DEVELOPMENT OF WHOLE BLOOD STAGE VACCINE FOR MALARIA TREATMENT VIA CRISPR-GENE EDITING TECHNOLOGY

SUMMARY

Malaria remains a major global health problem among mainly in pregnant women and children, which resulted in death of hundreds of thousands of humans. One of the greatest challenges for malaria treatment is drug resistance against antimalarial drugs. Therefore, eradication of malaria can only be achieved with the application of very potent and safe vaccines. However, there is no malaria vaccine currently available, and the most advanced subunit vaccine candidate has recently reported efficacy between 0-30% against clinical malaria cases. On the other hand, live attenuated whole parasites used in experimental vaccination trails induced complete sterile protection. However, the inefficiency of gene editing technologies has limited their use in generating live attenuated vaccines.

Four members (NT1, NT2, NT3, and NT4) of the nucleoside transporter gene family play an important role in the purine transfer from the host to human malaria parasites. In this thesis, we developed nucleoside transporter 1 deficient *Plasmodium yoelii* and *Plasmodium berghei* via CRISPR-Cas9 gene editing methods. The CRISPR/Cas9 system is an emerging genome-editing technology that is used to edit the gene for various living organisms. CRISPR/Cas9 system sheds light on *Plasmodium* (*P. yoelii*, *P. falciparum*, etc.) to modify the targeted genes based on homologous recombination. We also generated a mixed live attenuated blood-stage malaria vaccine model using that NT1 deficient plasmodium strains. Equally mixed *Pbnt1(-)* and *Pynt1(-)* parasites in single subcutaneous fresh or frozen doses were injected in a group of mice and conferred sterile protection against intravenous infectious blood-stage challenge with wild-type parasites of *P. berghei* ANKA and *P. yoelii* 17X-NL strains. This data may indicate that a single subcutaneous sub-patent dose of two species of genetically-growth-attenuated parasites, can protect humans against two *Plasmodium* spp. infections. NT1 knockout parasites could be developed in cultures provided with supra-physiological concentrations of purine and shipped to endemic areas in frozen-stock doses stored in liquid nitrogen.

In this thesis, we also evaluated the role of the nucleoside transporter 4 gene (NT4) in the *Plasmodium* life cycle as a potential malaria vaccine target. Herein, NT4 deficient *P. berghei* parasites were generated, and in the erythrocytic stage, significant differences have not been observed. However, oocyst egress and sporozoite invasion of salivary glands are restricted in the mosquito stage. Moreover, the *Pbnt4(-)* salivary glands and hemolymph sporozoites did not develop infectivity. As a result of these results, the NT4 gene could be a promising target for the next malaria transmission-blocking studies.

Keywords: Malaria, *Plasmodium berghei*, *Plasmodium yoelii*, CRISPR-Cas9, Blood-stage vaccine, Nucleoside Transporter 1, Nucleoside Transporter 4