GENERATION OF PLASMID-BASED EUKARYOTIC MODEL TO INVESTIGATE BIOLOGY OF CRIMEAN CONGO HEMORRHAGIC FEVER VIRUS NUCLEOPROTEIN AND GLYCOPROTEINS

SUMMARY

RNA viruses are the main cause of many pandemics that shape human history by causing the death of millions of people. The rapid adaptability of RNA viruses, which allows them to circulate for thousands of years, and the increased contact of humans with wild animals can trigger the emergence of virus variants that can cause new pandemics. Virus-like particle studies (VLPs) to elucidate unknown mechanisms in the virus life cycle and pathophysiology of disease are of vital importance in the development of effective antiviral strategies against these viruses that affect human life. The lack of genomic material that can cause virus infection paves the way for these viruses to be studied in BSL-2 laboratories, which are easily accessible to many researchers around the world. In recent years, the World Health Organization (WHO) has published a list of infectious agents that should be primarily investigated. Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viruses, which are also clinically important for our country, are included in this list. From this point of view, in this thesis, various viral protein expression studies have been carried out to develop different VLP systems that can both study the biology and immunology of CCHFV and form the basis of vaccine candidate development studies for SARS-CoV-2. For this purpose, firstly, CCHFV viral proteins were produced in Huh-7 cells via pcDNA 3.1 plasmid, and their expression was confirmed by both post-transcriptional and post-translational analyses. Furthermore, immunological analyzes by in-house ELISA revealed that various viral protein combinations may promote the production of different CCHFV proteins. In addition, a unique ambisense minigenome system has been developed. The inclusion of this system in future VLP studies might result in the generation of a transcription and entry competent VLPs for CCHFV Kelkit 06 strain. Second, four different plasmids carrying multiple expression cassettes in various combinations were constructed to produce four different VLP-based vaccine candidates against SARS-CoV-2 in *P. pastoris*.

In conclusion, the experiments conducted in this thesis have shown expression and detection of immunologically significant viral antigenic proteins of CCHFV and SARS CoV-2, two of the most serious human viral pathogens of our time, in eukaryotic cells. These studies have established a critical infrastructure at our laboratory which will be imperative for future studies. It appears that for generation and demonstration at sufficient quantities and commercially meaningful VLPs from plasmid based expression systems, further optimizations are needed. It should be underlined that to address the questions on the biology and immunology of these viruses through VLP and minigenome approaches, these optimizations such as exhaustive trials with

different expression models, optimization of sequences, alternative transfection systems and stable expressions should be undertaken.

Keywords: RNA viruses, RNA virus infections, Arboviruses, Corona virus infections, COVID-19 vaccines