Ing. Lenka Belicová, Ph.D.
Department of Cell and Molecular Biology
Karolinska Institute
Stockholm, Sweden

2025-01-09

Opponent's review of the dissertation:
The Impact of Plectin Deficiency on Pathogenesis of Liver Diseases by Mgr. Kateřina Korelová

The dissertation investigates the role of plectin, a versatile cytoskeletal linker, in the context of cholestatic liver disease. The work is highly relevant, as mutations in the plectin gene have recently been linked to an early onset of a cholestatic liver disease. To elucidate how plectin maintains the structural integrity and function of liver epithelial cells, the thesis studied a mouse model with a liver-specific depletion of plectin, subjected to a variety of cholestatic injury models. By providing a detailed characterization of plectin distribution and architecture of the liver epithelial cells, the work uncovers the hepatoprotective role of plectin, particularly in cellular stress response induced by a cholestatic insult. The results deepen our knowledge in cytoarchitecture and mechanical stress adaptation, paving the way for further mechanistic insights into liver pathologies.

The work excellently addresses the author's aims, employing an impressive array of technically challenging methods that must have been executed with precision to yield conclusive results. This level of technical expertise deserves special recognition. Furthermore, the author integrates the latest advancements in the field, staying at the forefront of academic discourse, enhancing both the quality and impact of the work.

Although the work is generally well structured, with well-designed figures and clearly presented results, there are minor issues. There are several typos, grammatical and syntax errors throughout the text. On several occasions, the figure legends should describe clearer the number of animals and images per animal processed to help the reader to draw conclusions (e.g., Fig 37 -39). The quantification of bile canaliculi is unfortunately not sufficiently described (e.g., Fig 37 and Methods section).

Formatting recommendations: Bar charts are predominantly used to present continuous data in the thesis; however, bar charts have drawbacks, e.g., they conceal

E-mail: lenka.belicova@ki.se | **Visiting address:** Department of Cell and Molecular Biology, Biomedicum, Karolinska Institute, Solnavägen 9, 171 65 Solna, Sweden

the data distribution. Individual measurements could be rather plotted in a dot plot over the bar chart. The author should consider using colour combinations in the figures that are colour-blind friendly (unlike red and green, e.g., in Fig 12).

Questions

1. Although the dissertation discusses mouse models for cholestatic liver injury, it is modest on the discussion of mouse models to study plectin.
    a. Could the author discuss pros and cons of the chosen liver-specific plectin KO mouse strain? At which point is the plectin KO achieved? What compensatory mechanisms could develop overtime that could possibly render the phenotype relatively mild in homeostasis? What phenotype could one expect if the plectin depletion was acute and/or transient? How this could be tested?
    b. Could the author further comment on the Figure 13: How is the residual mRNA expression explained? Why there is no detection of plectin in *Ple*$^{\Delta alb}$ liver lysate, assuming other cell types' plectin expression was unaffected?
2. Figure 17 is fascinating! Although the author interprets the keratin network in *Ple*$^{\Delta alb}$ hepatocytes is disorganized, the network still provides certain mechanical stability in unchallenged conditions. Could it be that the network assumes an alternative conformation which still maintains some of the functions?
3. The author hints to defects in polarized trafficking and organization of microtubules that could impact localization of bile acid transporters in *Ple*$^{\Delta alb}$ cells. Are there any plans to follow up on these topics and what experiments could provide more insights?
4. What mechanism would the author envision for plectin's role in the modulation of p38 signalling?

Conclusion

The submitted dissertation of Mgr. Kateřina Korelová meets all the requirements for a dissertation in the scope and quality of the obtained professional results (published/being published in prominent journals). I recommend the thesis for defence, and after successful defence, to award the degree of Ph.D.

Lenka Belicova

In Stockholm, 2025-01-09

E-mail: lenka.belicova@ki.se | **Visiting address:** Department of Cell and Molecular Biology, Biomedicum, Karolinska Institute, Solnavägen 9, 171 65 Solna, Sweden