Individual Doctoral Committee member's report – Doctor of Philosophy thesis

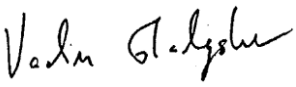
Name of Candidate: Aleksei Mikhalchenko

PhD Program: BioMed

Title of Thesis:

Supervisor: Vadim Gladyshev

Date of Thesis Final Review:

Signature: 

Date: 21-06-2018

Report

Aleksei Mikhalchenko carried out most of his doctoral research in my lab in the Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School. Based on his publication record and research completed, I think he should proceed with thesis pre-defense in June 2018 and final defense in October 2018. I will further address his accomplishments and offer insights on the quality of his work.

Publications. Aleksei has published two research papers, including one in Stem Cell Reports (as co-first author) and another one in Stem Cell Research (also as co-first author). This should formally be sufficient for the defense. In addition, there are two studies, currently in preparation for publication, where Aleksei is expected to be the first author. Overall, he contributed to 4 manuscripts, all with first or co-first authorship.

Thesis. His thesis includes a literature review and 3 studies. The literature review was fully written by Aleksei.

The first study, "Naked Mole Rat Induced Pluripotent Stem Cells and Their Contribution to Interspecific Chimera", represents an important development in the field. Naked mole rats (NMRs) are exceptionally long-lived, cancer-resistant rodents. Identifying the defining characteristics of these traits may shed light on aging and cancer mechanisms. In this study, Aleksei worked jointly with another lab member, Dr. Sang-Goo Lee, and they reported the generation of induced pluripotent stem cells (iPSCs) from NMR fibroblasts and their contribution to mouse-NMR chimeric embryos. They observed efficient reprogramming under N2B27+2i conditions. The iPSCs displayed a characteristic morphology, expressed pluripotent markers, formed embryoid bodies, and showed typical differentiation patterns. Interestingly, NMR embryonic fibroblasts and

the derived iPSCs had propensity for a tetraploid karyotype and were resistant to forming teratomas, but within mouse blastocysts they contributed to both interspecific placenta and fetus. Gene expression patterns of NMR iPSCs were more similar to those of human than mouse iPSCs. Overall, the study uncovered unique features of NMR iPSCs and reported a mouse-NMR chimeric model. The iPSCs and associated cell culture systems can be used for a variety of biological and biomedical applications.

As a follow up of that study, Aleksei reported the development of an iPSC line generated from immortalized NMR embryonic fibroblasts transduced with a doxycycline-inducible mouse OSKM polycistronic vector. This iPSC line was shown to express pluripotency-associated markers, form embryoid bodies, differentiate in vitro to the derivatives of three germ layers, and exhibit normal karyotype. The ability of iPSCs to differentiate in vivo was supported by the contribution to interspecific chimera upon injection into mouse blastocysts. This NMR iPSC line may be a useful tool in cancer and aging research.

In the second study, Aleksei prepared chimeric mouse-rat animals and characterized them. For this, he first developed rat iPSCs, characterized these cells, demonstrated generation of all three germ layers as well as teratomas when injected into mice. He then injected them into mouse blastocysts and produced chimeric mouse-rat animals. Some of these animals had chimeric coat coloring, which allowed easy visualization and analysis. An interesting observation resulting from this work is that chimerism has decreased with age of these animals. We expect that final experiments will completed and the manuscript will also be completed prior to final defense.

In the third study, Aleksei examined thermogenesis in the naked mole rat. These animals are considered poikilothermic. However, several observations argue against this idea. First, we found that UCP1 protein is functional in NMRs. These animals also lost much of their brown fat upon cold exposure. Moreover, NMRs responded to b-adrenergic receptor agonist by generating heat. Interestingly, they also activated lipogenesis in response to cold exposure. To complete this study, additional experiments in metabolic cages will be carried out shortly, and the study is expected to be completed and manuscript written prior to final defense.

Aleksei has worked hard on these projects. The nature of this research is that studies with stem cells take much time. Overall, he generated sufficient body of work for PhD defense.

Provisional Recommendation

☒ *I recommend that the candidate should proceed the Thesis Final Review*

☐ *The thesis is not acceptable and I recommend that the candidate be exempt from the Thesis Final Review*

The review report on doctoral thesis of Skoltech PhD student Alexei Mikhalchenko

PhD thesis by Aleksei Mikhalchenko is a high level study in the field of cellular, developmental and molecular biology. The study is divided into 3 parts, connected by the same idea of studying the unique animal, *Heterocephalus glaber*, or the naked mole rat (NMR). This rodent is characterized by exceptional longevity, far beyond naturally expected for the terrestrial animal of its size. Moreover, NMR is known to naturally resistant to develop cancer and shown no increase in mortality with age. In addition, it is a social animal, with reproduction delegated to a "queen" of the colony. Yet another remarkable feature of NMR is that it is the only known poikilothermic mammal, that means it do not maintain constant body temperature. Due to the specificity in reproduction, normal way of genetic manipulations with NMR is highly complicated. This makes necessary to develop a method of production and manipulation with NMR induced pluripotent cells, which was successfully described in the part I of the thesis. The main result of this part is not only the production of iPSC, but also the production of mice-NMR chimeric animal, although with rather limited persistence of NMR cells. To compare it with other inter-rodent chimerae, the part II of the thesis goes about development of mice/rat chimera. An important conclusion about decrease of chimerism with the age of an animal was made. Finally, the III part of the work is devoted to the thorough study of the molecular mechanisms of NMR thermogenesis, which is remarkably different in its regulation with that of other rodents (and other mammals).

In sum, the study is done on he high level of the best international standards and I would recommend it for the *Thesis defense*

Associate Professor Petr Sergiev