Reviewer report of the PhD thesis „The study of Xenopus tropicalis testis-derived stem cells" by Thi Minh Xuan Nguyen

The aim of the presented thesis was the identification of factors responsible for the induction of epithelial mesenchymal transition (EMT) of Sertoli cells (SC) that adopt stem cell-like phenotype and enable thus their broader differentiation potential.

The thesis has a standard structure and are organized into six Chapters. After short summary the author provides us with the comprehensive introduction to the testicular anatomy and development as well as the molecular mechanisms underlying EMT/MET. This part of is very well written and gives readers a solid background of the topic. The experimental part of the thesis consists of Material and Methods part and Results and Discussion chapters.

Author asked two main questions:
„Could EMT reprogram immature Sertoli cells back to their stem cell stage at which their differentiation potential increases?
What molecules are involved in the maintenance of immature SC phenotype? "

To answer them, the author employed the cell lines of Xenopus tropicalis testicular somatic cells (XtTSCs / XtiSCs) the model that has been established in the laboratory previously.

The results demonstrated that XtiSCs underwent EMT by inhibition of GSK-3 by CHIR99021. Mesenchymal like XtiSCs exhibit much broader differentiation potential (chondrocytes *in vitro* and cardiomyocytes *in vivo*). These cells also show directed migration capacity towards cervical cell line and site of injury in vivo. All those data establish XtiSCs as a great model to study mechanisms of EMT and underlying signaling pathways. Another important result was the observation that cytokeratin was retained in cell junctions of immature Sertoli cells together with β-catenin and the breakdown of the junction after acrylamide or CHIR99021 inhibition.

Xuan Nguyen clearly demonstrated that she is skilled experimental scientist, who is able to properly articulate scientific questions, analyze the data and present them in a clear form. Approaches that were taken are appropriate and reflect current trends in modern developmental biology.

As part of her thesis, author published/submitted two first author papers and she was co-author on one other paper in Open Biology.

Therefore, **I fully recommend to confer the PhD title to the candidate.**

For the discussion during the defense, I have only few minor comments/questions:

1. Cell culture media for ex vivo expansion of XtiSCs contained mouse recombinant LIF. It was required only for colony growth (Fig.12A). Is it really binding to xenopus LIFR?
2. Was there any particular reason for the selection of HeLa cell line to demonstrate the XtiSCs' capacity to directionally migrate in vitro?

3. Fig. 23 – Labeling of treatment in panels is missing
4. Abstract: CHIR99021 inhibitor is not the drug

Prague, 2.6.2019

Petr Bartůněk, PhD.
Institute of Molecular Genetics, v.v.i.
Vídeňská 1083
142 20 Prague 4

bartunek@img.cas.cz
Tel. 241 063 117
GSM 774 798 152