INSTITUTE OF GENETICS
AND ANIMAL BIOTECHNOLOGY
of the Polish Academy of Sciences

Anna Piliszek, PhD, DSc
Department of Experimental Embryology
Institute of Genetics and Animal Biotechnology
of the Polish Academy of Sciences
Jastrzębiec, ul. Postępu 36A,
05-552 Magdalenka, Poland
a.piliszek@igbzpan.pl

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Review of a master's thesis
by Andrea Hauserová, Bc
University of South Bohemia in České Budějovice, Faculty of Science,
entitled "Investigating functional p38-MAPK signalling during mouse blastocyst ICM cell-fate maturation"
supervisor: prof. Alexander Bruce, Ph.D.

The thesis addresses an important topic of regulation of preimplantation mammalian development, specifically the role of p38-MAPK (mitogen-activated protein kinase) in early lineage differentiation in mouse embryos, using isolated mouse inner cell masses (ICMs) as an experimental model. The role of p38-MAPK has not been previously studied using this approach, and the thesis reports novel findings.

The introduction is detailed and comprehensive, outlining current knowledge on preimplantation development, early lineage specification in mouse embryos and the role of transcription factors, receptor tyrosine kinases and p38-MAPKs specifically in these processes. The literature is correctly cited, although in some cases more detailed references would be recommended (these include more citations of original publications, rather than reviews, especially when specific findings are reported, such as ERK2 inhibition; citations of original discoveries of transcription factors role in preimplantation mouse development; citation of Nichols et al., 2009 as reference to small-molecule inhibition of ERK etc.). It

should be noted that "pluripotent ICM" mentioned in the introduction is an oversimplification, as pluripotency a feature of epiblast lineage, not ICM as a whole (for reference, see Nichols and Smith, 2009).

Materials and methods section is comprehensive and lists the details of all the procedures used. Additionally, more literature references for this section could be added. Dissection of 2-cell stage embryos seems unnecessarily mentioned, as they do not appear to be used in any of the experiments.

Results partially include recapitulation of some of the previous experiments, both from supervisor's research group and others, these however are fully justified and necessary for establishing proper techniques and controls for current experiments. The author convincingly proves that they have mastered immunosurgery procedure, which is the basis for majority of experiments reported in this thesis.

By analysing immunosurgically isolated mouse ICMs, the author successfully proves that p38-MAPK inhibition specifically and autonomously affects this compartment, reducing the number of GATA4+ PrE cells and the observed phenotype is not affected by TE maturation or blastocyst cavity expansion.

Interestingly, the results show that in case of isolated ICMs lower concentration of SB220025 p38-MAPK inhibitor specifically affects PrE differentiation. Additionally, a short, 12 hour window of p38-MAPK inhibitor sensitivity is identified in isolated mouse ICMs.

Author draws appropriate conclusions, and discusses their findings in the context of previous research, suggesting possible future experiments and potential mechanisms explaining observed phenomena (such as existence of uncommitted NANOG+ cells), proving their understanding of research material and available literature.

Questions and additional comments:

- Results section opens with methodological chapter describing details of immunosurgery procedure, entitled "Learning Immunosurgery: ICM isolation technique". While learning new methods is certainly an important part of researchers life, it is by itself not a research question. For this reason, this part of thesis might be better introduced as refining of a method, or control experiment, to better represent the experimental aspect of this endeavour. Next section (4.2) is

introduced in this way, and although it recapitulates previously published results, there is no doubt it is a necessary part of a current experimental scheme.

- Both NANOG and SOX2 are used as epiblast lineage markers (for example, fig. 7 and 8), however, only calculations for NANOG+ cell numbers are shown (NANOG/SOX2 in the text, but NANOG only in graphs). Can you provide results for SOX2 localisation, or speculate on the possible differences in expression patterns?
- The author used *in vitro* cultured mouse blastocysts as the source for ICM isolation, to avoid discrepancies in timing of lineage commitment between embryos. With this approach, *in vitro* cultured SB220025-inhibited whole blastocysts might have served as a more appropriate control. Can you provide comparison to *in vitro* cultured embryos, based on your own experiments or literature, or speculate on the possible effects of such approach?
- What are the specific effects of isolated ICM culture at higher concentrations p-38 MAPK inhibitor? How was cell death determined?
- Have the effects of equivalent (5 μM) concentrations p-38 MAPK inhibitor on intact mouse embryos *in vitro* been tested ?
- In the isolated ICM cultured in the presence of p-38 MAPK inhibitor, were all the cells expressing markers of one of ICM lineages, were there any co-expressing cells, or cells with no detectable marker?

In summary, the work presented in this thesis is original, of high scientific quality and addresses an important topic in the field of developmental biology. As such, it represents a valuable contribution to our knowledge of developmental mechanisms. Therefore, I recommend the thesis for successful defence.

dr hab. Anna Piliszek