ABSTRACT

Glutamate carboxypeptidase II (GCPII) is a transmembrane glycoprotein, which consists of short intracellular and transmembrane domains, and a large extracellular domain possessing carboxypeptidase activity. In the human body, GCPII fulfils a neuromodulatory function in the brain and facilitates folate absorption in the small intestine. In addition to the brain and small intestine, high level of GCPII is also present in the prostate and kidney. However, GCPII function in these tissues has not been determined yet. To study the role of GCPII in detail, several research groups attempted to inactivate GCPII encoding gene *Folh1* in mice. Surprisingly, the experiments led to rather conflicting results ranging from embryonic lethality to generation of viable GCPII-deficient mice without any obvious phenotype. This dissertation project aimed to dissect the discrepancy using alternative strategy for gene modification.

For this purpose, we designed TALENs that specifically targeted exon 11 of *Folh1* gene and manipulated mouse zygotes of C57BL/6NCrl genetic background. We analysed all genetically modified mice of F0 generation for presence of TALEN-mediated mutations and established 5 different GCPII-mutant mouse colonies from founder mice that altogether carried 2 frame-shift mutations and 3 small in-frame deletions. We thoroughly characterized all 5 colonies and found out that GCPII is not expressed in any of mice homozygous for *Folh1*-mutant variant. We were thus able to generate viable GCPII-deficient mice that breed normally and do not show any overt phenotype.

Produced GCPII-deficient mice were utilized for investigation of potential role of GCPII in the urogenital system. It was revealed that aged GCPII-deficient mice possess increased propensity to enlarged seminal vesicles, though the origin of this dilation is yet to be determined. In contrast, kidneys from aged GCPII-deficient mice did not display any pathological abnormalities and targeted metabolomics of mouse urine showed only 3 out of 193 measured metabolites discriminating GCPII-deficient and wild type mice.

In addition to dissecting the discrepancy found in the literature by showing that GCPII-deficient mice are viable, this dissertation project may set the direction for revealing the GCPII function in reproduction. Strikingly, supposed unimportance of GCPII for kidney function may open the door for GCPII-targeted anti-cancer treatment.