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## Klinika dětské hematologie a onkologie

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Ph.D. thesis by **Mgr. Barbora Vorlova** "Generation and Characterization of Glutamate Carboxypeptidase II (GCPII)-Deficient Mice" have 136 pages and 3 appendices. Thesis is focused on perspective and clinically relevant topic, adequate methods are used, all relevant aspects of the problem are discussed, the conclusions are clear and concise, relevant literature is cited and results were published in quality journals with higher IF - see. appendices. The thesis brings new scientific knowledge about GCPII. Regarding formal aspects, all the conditions are fulfilled, the language level is, if I can judge, very good and I did not find any typing error.

The aims of the thesis were: 1/ perform *Folh1* gene disruption in mice; 2/ analyze TALEN-mediated mutations and select mice carrying both the frame-shift mutations and deletions potentially resulting in only small deletion within the active site of GCPII; 3/ establish mutant mouse colonies and develop reliable genotyping protocol; 4/ characterize recombinant mouse GCPII in terms of its kinetic properties in NAAG hydrolysis reaction; 5/ prepare and characterize recombinant mutant variants of GCPII that would potentially be expressed in GCPII-mutant mice; 6/ characterize GCPII-mutant and GCPII-deficient mice; 7/ investigate the impact of GCPII disruption on reproductive tissue and renal function by examination of GCPII-mutant and GCPII-deficient mice. These objectives have been met.

I only have these minor comments on the thesis:

The chapter "Materials and methods" is, in my view, unnecessarily extensive.

It would also be appropriate to include the histopathology of the seminal vesicles in the thesis, it is only in appendix 2.

Statement "GCPII-deficient mice did not show any reproductive or overt phenotypic abnormalities." is not accurate- see. Fig. 26.

Statement "This would suggest that the function of GCPII in the kidney is not crucial." is not accurate, presented results do not allow for the assessment of other kidney functions, e.g. EPO production.

I have the following questions:

1/ On page 22 it is written: "Except of different types of cancer GCPII seems to be also overexpressed in inflammatory bowel disease (IBD). Indeed, it has been shown that *FOLH1* gene is upregulated in IBD". In which cells is GCPII upregulated in IBD.

2/ Is there anything known about prostate cancer in mice, can it be induced? If I know, only xenotransplants are used as models.

3/ Is known how GCPII knock out affects the growth of prostate cancer cells and astrocytic brain tumor cells?

The thesis demonstrates the ability of the author to independent creative scientific work.

**I recommend the thesis for defense and awarding Ph.D. title after a successful defense.**

Psáry, 3.10.2018

prof. MUDr. Tomáš Eckschlager, CSc.