CHARLES UNIVERSITY
Faculty of science

Posudek oponenta disertační práce/Opponents review

Studijní program: Fyziologie Živočichů

Study programme: *Physiology of Animals*

Student: **Mgr. Kateřina Korelová**

Školitel/Supervisor: RNDr. Martin Gregor, Ph.D.

Disertační práce: **Význam jaterní plektinové deficience pro patogenezi onemocnění jater**

Doctoral thesis: *The Impact of Plectin Deficiency on Pathogenesis of Liver Diseases*

Oponent/Opponent: Mgr. Jan Mašek, PhD

Text posudku/Review:

The dissertation focuses on the role of plectin, a cytoskeletal linker protein, in liver physiology and pathology. The study employs a conditional knockout (cKO) model to investigate the effects of plectin deficiency specifically in hepatocytes, achieved via the Alb-Cre driver system. The impact of plectin deficiency was assessed under both basal conditions and during cholestatic injury induced by three distinct experimental approaches: bile duct ligation (BDL), 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet, and cholic acid overdose.

The thesis (and published research articles) represents a significant contribution to experimental hepatology, particularly in elucidating the cytoskeletal mechanisms underlying liver function and response to injury. The research question is highly relevant, addressing the interplay between cytoskeletal integrity and cholestatic injury, a topic of growing interest in hepatology. The candidate has demonstrated a high level of experimental competence, particularly in the analysis of the plectin cKO model and in the selection of complementary experimental approaches to model liver injury.

The introduction section is written very well, providing a comprehensive overview of the liver's structure and function, the cytoskeletal role of plectin, and the relevance of cholestatic injury. Theoretical concepts are presented with clarity and flow, accompanied by well-chosen examples and illustrative cartoons. There was one notable weakness of the introduction – a very superficial summary of the existing cholangiopathies, where at least the basic distinction to genetic/viral/hepatotoxin-induced, and neonatal/adult would help the reader better understand the choice of the animal models. Without this link is the animal model section poorly anchored into the flow, and it made me wonder if the part reviewing the animal models wouldn't serve better in the methods section (as it could not cover all the numerous models available anyway). I would also appreciate an introduction to biliary system development, as Alb-cre recombines early on in the liver development and the observed effects of Plectin deletion might stem from disrupted liver development.

The results presented in the thesis are extensive, experimental design is robust, utilizing a combination of genetic and pharmacological models, together with state-of-art microscopy, to examine the role of Plectin in cholestasis. The use of three distinct injury models—BDL, DDC treatment, and cholic acid overdose—adds depth and versatility to the analysis. Each model tests different aspects of liver injury and regeneration: BDL leads to intrahepatic cholestasis and fibrosis. It is a classical model for studying chronic cholestatic insult. The dietary, DDC, model induces bile duct obstruction in the liver periphery, and the cholic acid overdose mimics acute, excessive, bile acid exposure,

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leading to rapid hepatocyte injury and apoptosis. It highlights the direct effects of bile acid toxicity on liver cells, providing insights into cytoskeletal contributions to cellular resilience.

The results are presented in a clear and logical sequence, with well-labeled figures and tables that support the text. Integrating histological, molecular, and biochemical data strengthens the conclusions drawn from the experiments. The candidate's technical proficiency is evident in the successful characterization of the plectin cKO model and in the execution of sophisticated histological and molecular techniques. The breadth of methodologies applied adds credibility to the findings. While there are overall only a few typos, and the figures are generally well-designed, I noticed two errors: Figure 14 includes a duplicate image, and Figure 25 a cropping error, which undermines the overall impression of rigor.

The discussion section adequately addresses the main findings and would have benefited further from more in-depth comparisons with previous studies in the field. For example, contrasting the role of plectin with other cytoskeletal proteins in liver injury could provide a broader context for the results. Importantly, as mentioned later in the comments, my suggestion for future work is to tune down the buzzwords to infuse the results with other qualities than what is supported by statistics.

In sum, the thesis is a well-executed and valuable contribution to the field of experimental hepatology. The candidate demonstrates a strong grasp of both theoretical concepts and experimental techniques. Despite minor errors in the figure presentation, the overall quality of the work is very high. The findings provide novel insights into the role of Plectin in liver function and its response to cholestatic injury, laying the groundwork for future studies on cytoskeletal contributions to liver disease. I am thus happy to recommend the acceptance of this dissertation for the award of the degree.

Otázky/Questions:

1. Figure 1. When looking at liver sections, would you say the HA tends to be on the opposite side of the portal triad to BD?
2. Page 18, when listing the cell types of the liver, would you say the list you provided is complete?
3. On Page 21, you describe the liver-specific deletion of b-catenin as a key cytoskeletal component of the liver that regulates cannalicular morphology, and bile secretion. Is the case with b-catenin that simple?
4. On page 37, you mention: „In healthy liver, the BECs play an important role in (I) bile modification and (II) forming a barrier." Would you know what is an additional, developmental, role of BECs?
5. On page 38, there is a whole paragraph on BEC primary cilia (ref. with Scheuerle et al., 2023), which focuses on a case report describing NUDCD2's role in fibroblasts. Was that an intention?
6. Comment: on pages 43-44, where you refer to Vartak et al 2016, describing BEC response to cholestasis. I suggest distinguishing cholestasis caused by failure of cholangiocytes and exogenously caused as the reaction of developmentally impaired cholangiocytes (ALGS) differs from the reaction of healthy BECs.
7. Comment: on page 45 you refer to BDL as „Initially developed for rats due to their lack of a gallbladder (Mariotti et al. 2018)" with a method established in 1984 (PMC2040968) the combination of „Initially", and contemporary ref, feels inappropriate.
8. The newly reported patients with plectin mutations develop cholestasis early after birth, why did you choose to study the effects of plectin deletion in adults, rather than neonatal mice?
9. Comment: Page 64., when presenting colocalization a Pearson correlation of the signal, or other quantification should be provided to back up the observation.

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10. Comment: In the future, be more modest with strong emphasizing words as crucial and indispensable, these were in multiple cases used without justification both in the introduction (page 49…This deficiency attenuates the ductular reaction and reduced bile duct proliferation, although K19 absence did not affect the severity of liver injury or fibrosis induced by CA or BDL (Chen et al. 2015). These findings highlight the crucial role of K19 in liver regeneration during cholestatic injury….), and discussion: "I demonstrate that cytoskeletal crosslinker protein plectin is essential for stabilizing the cytoskeleton of hepatocytes and BECs";
11. Comment: In the future, I recommend avoiding referring to results as differences, without support of statistics. Fig. 24B – „Additionally, while tBA levels increased in both groups, they remained higher in PleΔalb mice, suggesting a greater impact on bile acid homeostasis"; Fig. 29 („numerous necrotic foci in the pericentral region of PleΔalb mice compared to Plefl/fl controls (Figure 29B)."); „which correlates with the higher hepatocellular damage observed in plectin-deficient livers (Figure 29)"; Fig. 35 ", DDC treatment had minimal effect on the ductular area in PleΔalb mice, with values similar to untreated controls."; "We observed a significant increase in bile canalicular blebbing in PleΔalb mice compared to Plefl/fl controls at all time points analyzed (Figure 38)."
12. When interpreting the mild increase of collagen, and aSMA WB in Fig. 25 as a sign of fibrosis, have you considered neovascularization that can follow ductular reaction/cholangiocyte hyperproliferation?
13. Following the DDC treatment led to upregulation of A6 (S100 calcium-binding protein A6 (S100A6)) protein in cKO animals, but no change in CK19 staining. Could the two be unrelated, with A6 reflecting functional reaction in hepatocytes PMC10476764 possibly via its effect on p38 10.2217/fon-2017-0199, rather than ductular reaction?
14. Could the observed effect on E-cad after CA treatment: „PleΔalb lysates showed a 50% increase in E cadherin levels (Figure 33B), despite no obvious changes in the morphology of adherens junctions were observed (Figure 34A)" suggest altered zonation of the liver?
15. In Fig. 40, you report a significant reduction of AB/BC in pericentral and intermediate zones. How do you interpret the fact reduction is not progressive and remains the same from d1 to d14 after insult?
16. In discussion, I agree with your assessment: "Given the paucity of reported observations in knockout mice deficient for individual keratins, it seems implausible that the canalicular abnormalities observed in PleΔalb livers and in 3D cultures of primary hepatocytes lacking plectin can be attributed solely to disruptions in the keratin filament network", have you considered its role in desmosomes and hemidesmosomes PMID: 9389647?
17. Comment: When you write: "Previous studies have established a connection between resilience to cholestatic injury and the stability of the keratin filament network.", it's good to add the reference.
18. Given all the extensive work you did on plectin, would you say the "PLEC mutations who exhibited severe cholestasis and liver fibrosis due to impaired BSEP and MRP2 localization, highlighting the role of plectin in bile canalicular structure and function (Wu et al. 2019)." were a LOF or GOF of plectin function?
19. Fun question - Why mice, that are 100x smaller have liver lobules only ½ of the diameter of human lobules?

In Prague..24.1.2025...

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