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Review of diploma thesis of Andrea Hauserová – "Investigating functional p38-MAPK signalling during mouse blastocyst ICM cell-fate maturation"

Andrea Hauserová's master's project aimed to verify the cell-autonomous role of p38-MAPK signaling in the differentiation of primitive endoderm of the early mouse embryo. To do this, Andrea first needed to adopt a technique called "immunosurgery", in which she uses antibody-mediated cell lysis to deplete the trophectoderm of the embryo. The optimization and successful implementation of this method was itself a major achievement. In doing so, Andrea made very important and convincing controls on how the technique worked, which was crucial for the next steps - this alone convinces me of the high scientific standard of this work (here I suspect the excellent mentoring of her supervisor). Just to implement this method, Andrea had to master the demanding techniques of micromanipulation and subsequent analysis of the embryo, especially using confocal microscopy. The final experiment itself is already straightforward and extremely important for research on cell fate decision in early mouse embryo. In this sense, this work is very valuable, precisely done and of a high standard.

Overall, the written master's thesis corresponds to a high level of experimental work, everything is presented as it should be, I did not find any significant shortcomings, whether formal, linguistic or content, in the submitted text. There are grammatical errors here and there, but not too many, the text is clear, the structure is adequate. The only major error is the probable mix-up of Figures 7 and 8, is that correct? I greatly appreciated the clickable DOIs in the references. As for the introduction, it is extensive and covers a lot of information on the mechanisms of cell fate specifications in the early embryo. All in all, it helped me to orient myself to the subject, which indicates that the introduction was well written. What would perhaps have helped, and made the introduction even better, would have been the actual summary schemes for the actual text. In several places the author uses schemes adopted from the original literature, and while these do help to understand the specific details discussed in the text, these details are put into a larger context in the introduction. Here a self-summarizing scheme adapted for the purposes of this text would have been helpful. Picking up all the relationships and signaling is not easy and this would certainly help significantly. Also, long sentences are often used and at times it is a long list of stated facts, where it is not exactly easy to make sense of the issues presented. There are still reserves in the writing where Andrea has room to improve - this will surely come with further experience. The evaluation of the results and their presentation are adequate and clear, as is the discussion.

I evaluate the work, both experimental and written, as high quality and definitely recommend it for defense.

Questions for discussion during the defense:

1. Last sentence on page 39 - specifically how much "IS isolated ICM" material would be enough?

2. Page 35 - "*Furthermore the number of ICM cells expressing NANOG (Figure 21) (indicative of either EPI specification or potentially uncommitted ICM cells) was increased (from 7±2 to 14±3)*". Would it be possible to distinguish whether this is "EPI specification" or "uncommitted ICM"? The author partially answers this further in the text but it is not entirely clear to me, can she clarify or expand on this?

3. Can Andrea try to put the potential role of p38-MAPK into the larger scheme of cell fate specification, transcription factors and signaling involved so that we can see the bigger picture? (This might also help with the previous question).

4. In the thesis, the author uses chemical inhibition of p38-MAPK, which is certainly well validated and established. Yet, may I ask if the author might suggest verifying the effect of this inhibitor by some genetic manipulation? If not, why not, if yes, how? What limits, advantages or disadvantages, if any, would it have, how complicated would it be?

In Malovice, May 14th, 2023

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