Diploma thesis review

Miles Joseph Raishbrook BSc.

Title: **The impact of Fam84b in retinal homeostasis.** (2021)

Opponent: Jan Mašek, Ph.D.

Evaluation:

Evaluation criteria	Criteria description	Points (0-3)
1	overall scope of the diploma thesis, balance and structure of its parts	2
2	quality of literary research (number of used original sources, appropriateness of selection)	2
3	aptness of research questions and goals according to thesis assignment and literature research background	2
4	logical sequence of experimental work	1
5	materials and methods	3
6	experimental complexity	3
7	quality of experimental data processing (statistics, sample sizes, exclusion of outliers, choice of statistical/mathematical models)	2
8	interpretation of obtained results	2
9	aptness of results evaluation and comparison in the context of the current workplace and	2
10	Czech and English abstract quality	3
11	graphic design of text, figures, and tables	2
12	language and stylistic level, usage of valid nomenclature	3
13	correctness and completeness of legends for figures and tables (comprehensibility without regard to other text, explanation of abbreviations, units)	2
14	correct use of citation references (presence of non-cited facts, uniformity of the citation style, use of official citation formats)	2
Grade/Points	**Sum:**	31
1 (42-37)	**Excellent/Výborně**	
1,5 (36-31)	**Very good/Velmi dobře**	✓
2 (30-25)	**Good/Dobře**	
2,5 (24-19)	**Satisfactory/Uspokojivě**	
3 (18-13)	**Acceptable/ Vyhovující**	
4 (12-0)	**Unacceptable/Nevyhovující**	

Proposed grade: Very good/Velmi dobře

Evaluation summary:

Diploma thesis of Miles Joseph Raishbrook focuses on FAM84B protein and it functions in the homeostasis of the murine retina using whole-body knock-out of the *Fam84b*. The topic of the thesis is highly relevant due to its possible link to human retinopathies such as age-related macular degeneration.

Thesis introduction is quite extensive (over 20 pages) and provides a general overview of the IMPC phenotyping pipeline, describes the retinal architecture and some of its pathologies, followed by detail overview of known facts about FAM84B, its genetic locus and homologies. The similarity of FAM84B with LRAS is extremely important and the thesis would benefit from introducing it sooner, as it justifies the focus on the phenotypes in retina. An unnecessarily large part of the introduction is dedicated to Ras GTPases, leading to long sections of the text based on secondary literature (reviews), that is not correct and was avoidable.

The methodological part is extensive, and protocols are very detailed, illustrating an impressive range of different physiology/histology/molecular biology methods student used while working on the experimental part of the thesis. These include experiments with mice under anaesthesia, mammalian cell lines and bacteria, basics of PCR and cloning, Western blotting, and immunofluorescent and chromatic staining on sections followed by image acquisition. In fact, given the wide range of fairly complex methods, it becomes almost hard to believe all the work was done by the student, and a bigger clarity on this part would be helpful. Key piece information that was missing was the sequence of the CRISPR/Cas9 altered *Fam84b* of the in the *Fam84b*-/- mice - that is extremely important for the interpretation of the RNA *in situ* experiments and antibody-based experiments.

The results are thoroughly described and accompanied by figures of good quality, with appropriate legends, though at several instances, abbreviation sale bars were missing, and what is worse, the magnification differed between the WT and KO conditions (Fig. 25, 26). I was very surprised to see characterisation of the FAM84B expression so late in the results section (Fig. 25, results starting with Fig. 15), as it represents a crucial piece of data from which all the following analysis should stem from. Additionally, the characterization of the cells expressing FAM84B is not very deep, e.g no double staining was used to pinpoint the cell types expected to be affected in the *Fam84b*-/- mice, or staining of earlier stages to map the onset of the phenotype.

The strongest point of the thesis is a comprehensive analysis of the retinal layering and degeneration using an SD-OCT scanning of a big cohort of animals. These results point at retinal thickness phenotype and gradual degeneration of the retina during the mapped progression of the phenotype, in between w12-60. These results could bring even higher quality data if the same, defined cohort of mice was used across the experiment, as the author fairly points out in the discussion. It would be also very interesting to assess the possible differences between phenotypical progression in male and female mice.

It should be noted the author performed also experiments aiming at the molecular mechanism behind the *Fam84b* deletion. These were performed both on a relevant cell line (h-TERT RPE1) - co-IP and Mass spectrometry, and retinal (lens) tissue - Western blotting. The retinal data provide a possible link to FAM84B - Erk1 signaling, although, given the nature of the genetic manipulation, further experiments need to be performed to verify if it represents a direct interaction or not.

In the last section - discussion - are results well placed into the wider context of a wider context, and several appropriate experimental ideas are charted (TUNEL assays and BrDU labeling). Again an extensive part of the discussion is dedicated to the Ras-related part of the work, which is unfortunately linked to the least solid data, making the discussion less relevant than it could have been if the available data about Ras family members expression and KO phenotypes in retina, such as Ying et al., 2018 (Rab28-/- in retina), or Koso et al., 2019 (Ras overexpression using Pax6-cre) would have been taken in account.

In sum, the methodological aspect and use of the scientific language of the thesis are above average, but the thesis structure and experimental logic, interpretation of the experiments and their robustness (n/repetitions) do leave space for improvement. Needless to say that at the master thesis research-level control over these aspects lays on the shoulders of the supervisor. Taken together, I see a good potential for scientific growth of -soon to be- Msc. Raishbrook, and wish him all the best in his future career.

Questions and comments:

1) page 13. You mention advantages of inbred mouse strains - could think about some disadvantages?

2) page 29. You dispute the accuracy of the alignment f the FAM84B homologs taken from Jiang et al., 2019 (Fig. 10), what is your underlying reason and how would you validate it?

3) page 42. A more common term is “were incubated with” rather than “exposed”.

4) page 43. Could you provide more details on the RNA ISH probe design?

5) page 49. Out of curiosity, did you get 40ug of protein also from the 8 pooled lenses? Why you don´t see any Vinculin loading control on the blot (Fig. 29)?

6) pages 49-50. What is missing in the western blotting technique description?

7) page 58. Could the data used for Fig 18. be reused to answer the questions that arise from Fig.16 (patterns and symmetries of the damaged areas across retinas in different animals).

8) pages 61-63. Figs 21.,22. both lack scale bars and are used without quantification, thus have very low informative value.

9) page 64. In the Fig 23. the OS of the photoreceptors seem to be much thinner in the KO retina, is this just an artefact or a part of the phenotype?

10) page 65. Fig 24,. is based only on n=2 animals per genotype - this is a too low number, and even if it wasn´t it should be stated in the figure.

11) page 74. Could you think about a better negative control for your FAM84B staining of the WT tissue, than the -no antibody- condition?

12) page 75. Why have you optimized the RNA in situ, when you have a working antibody?

13) page 77. Fig 32 Have you tested the statistical significance of the downregulation?

14) page 78. Most of the first paragraph belongs to methods, not results.

15) page 82. Could you please provide the reference for the pigment expressing vessels (vessel cells) of the choroid (assuming melanocytes are not part of a vessel)?

16) page 83. I was very happy to see you pointed out the weakness in the design of the follow-up SD-OCT experiment.

17) How do you interpret the fact that no FAM84B was detected in the mass-spectrometry data, even though it was used as bait?

18) Related question - could you think about any confirmatory experiments that would solidify your data linking GRIPAP1 to FAM84B?

Jan Mašek, Ph.D.  Prague 28.1. 2021