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Re: Evaluation of the dissertation submitted by Razieh Amini

The doctoral dissertation with the title: “Genetic and physical regulation of ventricular development and Reissner fiber formation in zebrafish” submitted by Razieh Amini was carried out in the Laboratory of Neurodegeneration under supervision of Prof. Dr. Vladimir Korzh in the International Institute of Molecular and Cell Biology, Warsaw. The candidate is enrolled in the Warsaw PhD School in Natural and Biomedical Sciences.

The presented research focused on three main questions:

- The role of the voltage-gated potassium channel Kv2.1 in formation of Reissner’s fiber
- How do Shh and Wnt pathways in conjunction with cholesterol dependent membrane composition regulate secretory activity in roof and floor plate and how does membrane composition affect formation of Reissner’s fiber?
- How does body orientation and gravity affect Reissner’s fiber formation?

INTRODUCTION

The introduction leads to the main questions in sufficient detail, - providing a decent background. It identifies gaps in our knowledge as a basis for the in-depth research approaches in the next section. In general, the introduction is very well written and illustrated. In the accompanying papers the background is further deepened by the introduction of each of the four manuscripts.

I have some minor problems with some sections of the main introduction of the thesis. References are missing to back-up some statements made. Also as outlined below one statement appears contradictory and would require additional words for clarification.

Page 11

“These findings suggest that the Kcnc1/Kcng4b axis contributes to tissue integrity and secretion during development. Because neurogenesis depends on sustained, polarized secretion of Sspo by SCO, the Kv2.1 channel likely plays a coordinating role, linking tissue physiology to the controlled release of Sspo and the assembly of RF within the BVS.”

Quotations are missing for the link between neurogenesis and secretion of Sspo by SCO.

Question 1: What is the evidence that secretion of Sspo by SCO is stimulating neurogenesis?

Page 12

“Despite the absence of RF in humans, the role of soluble SSPO in neurogenesis remains debatable.”

I am confused: On the previous page neurogenesis depends on secretion of Sspo. Here, it is stated that humans do not form a RF. How do humans form neurons if previous statement were correct? Moreover, here the role of Sspo in neurogenesis is “debatable” while above on page 11 it is certain. Both statements lack quotations.

Question 2: How does the much larger CNS of humans compensate for lack of Reissner’s fiber. Is Sspo secreted in humans?

RESULTS

The results are presented in form of four manuscripts. Three papers were published or are submitted to peer-reviewed international scientific journals. One manuscript was made public in BioRxiv. For each publication, the applicant has listed the own contributions. The candidate is first-author of two publications - one published in Frontiers in Cell and Developmental Biology and the other in Microgravity.

In the first manuscript published in Frontiers in Cell and Developmental Biology, the candidate describes in detail the development of the Reissner’s fiber in zebrafish. Furthermore, it is shown how mutations in the two subunits of the voltage-gated channel Kv2.1 affect Reissner’s fiber development. By using immunohistochemistry and expression of a transgene, the candidate demonstrated opposing effects of the mutant subunits on transcript levels of key genes and on development of the Reissner’s fiber and the SCO.

Question 3: I am not sure whether I understand how mutations in the two different subunits lead to opposing effects. Could you please elaborate on this.

In the second manuscript published in Microgravity, the candidate addressed the role of gravity on formation of Reissner’s fiber. Artificial development in a vertical position causes defects in Reissner’s

fiber and altered expression of genes involved in mechanosensation. In addition, embryos showed a malformed body. Thus body orientation is an important factor affecting development.

A comment on the site:

“Data are shown as log2-transformed fold change relative to WT. Error bars represent propagated SD” This quote refers to Fig. 5 and is an example for other RT-PCR figures. Wildtype (WT) is a genetic term and refers to wild type genetic composition of the genome. The reference to wt in the quantitative RT-PCR expression analysis does therefore not make sense as both experimentally manipulated as well as control embryos have wild type genomes!! “Unmanipulated control” or “control-embedded embryos” or just “controls” would have been better terms.

Manuscripts 3 of which the candidate is second author deals with the phenotypic analysis of loss-of-function mutants in genes encoding the α subunit Kcnb1 and the modulatory subunit Kcng4b of the potassium channel Kv2.1. This manuscript was pre-published in BioRxiv and is under review in the Journal of Physiology. In this paper, it is shown that the mutants show locomotor and visual defects probably linked to impaired inhibitory circuits.

In Manuscript 4 published in Developmental Biology, Amini Razieh is co-author. Here, it is shown by mutant analysis that Kcng4b is involved in ear development. Loss and gain of function alleles have opposing effects on otolith development and kinocilia length.

In summary, the four manuscripts presented in the Results section provide a vast body of new insights into the role of the voltage gated potassium channel Kv2.1 in formation of Reissner’s fiber, in ear, vision and locomotor development and function. Moreover, the candidate showed that body posture is a key developmental factor in zebrafish.

DISCUSSION

The discussion puts the findings presented in the four manuscripts in a wider context of the existing knowledge and points at possible mechanistic explanations of the discoveries. In addition, this section provides further unpublished observations including graphs and figures to support further preliminary data not covered by the four manuscripts presented in the results section. One preliminary finding is that Kv2.1 appears to be linked to SCO development via Wnt/b-catenin signaling. Other evidence is presented that Shh signaling controls Reissner’s fiber development and that cholesterol metabolism is important in this process.

MATERIAL AND METHODS

This section describes the used methods and the used materials in sufficient detail to repeat the experiments. As such this section meets the standards of modern scientific conduct. Further details on the experimental procedures are given in the manuscripts compiled in the Results section.

FINAL STATEMENTS

This is a very well written and illustrated thesis that addresses important gaps in our knowledge. Thus the approaches and results are highly original which is also reflected in the impressive publication output in highly respected, international, peer-reviewed journals.

I, the undersigned, hereby state that the doctoral dissertation of Razieh Amini meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science (c.t., Journal of Laws of 2024, item 1571, as amended). I hereby recommend to the Doctoral Committee of the International Institute of Molecular and Cell Biology in Warsaw to admit Razieh Amini to the subsequent stages of the procedure for the conferment of the doctoral degree in the field of natural sciences, in the discipline of biological sciences.

Furthermore, I recommend to grant the distinction “summa cum laude”. The candidate has shown an impressive productivity using creative innovative approaches mastering a vast spectrum of experimental techniques. This thesis is well within the top 5% of dissertations submitted at KIT and Heidelberg University. This level of achievement is the prerequisite at these institutions to propose the distinction “summa cum laude”.

Yours sincerely,

