Reviewer assessment

Author of thesis: Jiří Hrubý

Title: Autophagy induction decreases the presence of endogenous P301S tau aggregates

Type of thesis*: Bachelor of Science

	Evaluation criteria	Grades						
		A	B	C	D	E	F	Non-evaluable
1	Scope of thesis, chapters proportion	×						
2	Review quality (i.e. quality and accuracy, number of references used)	×						
3	Objectives achievement	×						
4	Accuracy and completeness of figures and tables legends (i.e. understandability, consistency, abbreviation explanation, correct using of units)	×						
5	Accuracy of references using (i.e. absence of references quoted in text and list of references, formal stylistic consistency)	×						
6	Accuracy of summary in Czech and English	×						
7	Graphic quality of text and figures	×						
8	Language and stylistic quality, using of valid/ standard terminology and nomenclature	×						
9	Choice of appropriate experimental methods	×						
10	Comprehensibility and conciseness of used methods description	×						
11	Quality of experimental data processing	×						
12	Results interpretation		×					
13	Discussion (results summary and its implementation in the context of current research/knowledge)	×						

Note1: if impossible to apply, use “non-evaluable”
Note2: mark with “X”
Note3: final grade is based only on evaluable (A-F) items
*- select “Bachelor” or “Master of Science”

Final Grade (A-F)	A

Please, attach your comments and questions as well as reasons for your evaluation at the next page (pages)

Conclusion: The thesis is recommended ~~/not recommend~~ for defense

Olomouc , date: 26.6.2020

Signature: Viswanath Das, Ph.D.

REVIEWER'S COMMENTS AND QUESTIONS

The thesis by Jiří Hrubý for his Bachelor of Science degree studies the effect of autophagy induction by epigallocatechin gallate (EGCG) on the clearance of intracellular tau aggregates in a biosensor tau cell line after subjecting the cells to proteotoxic stress with aggregates of R3 peptides. The thesis is divided into appropriate chapters and sub-chapters. The introduction is clear and very interesting to read. There are about 40-45 references and the candidate cites all major studies from the literature in this field of research. The thesis objective is clearly defined and all experiments have been appropriately designed to address the objective. The figures are clear and have been concisely defined in the figure legends. The results are presented in 5 figures and the findings have been summarized in 1 page. I would have liked to see a bit more elaborated results and discussion of the results. Nevertheless, this is a nicely written work and presents interesting findings on the negative impact of R3 aggregates on pathways that are meant to clear misfolded protein aggregates under physiological conditions. Further, the results indicate clearly that the resumption of autophagy induction can have a significant effect on the clearance of toxic misfolded proteins. Jiří has done a tremendous job in putting together a good study and I congratulate him on the completion of his thesis. Not many students take up thesis work in English at the bachelor's level at our university. For this, I must specifically acknowledge his courage and willingness to work on this thesis in English.

A few questions/comments for the candidate:

Q1. No tau mutations have been reported in Alzheimer's disease but your cell model has P301S mutation associated with frontotemporal dementia. Why did you use the P301S-tau cell model and how does your finding with a tau-mutated model relate to AD?

Q2. How do you think the aggregates are taken up by the cells?

Q3. Does the clearance of intracellular aggregates by EGCG-mediated autophagy induction reduce cytotoxicity in cells?

Q4. In Figure 7A, you have chosen not to quantify and present an important part (the effect of monomeric R3). The treatment of cells with monomeric forms of R3 increases p62 levels. Further, it appears that cells can induce autophagy after 6 hours of treatment with monomeric R3 but not after 48 hours (LC3A/B conversion). My question is why does a longer (48 hours) treatment of cells with monomeric R3 (that has no β-sheet structure), increase p62 level, and inhibit autophagy induction? The stress and inhibition of LC3A conversion by monomeric R3 after 48 hours are similar to the effect of R3 aggregates.

Q5. In Figure 8, why do you see an increased p61 band in the absence of any treatment (lane 1 in blot B) compared to cells treated with R3 aggregates (lane 4 in blot B)?