Chikungunya virus nonstructural protein 1 as an antiviral target

Kovacikova, K.

APPENDIX

English summary
Nederlandse samenvatting
List of publications
CV

English summary

This PhD thesis is dedicated to the development of novel antiviral agents against Chikungunya virus (CHIKV). CHIKV is a reemerging mosquito-borne virus causing an arthritis-like disease that is characterized by abrupt fever, malaise, and chronic joint and muscle pain. The high morbidity associated with Chikungunya fever and the negative impact on human health underscore the need to develop an effective antiviral therapy and other control measures. This PhD thesis presents a series of experimental studies focused on the identification of novel small molecules with CHIKV inhibitory activity and the elucidation of their mode of action. Importantly, the results in this thesis demonstrate that CHIKV nonstructural protein 1 (nsP1) represents a suitable target for antiviral drug development. CHIKV nsP1 is a multi-enzymatic protein involved in CHIKV RNA capping and in the attachment of the replication complex to the intracellular compartments where viral RNA synthesis occurs, the so-called 'spherules'. The cap structure plays many important roles in the CHIKV replication cycle. It protects viral mRNA from degradation by host exonucleases and from recognition by the host innate immune sensors, and it enables efficient translation of viral mRNAs by host ribosomes.

The introduction of this thesis (**Chapter 1**) provides a broad overview on CHIKV biology, replication cycle and pathogenesis. This chapter further discusses CHIKV evolution and spread, transmission by mosquito vectors, and disease manifestations including acute and chronic complications. It concludes with an overview of important control measures such as vector control, vaccine development and treatment with antiviral drugs and monoclonal antibodies. **Chapter 2** provides specific insights into the development of small-molecule CHIKV inhibitors, placing emphasis on the target(s) of these compounds, their modes of action, and mechanisms of antiviral drug resistance. Topics of interest related to CHIKV antiviral drug discovery, including the choice of cell lines and animal models for CHIKV antiviral drug research, are also discussed. **Chapters 3-6** of this thesis present in-depth experimental findings regarding the identification and the mode of action of CHIKV nsP1-targeting compounds. More specifically, the rational design, selection and validation of 6'fluorinated-aristeromycin and 6'-fluorinated-homoaristeromycin analogues as CHIKV inhibitors are described in **Chapter 3**. The identification and the mode of action of two adenosine analogues, 6'-β-fluoro-homoaristeromycin and 6'-fluoro-homoneplanocin A (FHNA), are discussed in **Chapter 4**. Furthermore, this chapter describes the identification of CHIKV nsP1 as the viral target of these compounds by selection of compound-resistant variants and

production of recombinant mutants by reverse genetics. The antiviral effect of FHNA was also supported by enzymatic assays with purified wild-type and mutant Semliki Forest virus (SFV) nsP1 for confirmation of target specificity. **Chapter 5** presents a study on other potent inhibitors of CHIKV replication belonging to the CHVB series. These compounds with novel chemical moieties strongly inhibit early stages of CHIKV replication in a similar manner to the previously described MADTP series. Selection of escape mutants and reverse genetics identified CHIKV nsP1 as the viral target of the CHVB compounds. Their antiviral activity was also demonstrated in enzymatic assays with purified Venezuelan Equine Encephalitis virus and SFV nsP1. **Chapter 6** describes the results of a first-of-its-kind molecular docking study with a CHIKV nsP1 cryo-EM structure and CHIKV nsP1 inhibitors. It demonstrates that the inhibitors can be grouped into different functional classes based on their modes of action. Using the oligomeric CHIKV nsP1 cryo-EM structure, the CHVB and MADTP compounds are predicted to bind at the active site of the capping domain, while FHNA is predicted to bind in a secondary binding pocket in the membrane binding and oligomerization domain. These findings provide the basis for further exploration of the precise binding and inhibitory activity of these inhibitors in CHIKV-infected cells. Lastly, **Chapter 7** places the results from these studies in the context of the published literature and discusses the latest developments in the field as well as some unexpected findings.

In conclusion, this PhD thesis demonstrates the potential for developing combination therapy consisting of small molecule inhibitors with different targets for prevention and treatment of CHIKV infections. Furthermore, combination therapy could increase the barrier to resistance as the rapid emergence of drug resistance still remains a major obstacle in the development of effective antiviral therapy. Last but not least, arboviruses such as CHIKV will continue to (re)emerge in the future due to their unpredictable epidemiology, substantiating the need for the development of antiviral treatment in addition to effective vaccines.