interf. Cytokine Mediat. Res. 7, 9–25.

Corradì, H.R., Schwager, S.L.U., Nchinda, A.T., Sturrock, E.D., and Acharya, K.R. (2006). Crystal structure of the N domain of human somatic angiotensin I-converting enzyme provides a structural basis for domain-specific inhibitor design. J. Mol. Biol. 357, 964–974.

Daly, J.L., Simonetti, B., Klein, K., Chen, K.E., Williamson, M.K., Antón-Plágaro, C., Shoemark, D.K., Simon-Gracia, L., Bauer, M., Hollandi, R., et al. (2020). Neuporin-1 is a host factor for SARS-CoV-2 infection. Science 370, 861–865.

Desideri, G., Grassi, D., Croce, G., Bocale, R., Tiberti, S., Evangelista, S., Necozone, S., Di Orizio, F., and Ferri, C. (2008). Different effects of angiotensin converting enzyme inhibitors on endothelin-1 and nitric oxide balance in human vascular endothelial cells: evidence of an oxidant-sensitive pathway. Mediators Inflamm. 2008. https://doi.org/10.1155/2008/305087.

Dörge, H., Schulz, R., Belosjorov, S., Post, H., Van De Sand, A., Ko-nietzka, I., Frede, S., Hartung, T., Vinten-Johansen, J., Youker, K.A., et al. (2002). Coronary microembolization: the role of TNF-z in contractile dysfunction. J. Mol. Cell. Cardiol. 34, 51–62.

Emig, D., Salomonis, N., Baumbach, J., Lengauer, T., Conklin, B.R., and Albrecht, M. (2010). AltAnalyze and DomainGraph: analyzing and visualizing exon expression data. Nucleic Acids Res. 38, W755–W762.

Ferrario, C.M., Jessup, J., Chappell, M.C., Averill, D.B., Broznihtan, K.B., Tallant, E.A., Diz, D.L., and Gallagher, P.E. (2005). Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation 111, 2605–2610.

Fox, S.E., Akmatbekov, A., Harbert, J.L., Li, G., Quincy Brown, J., and Vander Heide, R.S. (2020a). Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. Lancet Respir. Med. 8, 681–686.

Fox, S.E., Li, G., Akmatbekov, A., Harbert, J.L., Laneira, F.S., Brown, J.Q., and Vander Heide, R.S. (2020b). Unexpected features of cardiac pathology in COVID-19 infection. Circulation 142, 1123–1125.

Guo, T., Fan, Y., Chen, M., Wu, X., Zhang, L., He, T., Wang, H., Wan, J., Wang, X., and Lu, Z. (2020). Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol. 5, 811–818.

Hakeem, H.A., Alsemari, M., Al Duhalib, Z., Ghonem, L., Alharbi, S.A., Almutairy, E., Bin Sheraim, N.M., Alsalhi, M., Alhijji, A., AlQahtani, S., et al. (2020). Association of angiotensin-converting enzyme inhibitors and angiotensin ii blockers with severity of Covid-19: a multicenter, prospective study. J. Cardiovasc. Pharmacol. Ther. 10.1177/1074248420976279.

Igase, M., Strawn, W.B., Gallagher, P.E., Geary, R.L., and Ferrario, C.M. (2005). Angiotensin II AT1 receptors regulate ACE2 and angiotensin-(1–7) expression in the aorta of spontaneously hypertensive rats. Am. J. Physiol. Circ. Physiol. 289, H1103–H1109.

Ishiyama, Y., Gallagher, P.E., Averill, D.B., Tallant, E.A., Brosnihan, K.B., and Ferrario, C.M. (2004). Upregulation of angiotensin-converting enzyme and angiotensin ii receptors by angiotensin ii receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation 111, 2605–2610.

Jobe, L.J., Meléndez, G.C., Levick, S.P., Du, Y., Brower, G.L., and Janicki, J.S. (2009). TNF-z inhibition attenuates adverse myocardial remodeling in a rat model of volume overload. Am. J. Physiol. - Heart. Circ. Physiol. 297, H1462–H1468.
Kang, Y.L., Chou, Y.Y., Rothlauf, P.W., Liu, Z., Soh, T.K., Cureton, D., Case, J.B., Chen, R.E., Diamond, M.S., Whelan, S.P.J., et al. (2020). Inhibition of PI3Kfyke kinase prevents infection by Zaire ebolavirus and SARS-CoV-2. Proc. Natl. Acad. Sci. U S A 117, 20803–20813.

Kelley, L.A., Mezulis, S., Yates, C.M., Wass, M.N., and Sternberg, M.J.E. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. Nat. Protoc. 10, 845–858.

Kim, H.N., Lee, J.H., Park, H.S., Yang, D.H., Jang, S.Y., Bae, M.H., Cho, Y., Chae, S.C., and Lee, Y.H. (2020). A case of COVID-19 with acute myocardial infarction and cardiogenic shock. J. Korean Med. Sci. 35. https://doi.org/10.3346/jkms.2020.35.e258.

Krown, K.A., Page, M.T., Nguyen, C., Zechner, D., Gutierrez, V., Comstock, K.L., Glembocki, C.C., Quintana, P.J.E., and Sabbadini, G. (2021). SARS-CoV-2 infects human pluripotent stem cell-derived cardiac cells predicts novel cytopathic features in hearts of COVID-19 patients. Biorxiv Prepr. Serv. Biol. https://doi.org/10.1101/2020.08.25.265561.

Kang, K., Sarkar, B., Zaslawsky, M., and Borchers, J. (2020). Replication of COVID-19 in human cardiac fibroblasts. Stem Cell Rep. 16, 2459–2472.

Nicin, L., Tyler Apblanap, W., Mellenitzin, H., Kettih, B., Tombor, L., John, D., Schmitto, J.D., Heineke, J., Emrich, F., Arsalan, M., et al. (2020). Cell type-specific expression of the putative SARS-CoV-2 receptor ACE2 in human hearts myocardial disease. JAMA Cardiol. 6, 116–118.

Kang, K., Sarkar, B., Zaslawsky, M., and Borchers, J. (2020). Replication of COVID-19 in human cardiac fibroblasts. Stem Cell Rep. 16, 2459–2472.

Natesh, R., Schwager, S.L.U., Sturrock, E.D., and Acharya, K.R. (2003). Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. Nature 421, 551–554.
vascular smooth muscle cells. J. Hypertens.

than losartan in blocking the angiotensin II-induced responses in EXP3174, a metabolite of losartan (Mk954, dup753) is more potent Rep. Med

cardiomyocytes are susceptible to SARS-CoV-2 infection. Cell

mugaswami, V., and Svendsen, C.N. (2020). Human iPSC-derived

Sharma, A., Garcia, G., Wang, Y., Plummer, J.T., Morizono, K., Aru-

and risk of Covid-19. N. Engl. J. Med.

Siddiqi, H.K., Libby, P., and Ridker, P.M. (2021). COVID-19 – a

Hankins, J., et al. (2018). ACE-inhibition induces a cardioprotective tran-

of angiotensin converting enzyme inhibitors and angiotensin II re-

Y.-C., Huang, X., Lin, L., et al. (2020). Association of inpatient use

-activators and risk of Covid-19. JAMA Intern. Med. 181, 279–281.

Varga, Z., Flammer, A.J., Steiger, P., Haberecker, M., Andermatt, R.,

Zinkernagel, A.S., Mehra, M.R., Schuepbach, R.A., Ruschitzka, F.,

Cheng, Z., Xiong, Y., et al. (2020). Clinical characteristics of 138

hospitalized patients with 2019 novel coronavirus-infected pneu-

China. JAMA 323, 1061–1069.

Wingler, L.M., Skiba, M.A., McMahon, C., Staus, D.P., Kleinhenz, A.L.W., Suomivuori, C.M., Latorraca, N.R., Dror, R.O., Lefkowitz, R.J.,

and Kruse, A.C. (2020). Angiotensin and biased analogs induce structurally distinct active conformations within a GPCR. Science 367, 888–892.

Xiong, T.Y., Redwood, S., Prendergast, B., and Chen, M. (2020). Co-

ronaviruses and the cardiovascular system: acute and long-term implic-}

ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a sin-

Zou, X., Chen, K., Zou, J., Han, P., Hao, J., and Han, Z. (2020). Sin-

in COVID-19. Lancet 355, 1417–1418.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., et al. (2020). Clinical characteristics of 138

hospitalized patients with 2019 novel coronavirus-infected pneu-

Du¨ sing, R., Christian, R., Wieczorek, A.J., and Vetter, H. (1993). D607–D613.

Spyroulias, G.A., Nikolakopoulou, P, Tzakos, A., Gerothanassis, L.P., Magafa, V., Manessi-Zoupa, E., and Cordopatis, P. (2003). Compar-

ison of the solution structures of angiotensin I & II implication with increased coverage, supporting functional discovery in genome-wide expression pro-

Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-

Cepas, J., Simonovic, M., Doncheva, N.T., Morris, J.H., Bork, P.,

et al. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 47, D607–D613.

Teragawa, H., Hondo, T., Amano, H., Hino, F., and Ohbayashi, M. (1996). Adverse effects of interferon on the cardiovascular system in patients with chronic hepatitis C. Jpn. Heart J. 37, 905–915.

Hao, P. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission63, 457–460.

Yakubova, A., Thorrez, L., Svetlichnyy, D., Zwarts, L., Vuilsteke, V.,

Laenen, G., Oosterlinck, W., Moreau, Y., Dehaspe, L., Van Houdt, J.,

et al. (2018). ACE-inhibition induces a cardioprotective transcrip-

Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., and Zhou, Q. (2020). Struc-

tural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 367, 1444–1448.

Yang, X., Yu, Y., Xu, J., Shu, H., Xia, J., Liu, H., Wu, Y., Zhang, L., Yu, Z., Fang, M., et al. (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir. Med. 8, 475–481.

Zhang, P., Zhu, L., Cai, J., Lei, F., Qin, J.-J., Xie, J., Liu, Y.-M., Zhao, Y.-C., Huang, X., Lin, L., et al. (2020). Association of inpatient use of angiotensin converting enzyme inhibitors and angiotensin II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. Circ. Res. 126, 1671–1681.

Zou, X., Chen, K., Zou, J., Han, P., Hao, J., and Han, Z. (2020). Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front. Med. 1, 1–8.