Opponent's evaluation report on PhD thesis

Title: The role of plectin deficiency in experimental colitis and colorectal cancer

Author: Petra Novotná

Supervisor: RNDr. Martin Gregor, Ph.D.

Opponent: RNDr. Lenka Libusová, Ph.D.

The PhD thesis presented by Petra Novotná was conducted in the Laboratory of Integrative Biology at the Institute of Molecular Genetics of the Czech Academy of Sciences. The candidate is a shared first author of a publication in the respected journal *Mucosal Immunology*. Additional work is anticipated to be published in another shared first author paper. Unfortunately, this manuscript in preparation is not included in the presented PhD thesis. Furthermore, Petra is a co-author of two other manuscripts currently in various stages of the publication process.

The thesis is written in good scientific English, structured traditionally and is presented in full. However, the Czech abstract appears to have been generated through machine translation from the English version, as evidenced by numerous "Czenglish" phrases such as "střevní epitelové buňky", "plectin", "Pozoruhodně, podrobná analýza…" and "kapacita adheze".

The Introduction presents a somewhat superficial overview of the topic. Additionally, the excessive use of abbreviations complicates the reading experience. Conversely, the overall quality of the chapter is significantly improved by the inclusion of well-chosen figures. The section discussing microtubules (p. 21) is inadequately addressed, incorrectly describing microtubules as 15 nm thick polar fibers and suggesting that α- and β-tubulins are encoded by only two genes in mammals.

Two aims of the research are clearly articulated, with associated sub-questions outlined. The Materials and Methods chapter covers a wide array of experimental approaches undertaken by Petra during her PhD studies. While most methods are

clearly described, I have concerns regarding chapter 3.4, which lacks clarity. Additionally, the reason for incubating homogenized colon samples at 60°C for 2 hours in the myeloperoxidase assay (chapter 3.18) is unclear. The specification of primers used for reverse transcription (chapter 3.20) states “random oligo(dT)18 primers” which is misleading.

The Results section is rich. It is divided into two parts corresponding to the stated aims, employing three distinct models: analyses of human patients, mouse models with plectin knockout (KO) in the intestine, and human cell lines with plectin KO. Although results vary across model systems, this diversity allows for a multifaceted exploration of the research questions.

The Discussion chapter contextualizes the findings within current literature and proposes potential applications of the experimental results in human medicine, particularly concerning inflammatory bowel disease and ulcerative colitis treatments. The thesis concludes with a summary and a comprehensive list of references.

Minor Comments on the Thesis

1. **Figure 12**: The reason for the statement that plectin signal is more fragmented in ulcerative colitis (UC) patients is unclear. Additional images supporting this claim would be needed. Moreover, qPCR results indicate plectin reduction only under severe inflammation conditions.
2. **Figure 19A**: This figure requires higher resolution to convincingly illustrate changes in keratin organization, as does **Figure 37**.
3. **Figure 19D**: Clarification is needed on how keratin 8 is specifically detected using a pan-keratin antibody.
4. **Figure 21A**: Which band in GAPDH signal of HSE fraction did you use for blot quantification? Was it a robust loading control for this experiment?

5. **Figure 25**: The main text of the Results section lacks commentary on this figure.
6. **Figure 40**: Quantification of actin fiber thickness is needed to support the claims made.

Questions for further discussion

1. Regarding the plectin deletion in the C-terminal portion of the molecule (between exons 26 and 31) in mouse epithelium, is a truncated protein produced? If so, does it resemble rodless plectin, which is associated with relatively mild phenotypes?
2. Why was exon 6 of plectin targeted in the employed cell lines, and how does this knockout compare to mouse models with deletions of exons 26 to 31?
3. For the $Ple^{\Delta IEC\text{-}ERT2}$ mice, how was the tamoxifen-induced loss of plectin in the intestinal epithelium evaluated? What percentage of cells lost plectin expression, and how leaky was the expression of Cre recombinase without tamoxifen induction? A proper negative control should include a mouse strain with inducible Cre recombinase fed by sunflower oil, not just $Ple^{fl/fl}$ mice.
4. Why do $Ple^{\Delta IEC\text{-}ERT2}$ mice exhibit greater crypt damage in the colon and less damage in the villi of the small intestine compared to $Ple^{\Delta IEC\text{-}ERT2}$? Is approximately one week of plectin removal in conditional KO mice sufficient to observe a fully developed phenotype, including compensation mechanisms?
5. Were there observed differences in the number or location of rare immune-related cell types in the $Ple^{\Delta IEC}$ colon or small epithelium, such as tuft cells or microfold cells?

6. Did you observe enhanced chromatin condensation in stretched cells (Fig. 33)? The study by Nagayama & Fukuei (2020) suggests stretched cells have more condensed chromatin, leading to increased resistance to UV-induced DNA damage, which contrasts with your findings.
7. The fraction of cells with p-γH2A.X foci varies significantly in your experiments, even among control cells (Fig. 34D,E vs. Fig. 36B – Caco cells 60% vs. 40%, RPE 35% vs. 18%). What accounts for this variability? Could the observed trends be the result of experimental variability?
8. Is cell polarity maintained in the intestinal epithelium of $Ple^{\Delta IEC}$ mice? Have any well-established polarity markers been assessed?
9. Do you consider long-term antibiotic treatment for patients with tissue fragility a feasible disease management strategy?

In summary, the thesis represents a substantial body of work demonstrating the significance of plectin in maintaining the mechanical integrity of the intestinal epithelium and its protective role against DNA damage and colorectal carcinogenesis. Despite the aforementioned comments, **I recommend this thesis for defense and the awarding of a Doctorate degree (PhD) to Petra Novotná.**

Prague, August 16, 2024 Lenka Libusová