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Understanding and Targeting Coronaviruses: exploring advanced cell culture models and host-directed antiviral strategies

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English Summary

Coronaviruses comprise seven human viruses, some of them the common cold viruses, only causing mild symptoms in healthy individuals, while SARS-CoV, MERS-CoV, and SARS-CoV-2 can potentially cause severe disease and deadly pneumonia. The outbreak of SARS-CoV-2 in 2019 and its rapid worldwide spread has made apparent the need for a fast response to newly emerging viruses and to have effective therapies available. Although vaccines against SARS-CoV-2 were developed at an unprecedented speed, early patients would have benefitted from antiviral drugs. The respiratory tract is the first entry point for coronaviruses, where epithelial cells are infected and also represent the first barrier of defense. Primary human airway epithelial cells that are cultured and differentiated at the air-liquid interface (HAE-ALI) represent an advanced cell culture model recapitulating the human lung epithelium better than mono-cell cultures.

In this thesis, four research projects conducted during the PhD track, are described and discussed, which focussed mainly on SARS-CoV-2. **Chapter 1** gives an introduction to the overall research topic of coronavirus biology and antiviral drug discovery, and the use of HAE-ALI to study both. **Chapter 2** describes the characterization of SARS-CoV-2 infection in HAE-ALI. Changes in the cellular composition, caused by culture time or drug treatment, impacted virus replication, and this correlated with the presence of the main susceptible cells, ciliated cells and goblet cells, as well as expression of virus cell-entry factors. Furthermore, the importance of having a diverse epithelium in the cultures was identified, where likely goblet cells play a supportive role in infection. The findings contribute to the understanding of the variable susceptibility to SARS-CoV-2 infection between individuals and across anatomical locations in the respiratory tract, and possibly in chronic lung diseases affecting the epithelium. **Chapter 3** then aimed to conduct a comparative study between SARS-CoV, MERS-CoV, and SARS-CoV-2, and two common cold coronaviruses. We employed the HAE-ALI cell culture model to decipher differences in the epithelial transcriptional response upon coronavirus infection. RNA sequencing data showed limited expression of interferon genes in infections with SARS-CoV, MERS-CoV, and SARS-CoV-2, as opposed to the common cold coronaviruses, which corroborated previous studies showing suppression of interferon responses by the these three coronaviruses. Furthermore, SARS-CoV-2 infection uniquely lacked the expression of a set of immediate early genes, which are expressed in response to stressors like infection. By utilizing the findings about one of these genes, NR4A1, an inhibitor was identified that blocks SARS-CoV-2 and MERS-CoV replication. **Chapters 4 and 5** describe two antiviral drugs that efficiently block the replication of SARS-CoV-2 and other coronaviruses. The first, R-Propranolol, is part of a drug that is approved for the treatment of hemangioma (benign vascular tumor), besides various

medical conditions like cardiovascular diseases. R-Propranolol was shown to reduce a pro-angiogenic factor, which was recently reported to be associated with an increased rate of severe lung pathology in COVID-19. Therefore, this drug could be an interesting candidate to investigate further as host-directed therapy to reduce vascular damage in COVID-19, caused by endothelial dysfunction and pathological angiogenesis. Additionally, a potent antiviral effect of R-Propranolol against SARS-CoV-2 and other coronaviruses was observed, which makes the drug an interesting antiviral with two potential angles of activity. **Chapter 5** describes a class of host-directed antivirals, glucosidase inhibitors, which inhibit endoplasmic reticulum (ER) resident alpha-glucosidases, important for protein folding in the ER and quality control. Many viruses, including coronaviruses, use the host's ER protein quality control machinery for their glycoproteins. For SARS-CoV-2, especially the spike protein, which is crucial for virus attachment and entry into the host cell, is heavily glycosylated and dependent on processing in the ER and Golgi. Several compounds were tested, belonging to two classes, iminosugars and cyclitols. While iminosugars have been studied for decades as potential antiviral drugs, we identified 1,6-*epi*-cyclophellitol cyclosulfate, a candidate of a new class of glucosidase inhibitors, as superior due to its high specificity for ER alpha-glucosidase II and potent antiviral efficacy. Inhibition of ER alpha-glucosidases led to a reduction in spike protein generation and subsequently to a reduced production of infectious virus particles. In addition to SARS-CoV-2, 1,6-*epi*-cyclophellitol cyclosulfate also blocks the production of SARS-CoV and MERS-CoV progeny, rendering this class of compounds promising broad-spectrum antivirals. In the final chapter, the main findings of the research projects are discussed in the context of recently published studies. Furthermore, the current landscape of SARS-CoV-2 host-directed antiviral therapy and the benefits of using the most relevant cell culture models in antiviral drug discovery are discussed.