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Thesis Changes Log

Name of Candidate: Aleksei Mikhalchenko

PhD Program: Life Sciences

Title of Thesis: Comparative Biology of Aging through the Lens of Induced Pluripotent Stem Cells

Supervisor: Prof. Philipp Khaitovich, Skoltech

Co-Supervisor: Prof. Vadim Gladyshev, Harvard Medical School

Chair of PhD defense Jury: Prof. Olga Dontsova ***Email***: *o.dontsova@skoltech.ru*

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The thesis document includes the following changes in answer to the external review process.

Reviewer comment 1:

In the comparative gene expression analysis of iPSCs of the three studied species (page 69) clustering in additional to PCA would be desirable. In the same section, nothing is said about genes with deceased expression: is it due to the assumption that they reflect fibroblast specificity and hence are not interesting? — this needs at least to be discussed. Analysis of commonalities between human, mouse, and NMR DEGs ignores the fact that there are much less observed DEGs in mouse than in human and NMR. This requires an explanation (e.g. could that be due to differences in library sizes influencing statistical significance of fold differences?); anyhow, the number of DEGs should be accounting for when the overlaps in DEGs and GO categories / pathways in pairs are analyzed. A nice addition to Fig. 17 could be a panel with PCA for all species: would samples cluster first by species and then by cell type or vice versa?

Author:

Thank you very much for your comments! Correlation matrix (added as Fig. 17E) showing similarities among all mouse, naked mole rat, and human samples in regards of their gene expression revealed several interesting observations. First, NMR fibroblasts were closer to NMR iPSCs than to mouse and human fibroblasts. Second, similarities in fibroblasts and iPSCs were higher within naked mole rat species than within human or mouse. This is supported by other data obtained in Dr. Gladyshev lab, showing that in naked mole rat, various cell types are more similar to each other that respective cell types in other rodent species. Finally, the correlation matrix suggests that NMR iPSCs have more similarities with mouse iPSCs rather than with human ones.

PCA for all three species was added (Fig. 17F). It showed that common differences separating mouse, naked mole rat, and human fibroblasts from respective iPSCs were more clear than differences among species. Thus, samples clustered first by cell type and only then than by species.

Reviewer comment 2:

On the technical side, the quality of some figures and tables is far from perfect. It looks like they have been copy-pasted from the papers in the pixel format, thus lowering the resolution instead of being reproduced / reformatted directly.

Author:

Due to the large size of the original file with high quality figures, its upload to Canvas was unsuccessful. For this reason, the file was compressed and only then uploaded. Uncompressed file should be available on Skoltech website.

Reviewer comment 3:

The claim that BAT is used up to compensate for lower temperatures (pg 123-125), measuring BAT mass before and after cold exposure for 24 hours, is not necessarily controlled by a similar measure in WAT mass. It could be that BAT consumption is not different from WAT usage and could be due to energy

metabolism. Higher BAT than WAT consumption upon cold exposure would indeed support intact BAT function in naked-mole rat thermogenesis.

Author:

We performed and added (Fig. 36) additional experiments indicating robust endogenous BAT thermogenesis in naked mole rats.

Reviewer comment 4:

Specify whether iPSCs were generated from LT-immortalized cells. It is stated that embryonic fibroblasts were immortalized, while whether the other cells contained LT is unclear.

Author:

Only naked mole rat embryonic fibroblasts were LT-immortalized. We also used non-immortalized embryonic fibroblasts, as well as fibroblasts isolated from tissues of adult individuals (kidney, skin, testis). All these cells were not immortalized. Thus, iPSCs were generated from various fibroblasts, among which only embryonic fibroblasts were LT-immortalized.

Reviewer comment 5:

Elaborate on the unexpected finding of wide spread tetraploidy in naked mole rat iPSCs. Was it connected to LT antigen?

Author:

We observed a propensity for a tetraploid karyotype in both naked mole rat fibroblasts and iPSCs. This seems not to be related to LT antigen as both LT-immortalized embryonic fibroblasts and non-immortalized embryonic fibroblasts as well as respective iPSCs showed such propensity. Among all fibroblasts (embryonic, immortalized embryonic, and adult), only adult fibroblasts exhibited primarily normal karyotype. Interestingly, iPSCs generated from adult fibroblasts increased their tetraploidy. These findings support the idea that naked mole rats may rely on the increased use of tetraploid cells.

Reviewer comment 6:

Since aborted embryos has higher percentage of naked mole rat cells, does it indicate the difficulty in forming full-term chimeras? What may be the reason?

Author:

Seems like it does. First, naked mole rats are characterized by slower development (~70 days gestation period) compared to mouse (19-21 days). Second, the body temperature of naked mole rats at thermoneutrality is about 32 °C. The appropriate temperature for NMR cell culture is also known to be 32 °C, not 37 °C as in case of mouse. Although we showed that naked mole rat iPSCs could differentiate to functional cells of all three germ layers at higher temperature (37 °C) *in vitro*, the number of survived and

differentiated cells were lower compared to that at 32 °C. All of these might contribute to diminished chimeric phenotype of NMR-mouse fetal chimeras over the time of embryo development and, thus, explain why we see higher rate of chimerism in embryos aborted prior to E13.5.