

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Alexander Tyshkovskiy

PhD Program: Life Sciences

Title of Thesis: Molecular Signatures and Mechanisms behind Lifespan Extensions

Supervisor: Prof. Philipp Khaitovich

Co-Supervisor: Prof. Vadim Gladyshev

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Date of Thesis Defense: 23 October 2018

Name of the Reviewer:

I confirm the absence of any conflict of interest

Signature:



Date: 09-09-2018

Reviewer's Report

This is a good thesis.

Ch. 1, the review part of the thesis is logical and complete, and lays a solid foundation to the performed research.

Ch. 2, about the effect of gamma-radiation on fibroblasts of mouse and naked mole rat, contains interesting observations about specifics of this response in the two species. The transcriptome analysis is nicely complemented by physiological observations about cell fate following radiation. The discussion puts the observation in the context of published studies and sets possible directions for further research.

Results of PCA analysis are described, but a figure would be useful (p. 45). Similarly, it would be useful to provide the global correlation heatmap, and not only the species-wise ones in Fig. 4. In fig. 6A, no scale is given in the right panel: even if it is the same as in the left one, it would be useful. At that, the number of overrepresented GO categories is large, presumably due to nested categories (p. 50). A schematic representation showing the interconnection of these categories would be of interest.

Ch. 3 describes dependence of the methylation status on age and calorie restriction in the mouse model. Regression models were applied to identify different classes of sites and their prevalence in functionally different genome regions. Probably the most interesting results of this analysis is the negative correlation between methylation changes during aging and calorie restriction. At that, a couple of additional analyses would be useful. It might be interesting to supplement the regression analysis with clustering of

methylated sites by their age dependence patterns and analyzing functional characteristics of sites (and relevant genes) in these clusters as well as their methylation upon calorie restriction. It would also be nice if the analysis of methylation were supplemented by the analysis of gene expression. Finally, the analysis of the human data seems sort of auxiliary, confirming the mouse observations; a more detailed comparison of homologous sites (more exactly, sites in homologous regions, e.g. genes and promoters) might be of interest.

Student's t-test was applied, but it is not clear whether the distribution had been tested for normality (p. 64). Does the observation that decreasing-methylation sites are initially hypermethylated and increasing-methylation sites are initially undermethylated (pp. 71-72) mean that the variance of methylation (over all changing sites or even over all sites) decreases with age? – to some extent this is demonstrated using Shannon's entropy in 3.2.4, but a direct test also might be relevant.

In Ch. 4 various types of life-extending treatments were compared. The author listed common and specific transcriptome responses to the treatments, observed discordance between gene/pathway expression changes and lifespan effect between sexes, and demonstrated that certain life-extending treatments have a feminizing effect on males and the masculinizing effect on females at the transcriptome level. The latter observation can be interpreted as convergence of transcriptomes between sexes for certain life-extending treatments. On the other hand, these effects do not cause life extension per se.

It is not clear why the schemas for treatment (including age of sample collection, mouse strains, sex composition) were different for the original (not from published sources) experimental data. Differential gene expression was assumed with fold change 1.5, unlike previous analyses, where the threshold $FC > 2$ was used — while such thresholds are necessarily arbitrary, some motivation still is desired. Definition of z-score via log p-value (pp. 97-99) is somewhat unconventional, as usually z-score is just the difference with the mean in SD units.

Overall, the results of the study are scientifically sound and interesting. The thesis is largely well-structured, and clearly written, but requires spellchecking and some language and style correction. Statistical analyses are convincing; however, as a general remark, it might useful to provide FDR in addition to the adjusted p-value. The results are published in good journals and the publications satisfy SkolTech PhD requirements.

Provisional Recommendation

I recommend that the candidate should defend the thesis by means of a formal thesis defense

I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report

The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense

