Review on the PhD thesis submitted by Mgr. Petra Novotná entitled: "The role of plectin deficiency in experimental colitis and colorectal cancer"

The doctoral thesis submitted by Petra Novotná investigates the role of Plectin in the formation of intestinal barrier, homeostasis and colitis (Aim1). Additionally, the focus is directed to understanding how Plectin contributes to mechanical stress-driven DNA damage and formation of colon cancer (Aim2). In general, the thesis is well written, adding new and interesting pieces to the complex mosaic of intestinal homeostasis, colitis and cancer development from the angle of cellular contacts and cytoskeleton. The thesis clearly shows that loss of plectin from the intestinal epithelium is connected to altered cellular contacts and cellular architecture, makes the intestine prone to colitis and colon cancer. Nevertheless, I have some comments regarding the quality and clarity of the thesis. The questions added to the end of this review should not be considered as criticism, but they rather reflect my curiosity concerning results, their interpretation and experimental design.

The Introduction summarizes the current knowledge concerning the composition of cellular junctions with ECM or other cells and how they are interconnected to cytoskeleton. Although the introduction covers all relevant aspects it seems to be imbalanced a little bit. The major attention is directed to proteins constituting cellular junctions and forming cytoskeleton (as a kind of list or atlas). On the other hand, parts introducing colitis (UC/IBD) and colon cancer are very short and shallow. What I was missing was the information about immune cells involved in colitis (in results section they are checked), type of inflammation that is induced by immune cells etc. Concerning colon cancer, at least the heterogeneity and the existence of various types of colon cancer (for example CMS classification; https://doi.org/10.1038/nm.3967) should be mentioned. The characteristic features of colitis associated cancer should be highlighted. Additionally, the connection between cellular junctions, cytoskeleton and signaling pathways modulating transcription and thus cellular fate is mentioned very rudimentary (1.2.7.1.), although later discussed within the discussion. For me as a person with certain knowledge in the field of intestinal and epithelial biology the Introduction was clearly written and easy to follow. What prevented the smooth reading of this section was an abuse of abbreviations. Sometimes less means more. Many terms could be (even repeatedly) mentioned in full wording (e.g. type of type of junctions, proteins families, type of filaments) – as there is no restriction in the term of words count for the thesis (I assume). Minor point: *Figure3* (and some other figures) – as there is no reference indicated, I assume the figure was created solely by the author. Was any licensed software used to generate such a figure (e.g. Biorender, Adobe Illustrator)? If so, such information should be indicated at least in Material and Methods.

The Material and methods section describes sufficiently all experimental approaches used by the author. However, in my opinion, certain parts will benefit if they describe the experiments in more detail. As a strategy against reproducibility crisis many journals prefer full method section, without sentences "…as described in" or "…as described previously". It means this section should allow the reader to repeat the experiment(s) without further literature search and all necessary details should be indicated (e.g. how exactly DAI was calculated). It would be helpful to add a table summarizing all used material (with catalog numbers or other unique identifiers), cell lines, experimental animals, software, deposition of data etc. Concerning experiments based on patient material - no indication of ethical approval (or patient consent) is indicated. Similarly for animal-based experiments there is no information about experimental approval

and if these experiments followed Basel declaration. Minor point – "DSS" is missing in 3.4 and 3.6 (it is just indicated 2% (TdB Consultancy)).

The Results section is divided into two parts according to aims. Results solving Aim1 were published in Mucosal Immunology, that is a renowned journal within the field. This is a sign of the high standard of research performed by the author. The results concerning Aim2 represent mainly unpublished data. The author mentions two "manuscript in preparation", but in my opinion it does not mean anything, unless they are at least uploaded to preprint servers. Generally, it would be worth showing quantifications in the case of some stainings. Within the Aim1 the use of Caco-2 and hCC cells is mentioned, but only results based on Caco-2 are shown (or I simply could not find those for hCC). The histology (and general cellular analysis) of $Ple^{\Delta IEC\text{-}ERT}$ is not shown in thesis, although this is not exactly the same model as $Ple^{\Delta IEC}$, (yes, some H&E staining is in supplementary material of the publication). In contrast to the author, I would be very careful in determining stem cells in colonic crypts just based on their cellular position (Figure 28 and others). The author neither did not use any cellular tracer nor did not check the expression of stem cell markers. Stem cells in colon are not restricted just to the real bottom of the crypts. On the other hand, dividing the crypt to (arbitrary) zones is fine. Sometimes the magnification and resolution of immunostainigs do not allow to agree or disagree with author's statements (e.g. Figure 37). In this case, I simply could not see a big difference between wt and KO and/or stretched/unstreched. It may help to add arrows/arrowheads to point to changes/abnormalities/phenotype or to add the drawing depicting what to check/look at. The Discussion puts the acquired data into the context of the previously published literature. It helps to understand the results in a broader context, but sometimes is more like a combination of results section with introduction. When the discussion starts to be interesting and inspiring (e.g. p122) it suddenly stops without going further. Some aspects as striking difference between small intestine and colon (in the case of $Ple^{\Delta IEC}$) are discussed very superficially. On the other hand, I agree that not everything could be discussed, and the thesis is rich in data. Conclusions represent a clear and nice summary of the thesis. Just minor point: p62 it is mentioned…" ATB treatment revealed that inflammation also contributes to DNA damage in $Ple^{\Delta IEC}$ mice.", but just $Ple^{\Delta IEC\text{-}ERT}$ animals were treated with ATB.

Taking together I congratulate the author for very good doctoral thesis and despite the presence of certain points that can be improved I recommend the thesis to the committee as a basis for PhD graduation.

Questions

1) Why only mouse males were used for the experiments (as mentioned in 3.2)?
2) An extensive tamoxifen gavage as mentioned in 3.2. (twice a day, for three days – every second day), may have an impact on GI tract itself (surely on stomach). Did you check control group (e.g. $Ple^{fl/fl}$, no CreERT driver) administered by tamoxifen for any intestinal abnormalities?
3) What is the average frequency of reduced/lost/mis-patterned Plectin in UC patients? Most cells still seem to be localized in UC tissue the same as in healthy ones. Do you have any idea what stays behind such a scattered pattern? Did you check or is it known from literature if other components of desmosomes, hemidesmosomes or apical cell-cell junctions are lost (or aberrantly localized) in UC patients in similar way/pattern (in the same cells)?
4) In your murine experimental models, you completely eliminated Plectin from epithelial cells, whereas in UC patients only a few cells lost the Plectin expression. To which extend is your mouse model comparable to human UC? Did you try partial recombination in the case of murine epithelium?

5) Did you perform any co-staining of K67+ cells (i.e. Ki67 and other markers)? Just the expression of Ki67 does not necessarily determine TA cells, especially in the case of damaged/regenerating epithelium. These could be for example proliferating secretory cells (hyperplastic), reactivated reserve stem cells etc.
6) ATB treatment strongly reduced the epithelial damage (detachment from BM) in colon, but not in the small intestine (Figure 24). If the detachment from BM is promoted by inflammation so much, to what extent does it depend on Plectin (in colon)? How much are the observations in full KO ($Ple^{\Delta IEC}$) concerning colon relevant, if the animals suffered by inflammation? What could be the cause of the different impact of ATB treatment on small intestine and colon? Did you probe intestinal barrier function upon ATB treatment in $Ple^{\Delta IEC\text{-}ERT}$ animals?
7) Was any batch-correction pipeline (software package) used for the expression meta-analysis (4.2.1 and 3.22)? Did you perform data integration of relevant datasets? Btw. exact identification of datasets (e.g. GEO numbers) should be indicated.
8) Figure31D indicates there is no difference concerning the level of DNA damage (determined as p-gH2A.X^+ foci number or area of foci) between wild type and $Ple^{\Delta IEC\text{-}ERT}$ animals. The only exception is Z3, but in this case ATB treatment eliminated such a difference. This may suggest that the level of DNA damage does not depend on the presence or absence of Plectin at all. Can you please comment on this?
9) My apology, but at current magnification and resolution I am not able to recognize the impact of Plectin inhibitor on K8 pattern as described in text on page 95 (Figure 33C). Btw. Why did you use the inhibitor instead of the more elegant approach based on organoids isolated from $Ple^{\Delta IEC\text{-}ERT}$ animals? In this case you may have one type of organoid – control untreated, Plectin-KO will be acquired by 4-OHT treatment.
10) Figure 33E – what p=0.13 (unsignificant) does refer to?
11) Caco-2 cells are derived from human CRC, they harbor mutation in APC that induce DNA damage (elevated number of p-gH2A.X^+, DOI: 10.1016/j.celrep.2017.05.051). Moreover, they harbor mutated p53. Do these cells represent a relevant model to study DNA damage upon mechanical stress? Do the data based on Caco-2 cells reflect the situation in normal gut? You used RPE cells, but those are from completely different type of epithelia as also reflected by different type of ECM you used for coating. Additionally, colon cancer cells are often in hybrid/partial EMT state, thus cellular junctions might be compromised? Did you check this aspect?
12) In the discussion you mention the possible connection between Itgb4/plectin and RhoA-ROCK-MLC ; FAK-PI3K or Hippo-YAP/TAZ. Did you check any impact of Plectin-KO on these pathways?
13) In the discussion you suggested a possible combination of liquid diet and ATB treatment to reduce colitis symptoms (associated with cellular junctions). What do you think about the long-term impact of ATB on intestinal microbiome? Could it be any alternative instead of ATB (NSAID, cytokines or their inhibitors….)?

In Zurich, 2nd August 2024 RNDr. Tomáš Valenta, PhD.