Journal of the American Heart Association

RESEARCH LETTER

Differential Response of Engineered Human Cardiac Tissues to Delta and Omicron COVID-19 Virus

Qiao Zhang, PhD; Ren-Zhi Zhan, PhD; Marisa Patsy, BS; Binjie Li, MS; Yifan Chen, BA; Barbara D. Lipes, PhD; Nenad Bursac, PhD; George A. Truskey, PhD

Cardiac damage by SARS-CoV-2 may involve viral infection of cardiomyocytes, which express angiotensin-converting enzyme 2 (ACE2) to which SARS-CoV-2 binds and enters cells, systemic effects such as inflammation and cytokine storm, or a combination of mechanisms.[1] Delta and Omicron variants of the Spike protein have higher potency and infectivity relative to the early D614G variant,[2] yet the mechanisms of entry of these variants into cardiomyocytes have not been studied. To address this knowledge gap, we used human induced pluripotent stem cell-derived cardiomyocytes (iCMs) to fabricate tissue-engineered 3-dimensional (3D) cardiac muscle bundles (cardiobundles) as a model system to test SARS-CoV-2 viral infection and potential drug candidates. Relative to 2-dimensional culture, cardiobundles show advanced levels of structural and functional maturation including longer sarcomeres and higher conduction velocity and contractile force.[3] To test the hypothesis that Spike protein variants influence viral entry into cardiobundles, we fabricated a pseudovirus with a lentivirus (Pseudo-V) *PAX2* backbone (Addgene 12 260), transfer plasmid containing GFP (green fluorescent protein) and luciferase reporters (Addgene 72 485), and plasmids encoding COVID-19 Spike proteins.

SARS-CoV-2 enters host cells after binding to ACE2. The Spike protein is then cleaved by either transmembrane serine protease 2 (TMPRSS2; cell surface entry) or cathepsin L (endosomal entry).[4] iCMs express ACE2 at both protein (Figure 1Ai) and mRNA levels (Figure 1Aii). ACE2 mRNA levels in iCMs were higher than wild-type 293 cells but much lower than in 293/17 cells that overexpress ACE2 (293ACE2, a positive control). Consequently, D614G Pseudo-V infection of iCMs was low and was not improved by polybrene (Figure 1B). (Likewise, Pseudo-V infection of 293ACE2 cells was unaffected by polybrene). Entry of D614G pseudovirus was blocked by treatment of iCMs with Spike protein and drugs that target cathepsins (E64D) and endosomal acidification (chloroquine) (Figure 1C). SID26681509, a cathepsin L inhibitor, blocked viral entry in 293ACE2 cells but was much less effective in iCMs (Figure 1C). An ACE2 analog (Captopril) and TMPRSS2 inhibitors camostat and nafamostat did not affect viral entry. Thus, virus expressing the D614G Spike protein entered iCMs via an endosomal pathway.

The data that support the findings of this study are available from the corresponding author upon reasonable request. Human cells were obtained from commercial sources and the institutional review board determined that their use was exempt.

To test how iCMs respond to different strains of Spike protein, we replaced the D614G spike plasmid with plasmid containing sequences for Delta

Key Words: ACE2 ■ cardiomyocytes ■ iPSCs ■ SARS CoV2 virus ■ tissue engineering

Correspondence to: George A. Truskey, Duke University, Department of Biomedical Engineering, 101 Science Drive, Room 1427, Durham, NC 27708. Email: george.truskey@duke.edu

This article was sent to Kevin F. Kwaku, MD, PhD, Associate Editor, for review by expert referees, editorial decision, and final disposition.

For Sources of Funding and Disclosures, see page 3.

JAHA is available at: www.ahajournals.org/journal/jaha

(B.1.617.2, Addgene 172320) or Omicron (BA.1, Addgene 180375) variants. Although Omicron Pseudo-V showed higher infection in 293ACE2 cells, Delta Pseudo-V more effectively infected CMs in 2-dimensional cultures (Figure 1Di and 1Dii). Because cardiobundles provide a more physiological 3D environment in which virus moves through the interstitial space to infect cells, we next examined use of cardiobundles as an in vitro platform for SARS-CoV-2-related drug testing. We first pretreated iCMs with E64D or chloroquine for 30 minutes and then added Omicron or Delta Pseudo-V to the cell suspension for 1 hour before mixing with fibrinogen (Figure 1Ei). Thrombin was then added to initiate gelation. The differential response of CMs to Delta and Omicron Pseudo-V observed in 2-dimensional persisted in 3D culture (Figure 1Eii and 1Eiii). Chloroquine effectively reduced Delta viral infection, whereas E64D significantly reduced infection from both Delta and Omicron Pseudo-V. To test if Omicron and Delta Pseudo-V directly infect intact cardiobundles, we fabricated cardiobundles, pretreated them with or without E64D for

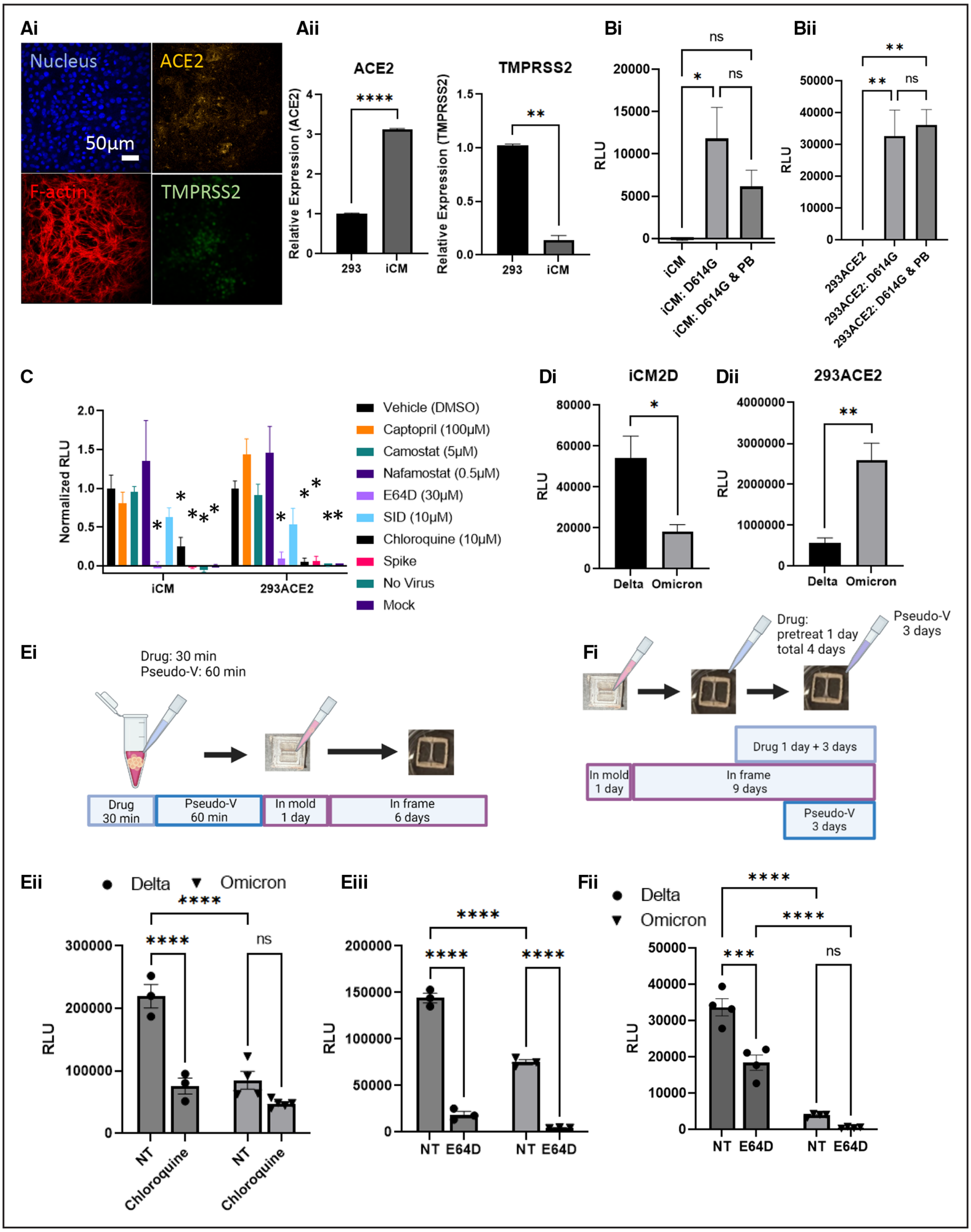

Figure 1. Characterization of ACE2 and TMPRSS2 levels and Spike protein pseudovirus entry into induced pluripotent stem cell-derived human cardiomyocytes.

A, **A**i, Immunostaining of cell nuclei (blue), angiotensin converting enzyme 2 (ACE2, yellow), F-Actin (red) and transmembrane serine protease 2 (TMPRSS2, green) in induced pluripotent stem cell-derived human cardiomyocytes (iCMs). iCMs were derived from the DU11 induced pluripotent stem cell cell line, reprogrammed episomally from BJ fibroblasts (ATCC CRL-2522) at the Duke University iPSC Core Facility. **A**ii, reverse transcription quantitative polymerase chain reaction of ACE2 and TMPRSS2 expression levels in iCMs and wild-type 293 cells (n=3, *****P*<0.0001, ***P*<0.01, Student *t* test). **B,** Luciferase reading of iCMs (i) and 293ACE2 (ii) cells with or without D614G Pseudo-V. Addition of polybrene (PB) along with Pseudo-V did not enhance viral entry into iCMs or 293ACE2 cells (n≥3, **P*<0.05, based on ANOVA with post hoc Tukey's test). iCMs were transduced with 5x Pseudo-V and 293ACE2 cells were transduced with 1x Pseudo-V. **C,** Effect of inhibitors of ACE2 (Captopril, 100 μM), TMPRSS2 (Camostat, 5 μM and Nafamostat, 0.5 μM), cathepsins (E64D, 30 μM and SID26681509, 10 μM), and endosomal acidification (chloroquine, 10 μM) on D614G pseudovirus entry into iCMs and 293ACE2 cells (n≥3, **P*<0.05 based on ANOVA with post hoc Dunnett's test). Drug concentrations used were based on published results[1] and preliminary studies. **D,** Entry of pseudovirus expressing B.1.617.2 Delta or BA.1 Omicron Spike protein into iCMs (**Di**) or 293ACE2 (**Dii**) as detected by chemiluminescence (n=6, **P*<0.05, ***P*<0.01 based on Student *t* test). **E**, **Ei,** schematic of the experimental timeline for viral infection of 3-dimensional (3D) cardiobundles before bundle assembly. Drug (NT: not treated vehicle control) was first added to the cells for 30 min and then Pseudo-V was added to cells for another 60 min. Then the cells were poured onto the molds for fabrication of cardiobundles. **Eii** and **Eiii**, Luciferase reading of 3D cardiobundles treated with chloroquine, 10 μM (**Eii**) or E64D, 30 μM (**Eiii**) infected with Delta or Omicron compared with NT (n≥3, *****P*<0.0001 based on 2-way ANOVA test with post hoc Tukey's test). Schematic of the experimental timeline was created with BioRender.com. **F**, **Fi,** schematic of the timeline for viral infection and drug treatment of cardiobundles. 3D cardiobundles were first fabricated and matured for 6 days. Drug (NT: vehicle) was added to the medium at day 6 for the following 4 days and virus was added to the medium at day 7 for the following 3 days. **Fii,** Luciferase reading of cardiobundles treated with chloroquine, 10 μM (Eii) or E64D, 30 μM (**Eiii**) infected with Delta or Omicron compared with NT (n=4, *****P*<0.0001, ****P*<0.001 based on 2-way ANOVA test with post hoc Tukey's test). Schematic of the experimental timeline was created with BioRender.com.

1 day and then added Delta or Omicron Pseudo-V into the surrounding culture medium for 3 days (Figure 1Fi). We confirmed that Delta Pseudo-V has higher infectivity than Omicron whereas E64D reduced the Delta viral entry significantly (Figure 1Fii).

Because D614G, Delta, or Omicron Spike proteins enable infection of iCMs, they may increase risk of cardiovascular disease, even in patients without hospitalization.[5] Only chloroquine, which causes endosomal acidification preventing viral disassembly, and E64D, which inhibits cathepsins, significantly reduced Spike protein Pseudo-V entry. This suggests that SARS COV-2 entry in CMs is through endosomal entry instead of cell surface entry, shown as a mechanism of infection in lung epithelial cells.[4] Furthermore, although Omicron variant Pseudo-V more efficiently infects 293ACE2 cells, Delta variant Pseudo-V shows greater infection in iCMs. The higher affinity of Omicron to ACE2 allows more viral entry into the 293ACE2 cells[2]; however, the limited amount of ACE2 on the iCM surface alters the viral entry process. Although high expression levels of TMPRSS2 in gut and lung epithelial cells result in impaired Omicron viral entry relative to Delta viral entry,[4] iCMs do not express high levels of TMPRSS2 (Figure 1Aii), which further supports endosomal pathway as the dominant mechanism of cell entry. The mechanism for increased uptake of the Delta Pseudo-V relative to the Omicron Pseudo-V in iCMs may be cell-type specific and warrants future investigation. Although pseudoviruses only allow study of the viral entry process, our results show that cardiobundles can be used to screen novel drug candidates against viral entry in a more predictive manner than conventional 2-dimensional -cultures with overexpressed ACE2 (Figure 1E and 1F).

ARTICLE INFORMATION

Received January 3, 2023; accepted May 1, 2023.

Affiliations

Department of Biomedical Engineering, Duke University, Durham, NC (Q.Z., R-Z.Z., M.P., B.L., Y.C., N.B., G.A.T.); and Department of Medicine, Duke University School of Medicine, Durham, NC (B.D.L.).

Acknowledgments

We thank Michael Farzan (the Scripps Research Institute) for providing us the 293T/ACE2 cells and Nicole Doria-Rose (Vaccine Research Center) and David Montefiori (Duke University) for providing us the D614G spike protein and TMPRSS2 plasmids.

Sources of Funding

This study was supported by National Institutes of Health grants UH3TR002142 (G.A.T), 3UH3TR002142-04S1 (G.A.T), U01HL134764 (N.B), and R01HL164013 (N.B).

Disclosures

None.

REFERENCES

1. Chung MK, Zidar DA, Bristow MR, Cameron SJ, Chan T, Harding CV 3rd, Kwon DH, Singh T, Tilton JC, Tsai EJ, et al. COVID-19 and cardiovascular disease: from bench to bedside. *Circ Res*. 2021;128:1214–1236. doi: 10.1161/CIRCRESAHA.121.317997
2. Bowen JE, Addetia A, Dang HV, Stewart C, Brown JT, Sharkey WK, Sprouse KR, Walls AC, Mazzitelli IG, Logue JK, et al. Omicron spike function and neutralizing activity elicited by a comprehensive panel of vaccines. *Science*. 2022;377:890–894. doi: 10.1126/science.abq0203
3. Jackman CP, Carlson AL, Bursac N. Dynamic culture yields engineered myocardium with near-adult functional output. *Biomaterials*. 2016;111:66–79. doi: 10.1016/j.biomaterials.2016.09.024
4. Meng B, Abdullahi A, Ferreira I, Goonawardane N, Saito A, Kimura I, Yamasoba D, Gerber PP, Fatihi S, Rathore S, et al. Altered TMPRSS2 usage by SARS-CoV-2 omicron impacts infectivity and fusogenicity. *Nature*. 2022;603:706–714. doi: 10.1038/s41586-022-04474-x
5. Xie Y, Xu E, Bowe B, Al-Aly Z. Long-term cardiovascular outcomes of COVID-19. *Nat Med*. 2022;28:583–590. doi: 10.1038/s41591-022-01689-3