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MASTER'S THESIS REVIEW

Title: „Investigation of the gene *Dynactin subunit 2 (Dctn2)* in regulating the frequency of asymmetric cell divisions during mouse preimplantation embryonic development."

Author: Bc. Michaela Kubíčková

Opponent: Andrej Šušor, PhD.

Summary:
The author of the master's thesis Michaela Kubíčková performed experimental work in the well-known laboratory of the Dr. Alex Bruce. This lab is focused to the study of the preimplantation development of the mouse embryos which also shows publication record.
The diploma thesis is focused to the lineage contribution of the daughter cells under the regulation of the single gene product, DCTN2. Author found that downregulation of the expression of the DCTN2 leading to the reducing number of formation of inner cells in the 16 cell mouse embryos.

I have to conclude that study topic is very interesting. The team selected appropriate and interesting gene candidate which clearly contributes to the formation of the early embryo physiology. I have to highlight the difficulty of the study topic and used model. Despite of this the student excellently managed this uneasy task which leads to presented results. Moreover, Michaela Kubíčková adopt number of methods which most likely will be beneficial for her future. The study nicely presents positive correlation of the expression of the gene of the interest and its role in formation of the early embryo.

Question for defense:
It is known that impairment of the expression of some single genes leads for example to the miscarriage in the mammals including humans. Do we know something about abnormal expression (deletion, mutation) in the *Dcntn2* gene in the human population or in the animal kingdom?

Conclusion:
The work meets the goal and results in a valuable experimental study and I am expecting that finding will be presented to the readers of the peer viewed international scientific journal.
The author of the thesis proved her ability to perform research and to achieve scientific results. I recommend this thesis for presentation with the aim of receiving the Master's degree. My grading is excellent (A or 1).

Sincerely,

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Andrej Šušor, PhD.

Libechov 14.5.208

Warsaw, 20 May 2018

Dr. Katarzyna Teresa Filimonow
Institut of Genetic and Animal Breeding,
Jastrzębiec, Poland

Review of Master thesis: „Investigation of the gene *Dynactin subunit 2 (Dctn2)* in regulating the frequency of asymmetric cell divisions during mouse preimplantation embryonic development" Bc. Michaela Kubíčková

In the presented master thesis Michaela Kubičková described study focused on the role of the *Dctn2* gene expression in the preimplantation mouse embryonic development. The aim of the study was to understand how expression of *Dctn2* regulates cell fate in the first lineage specification in the embryo. In the 8-cell stage all blastomeres are morphologically identical with apical-basolateral polarisation, having access to the environment. In the next round of division these blastomeres can divide symmetrically, giving to polar cells or assymetrically, giving one cell which stays outside and polarised and one cell which becomes inner apolar blastomere. So at 16-cell stage some blastomeres are positioned inside the embryo, meaning they are enclosed by other blastomeres and non-polarised. So as a consequence of this cleavage two population of blastomeres are distinguished in the embryo – outside, polar blastomeres and inside, apolar. In the next round of division (8→16) inner blastomeres are giving more inside cells and outer blastomeres are again dividing symmetrically or asymmetrically. As a consequence of this two round of divisions the 32-cell stage embryo is built from inner cells which are forming ICM (inner cell mass); and outer cells which make first extraembryonic lineage – trophectoderm. In the further development from the ICM epiblast specifies – embryonic lineage making the embryo proper; and second extraembryonic lineage - primitive endoderm, which builds yolk sac. Trophectoderm is giving rise to the placenta. Michaela Kubičková presented how Dnct2 regulates spatial distribution (outside/inside) of blastomeres within 8→16 transition.

The thesis has classic structure, with introduction, material and methods, results and literature sections. All together the thesis has 72 pages with 21 figures, what seems to be close to maximum volume for the thesis for Master degree. The introduction section is very

extensive, giving detailed, but clearly presented background of the research she presents in the master thesis. In my opinion Bc. Michaela Kubíčková combined very well historical and the current knowledge about mouse embryo development, selecting and describing the most important issues. In the next section almost all materials and methods were described in details, with appropriate literature. The only oversight I have found is information of the dsRNA used as a control in the experiments in which Dctn2 was downregulated. Information that control dsRNA was made against GFP is given in the last part of materials and methods section (3.6), instead of 3.4 and 3.5, parallel with information about dsRNA against Dctn2. Also I have not found details about how this control dsRNA was prepared. Except this one oversight I find this section written in a very detailed and understandable way, which allows other experimenters to reuse the methodology. In my opinion that is the only way to describe material and methods in scientific literature as a master thesis is. What I find outstanding in the presented thesis is a variety of methods that was used. It is not so common that master student perform research with wide-ranging methodology. The results section was prepared with due care about composition and clear presentation of data. One would consider that presenting results from literature should be done only in the introduction or discussion, but not in the results section. As I assume figure 10 was presented in this section for a convenience of a reader to compare results achieved by Michaela Kubíčková with data presented in the literature. The discussion, consequently with other sections, is very clearly confronting current knowledge with results described in the thesis. Michaela Kubíčková shows the influence of her results on the field but without over-interpretation of her data or speculation. The last part of the thesis is literature, prepared in the standard composition, allowing the reader to search for the particular publication without difficulties.

Michaela Kubíčková presented experiments in which she revealed the role of Dctn2 in the generation of inner cells during division of blastomeres between 8- to 16-cell stage. Clonal downregulation of Dctn2 caused no generation of inner cells by the progeny of injected blastomere. In further research Michaela Kubíčková showed how mTOR inhibition causes downregulation of Dctn2. According to results obtained in Bruce group these results suggests involvement of mTOR pathway in the regulation of inner/outer cells composition in the cleavage 8→16 through Dctn2. However rescue experiment seems to be required to fully confirm this conclusion. It seems extremely interesting how this regulation of generating inner cells occurs. What is the exact mechanism how Dctn2 changes cell machinery? How exactly Dctn2 plays role in the outside/inside positioning of blastomeres? Results presented by Michaela Kubíčková are

giving new insights into mechanisms regulating generation of the first specification of blastomeres in the mouse embryonic development and are good starting point for further investigation. I would like to ask then what kind of experiments Michaela Kubíčková would perform to answer these questions.

In my opinion the presented thesis deserves for the highest mark, however my role as an opponent is to find oversights and/or discuss particular issues. First I would like to discuss experiments with downregulation of Dctn2 by microinjection of dsRNA. The injections was performed on the 2-cell stage, however according to RNA level data for Dnct2 published by Wang mRNA for Dctn2 appears at 8-cell stage. Wouldn't be more accurate to inject blastomeres at this stage though? I bear in mind that injection into 4-cell stage is slightly more difficult than into 2-cell stage embryo. Moreover, after injection dsRNA against Dctn2 into 2-cell stage the downregulation at 16-cell stage was on the level of 15% meaning that the method used by Michaela Kubíčková was very efficient. Other issues about microinjection are if the concentration of injected dsRNA or mRNA was validated and why global downregulation was performed by injection into 2-cell stage not zygote.

Another issue I would like to bring up is the housekeeping gene selected for qPCR. Michaela Kubíčková had chosen *H2afz* gene, however from the work of Mamo et al., (2007) we know that *H2afz* is an appropriate housekeeping gene from zygote till 8-cell stage embryo, but not in a blastocyst. Unfortunately in this publication there is lack of analysis of this gene between 8-cell stage and blastocyst. Was then, for research presented in the thesis, characterized expression the level of the *H2afz* between 8-cell and blastocyst stage? I did not find as well information about number of embryos were used for a validation if dsRNA downregulates *Dcnt2*. There is information about technical repeats in the material and methods section, but it is given without number of embryos used for this validation and without information about biological repeats.

Michaela Kubíčková tried to visualize Dctn2 protein in mouse embryos. In the literature it is difficult to find immunostaining for Dctn2, except HeLa cells staining in Morris et al. publication (2015). From the experience I know that there are proteins which visualization in the embryo is difficult or even impossible in comparison with cell culture material. Seems that similar difficulties are described in the presented thesis. Michaela Kubíčková used another antibody than Morris and specificity of the antibody she used is not clear. However Michaela Kubíčková

shows cortex localisation of Dctn2 by injecting Dctn2-Venus construct. What is worthy to mention the construct includes 5'UTR motif, useful for mTOR experiments.

The statistical analyses was performed in the thesis was made on the number of inner and outer cells after dsRNA injection to downregulate *Dctn2* expression. The test used was two-tailes Student's t-test, however maybe confidence intervals should be considered as a more accurate method.

Despite of some oversights mentioned above, I find both the research made by Michaela Kubíčková and its description in the thesis performed on a high level (degree-1). I am not able to come for the M. Kubíčková's defence, for a substansive list of questions I had Michaela has answered to me in writing. I am more than satisfied with her answers. If there is official opportunity at University of South Bohemia to give distinction for an exceptional master thesis I strongly suggest Michaela Kubíčková for this award.

Katarzyna Filimonow

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