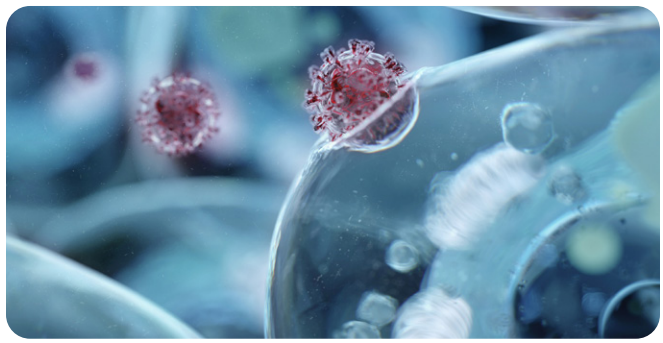
hESC-Derived cardiomyocyte screening platform identifies novel inhibitors of SARS-CoV-2 viral entry

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes COVID-19, the respiratory illness responsible for the COVID-19 pandemic. Studies have shown that patients infected with SARS-CoV-2 with cardiovascular comorbidities are more susceptible to severe infection and thus the case fatality rate in these patients rises from 2.3% to 10.5%. Interestingly, a recent study showed that expression of the genes involved in viral entry were upregulated in aged heart muscle cells (cardiomyocytes), which might explain why older people appear to be particularly susceptible to the cardiovascular complications associated with COVID-19.

To further understand the pathology of SARS-CoV-2 in cardiomyocytes, a team of researchers led by Sanjay Sinha and Anthony P. Davenport, from the University of Cambridge in the UK, have developed a screening platform using human embryonic stem cell-derived cardiomyocytes (hESC-CMs).[1] The aim of the study was to confirm that their model expressed the protein machinery critical for SARS-CoV-2 infection and screen for novel therapeutic agents that inhibit infection. The new platform helped to identify two potential inhibitors of SARS-CoV-2 infection.



Detecting host cell proteins and genes associated with SARS-CoV-2 viral infection

The entry of SARS-CoV-2 into host cells is dependent on high-affinity binding of the viral spike (S) glycoprotein to the cell surface receptor angiotensin-converting enzyme 2 (ACE2). The S protein is primed through proteolytic cleavage of S1/S2 and S2′ sites, which is mediated by the transmembrane protease, serine 2 (TMPRSS2), also present at the cell surface. Other host protein components critical for SARS-CoV-2 infection include the endosomal proteases furin and cathepsins, which are involved in S1/S2 cleavage and endosomal processing, respectively.

To confirm the presence of these host cell proteins in their model, hESC-CMs were plated in CellCarrier-96™ Ultra Plates (Revvity) and immunolabelled with primary antibodies raised against ACE2, TMPRSS2, B^0AT1, cathepsin B, cathepsin L, and furin. Fluorescent confocal images were acquired using the Opera Phenix® High Content Screening System (Revvity) (Figure 1).

revvity

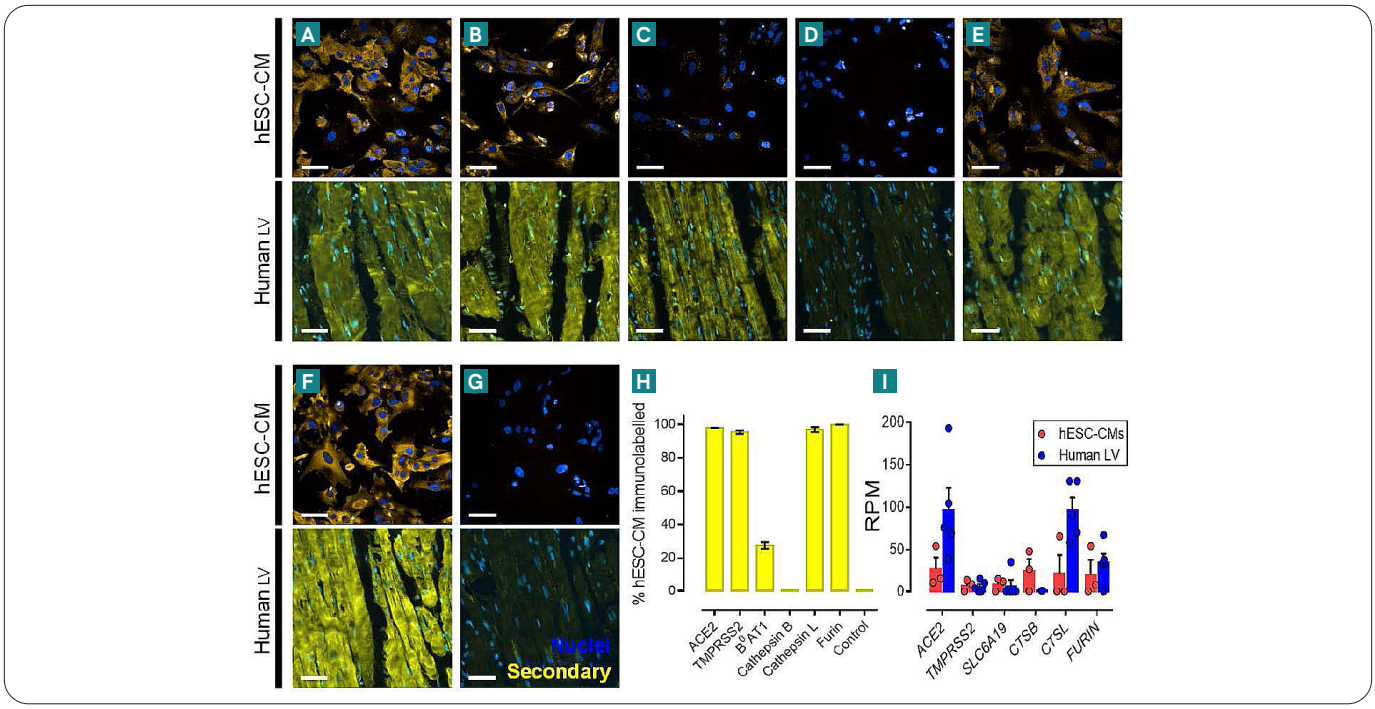


Figure 1: **Detection of host cell proteins and genes associated with SARS-CoV-2 viral infection. a-f:** Representative fluorescent confocal images (n=3 independent experiments performed in duplicate) of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) (upper) and representative fluorescent images (n=6 from six different donors) of human left ventricle (human LV) tissue sections (lower). Both cells and tissue were fixed with 4% formaldehyde and immunolabelled with primary antibodies raised against ACE2 (**a**), TMPRSS2 (**b**), B0AT1 (**c**), cathepsin B (**d**), cathepsin L (**e**), and furin (**f**), before visualization with secondary antibody conjugated to Alexa Fluor 555 (yellow) and Hoechst 33342 nuclear marker (blue). **g**: shows control cells (upper) and tissue (lower) treated with secondary antibody only and Hoechst 33342 nuclear marker. Scale bars show 50 µm. **h**: Graphical data showing the percentage of the observed hESC-CM population positively immunolabelled (above background) after visualization with secondary antibody targeting primary antibodies raised against the outlined protein targets. **i**: Graphical data showing the reads per million (RPM) ± SEM for expression of viral entry and processing genes in hESC-CMs (n=7 replicates across three distinct differentiations) and human LV (n=5 individuals). SLC6A19, CTSB, and CTSL are the genes that encode B0AT1, cathepsin B, and cathepsin L, respectively.

Analysis revealed that over 95% of the cells showed immunolabelling for proteins associated with infection, namely ACE2, TMPRSS2, cathepsin L, and furin. B0AT1, which is a neutral amino acid transporter whose surface expression is critically regulated by ACE2, was observed in 27.6% of the cell population, while cathepsin B signal was not observed. When the study was repeated in human left ventricle tissue sections, immunolabelling was detected for all proteins except cathepsin B.

Gene expression analysis confirmed these findings, with all corresponding genes for the proteins associated with SARS-CoV-2 infection expressed in hESC-CMs and human left ventricle tissue, except the gene that encodes cathepsin B (CTSB) in ventricular tissue.

Finally, the researchers infected hESC-CMs with SARS-CoV-2 and demonstrated titre- and time-dependent levels of infection in the model, confirming viral entry of SARS-CoV-2.

Pseudotyped lentiviral infection of hESC-CMs

In the next stage of their investigation, the researchers validated infection of hESC-CMs using a quantitative automated system in conjunction with a SARS-CoV-2 spike pseudotyped GFP-expressing lentivirus. Virus treated hESC-CMs were visualized on CellCarrier-96 Ultra Plates (Revvity) using the Opera Phenix High Content Screening System (Revvity) and imaging confirmed high rates of infection in the control cell populations (64.9% and 61.8% in media and DMSO, respectively).

Screening for novel therapeutic agents that inhibit SARS-CoV-2 infection

To screen for novel agents that inhibit infection, the researchers treated wells with compounds targeting the proteins involved in SARS-CoV-2 viral entry and processing. Fluorescent confocal images of hESC-CMs pre-treated with small molecule inhibitors (camostat, benztropine, and E64d), a peptide antagonist (DX600), and an ACE2 antibody (ACE2 Ab) revealed significant reductions in levels of infection (Figure 2).

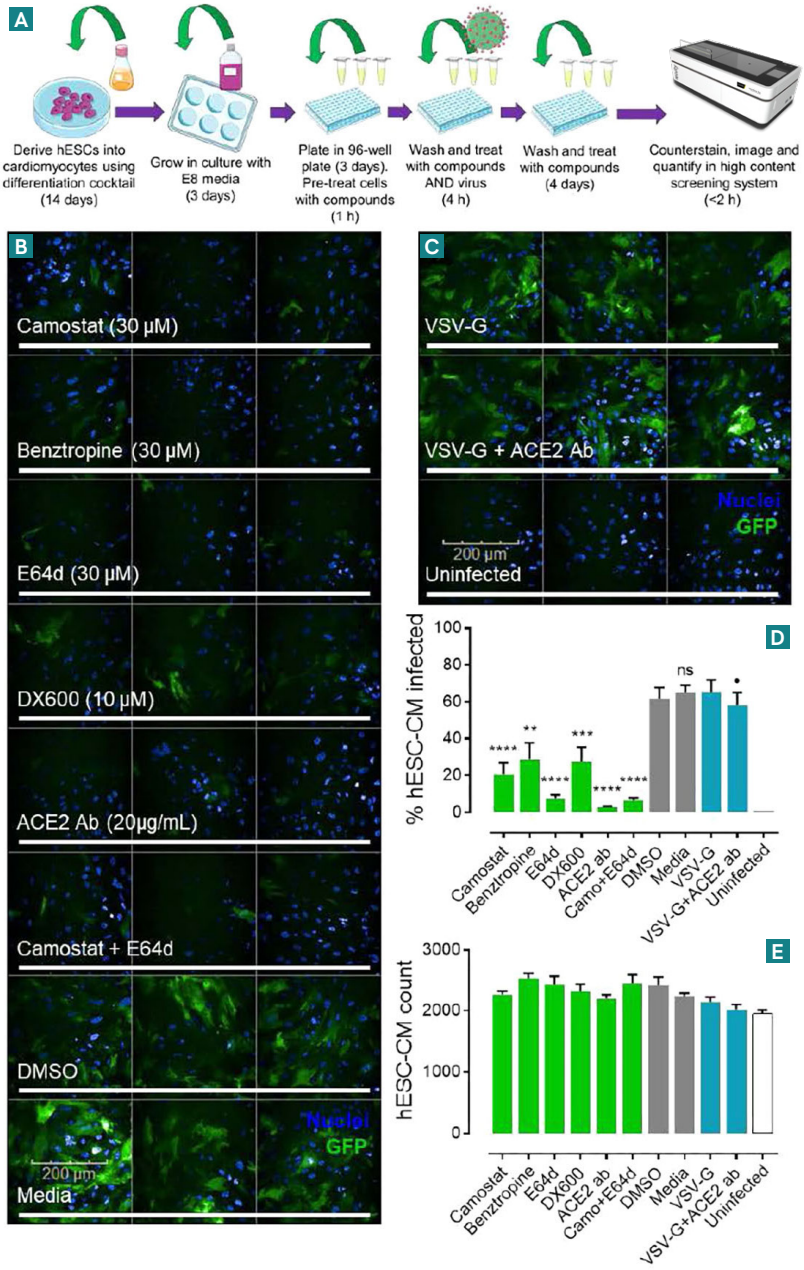


Figure 2: **Pseudotyped lentiviral infection in hESC-CMs. a**: Schematic showing the experimental workflow in brief for generating human embryonic stem cell-derived cardiomyocytes (hESC-CMs) and taking them into the pseudotyped lentiviral infection drug screen before conducting quantitative imaging. Schematic was generated using templates from Servier Medical Art. **b**: Representative fluorescent confocal images (n=2 independent experiments performed in triplicate) of hESC-CMs pre-treated with small molecule inhibitors (camostat, benztropine, E64d), peptide antagonist (DX600), or antibody (ACE2 Ab) targeting protein components involved in SARS-CoV-2 infection. Control cells were treated with DMSO (0.6 %) or media. Cells were treated with drugs for 1 h before incubation with SARS-CoV-2 spike pseudotyped GFP-expressing (green) lentivirus for 4 h. After removal of viral particles, cells were washed and maintained in the presence of drugs for five days before fixation with 4% formaldehyde and staining with Hoechst 33342 nuclear marker (blue). Scale bar shows 200 µm. **c**: Representative fluorescent confocal images (n=2 independent experiments performed in triplicate) of control human embryonic stem cell-derived cardiomyocytes (hESC-CMs) treated with VSV-G pseudotyped GFP-expressing (green) lentivirus, in the absence (upper) or presence (middle) of antibody (ACE2 Ab). Uninfected controls were not treated with viral particles (bottom). Again, cells were stained with Hoechst 33342 nuclear marker. **d**: Graphical data showing the percentage of observed hESC-CMs infected with either SARS-CoV-2 spike or VSV-G (control) pseudotyped lentivirus in the presence of drugs or DMSO (0.6 %) as indicated. Uninfected cells were not treated with viral particles. ** = $p<0.005$; *** = $p<0.0005$; **** = $p<0.00005$; and ns = no significant difference (as determined by one-way ANOVA) for each condition versus the DMSO treated control cells. ● = no significant difference for condition versus the VSV-G control. **e**: Graphical data showing the overall count of observed hESC-CMs for each condition, as indicated. No condition showed a count significantly different (as determined by one-way ANOVA) from the DMSO treated control cells.

Specifically, the screen successfully identified benztropine and DX600 as novel inhibitors of SARS-CoV-2 viral entry; these compounds reduced pseudotyped infection levels to 28.9% and 20.5%, respectively. Benztropine is a small molecule inhibitor of the potential ancillary protein for viral entry, B0AT1, and is used clinically as an adjunct in the therapy of all forms of parkinsonism. DX600 is a highly selective peptide that has not been tested previously as a viral entry inhibitor. The researchers also confirmed that camostat, E64d, and ACE2 Ab, all of which have previously been shown to have inhibitory effects against SARS-CoV-2 infection, reduced infection levels to 20.5%, 7.8%, and 2.9%, respectively.

Finally, the overall cell count of observed hESC-CMs for each condition revealed no significant difference between treated hESC-CMs and the DMSO-treated control cells, confirming that treatments were unlikely to be toxic. Uninfected cells and those cells treated with pseudotyped lentivirus were also present in comparable numbers, suggesting the viral inoculation was not toxic.

Conclusion

The present study identified and validated a qualitative and quantitative screen using hESC-derived beating cardiomyocytes that express SARS-CoV-2 host entry proteins. The study also demonstrated that the hESC-CMs, which are widely available, were viable and can be handled reliably in 96-well plates, the minimum format usually required for high-throughput screens. Two novel inhibitors of SARS-CoV-2 viral entry, benztropine and DX600, were identified and the inhibitory effects of camostat, E64d, and ACE2 Ab were confirmed.

These results provide a platform to generate high-quality data in pre-clinical studies and justify translational research in animal models of acute respiratory distress syndrome and the repurposing of current drugs for clinical trials.

References

1. Williams T, Colzani M, Macrae R, Robinson E, Bloor S, Greenwood E et al. (2021). Human embryonic stem cell-derived cardiomyocytes express SARS-CoV-2 host entry proteins: screen to identify inhibitors of infection. bioRxiv. https://doi.org/10.1101/2021.01.22.427737

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com