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Referee's Report on the PhD thesis by Mgr. Martin Ondrej entitled Radiosensitization of cancer cells by modulation of autophagy: Phosphoproteomic analysis

The aim of the dissertation thesis of **Mgr. Martin Ondrej** was to analyse phosphoproteomes employing several techniques of molecular biology to examine the underlying molecular mechanisms and signalling pathways involved in the regulation and inhibition of the autophagic process.

Due to the fact, that all particular work was already published in articles in impacted journals (first and second quartiles), the dissertation is rationally compiled into a commented set (totally 157 pages including all enclosed publications, 46 pages of own dissertation thesis). The work is divided into 2 results areas (1. Mechanisms associated with the inhibition of autophagy using the autophagy inhibitor—Lys05 and 2. Optimization of phosphoproteomic analyses)

It is supplemented by **five publications associated with the topic of dissertation thesis** articles in impacted journals, one of which is the review and the other an original research article (in 3 out of them the candidate is the first author). In the introductory part of the dissertation (pp. 10- 18), the author provides a detailed overview of the autophagy in oncogenesis and the molecular and cellular mechanisms and signalling pathways behind the autophagy. In the appendices to the thesis, a review article "To live or let die: unclear task of autophagy in radiosensitization battle." by **Ondrej M**, Cechakova L, Durisova K, Pejchal J, Tichy A, published in the "green journal" Radiotherapy and Oncology is attached, which corresponds to the ideas presented in the first part of thesis introduction.

The next section provides a detailed description of experimental models and methods used for experiments involving combined treatment of H1299 cells with novel inhibitor Lys05 and IR leading to concentration and dose-dependent decrease in cell proliferation. The corresponding results section presents the individual outcomes of the dissertation experiments: combined effect of autophagy inhibitors and IR on cell proliferation, effect of the treatment on autophagy related signalling molecules and morphological changes in affected cells. The results are also presented in the appendix publication 2 "A Potent Autophagy Inhibitor (Lys05) Enhances the Impact of Ionizing Radiation on Human Lung Cancer Cells H1299." by Cechakova L, **Ondrej M**, Pavlik V, Jost P, Cizkova D, Bezrouk A, Pejchal J, Amaravadi RK, Winkler JD, Tichy A. published in International Journal of Molecular Sciences 2019; 23;20(23) and in the appendix publication 3„Lys05 – A promising autophagy inhibitor in the radiosensitization battle: Phosphoproteomic perspective". **Ondrej M**, Cechakova L, Fabrik I, Klimentova J, Tichy A. *Cancer Genomics and Proteomics*. 2020;17(4):369-382.

Following section is dedicated to the optimization of phosphoproteome analysis and is also presented in appendix publication 4 „Radio-sensitizing effects of VE-821 and beyond: Distinct phosphoproteomic and metabolomic changes after ATR inhibition in irradiated MOLT-4 cells". Šalovská B, Janečková H, Fabrik I, Karlíková R, Čecháková L, **Ondrej M**, Link M, Friedecký D, Tichý A. PLoS One. 2018;13:e0199349. and appendix publication 5 „Fractionation of enriched phosphopeptides using pH/acetonitrile-gradient-reversed-phase microcolumn separation in combination with LC–MS/MS analysis." **Ondrej M**, Rehulka P, Rehulkova H, Kupcik R, Tichy A. International Journal of Molecular Sciences. 2020;21(11):3971

The methodological level of the work is high, it is obvious that the author has mastered advanced modern methodologies of cell analysis, mass spectrometry and genomic based techniques. The range of techniques involved in this thesis demonstrates the versatility of candidate and his working place. The data are presented in an incisive way, supported by graphs and schemes. It is obvious that the author has collected enough experimental data implicating subsequent drawing of valid conclusions. In the discussion parts, the author presents his experimental findings and conclusions in the context of known facts and considers the possibilities of influencing the molecular mechanisms of autophagy in the context of potential cancer treatment.

Opponent's questions and comments:

1. The candidate proved outstanding methodological level of the work involving optimization of phosphoproteomic work, demonstrated in details in Publication Nr. 5. However, in publication – Nr. 3, no phosphoproteome prefractionation was employed resulting in identification of probably lower number of phosphopeptides. Could you asses the cost-effectiveness of implementation the prefractionation step?

2. Could you estimate the potential of administration of Ly05 in the clinical practice in the treatment of IR resistant form of non-small cell lung cancer.

3. Due to the structural similarity and mechanism of action of Lys05 with hydroxychloroquine, could you predict the potential of Lys05 in the treatment of SARS-CoV2?

Conclusion:

The dissertation proved the **Mgr. Martin Ondrej** is not only a skilled experimenter, but can also interpret the data well in the context of current knowledge and is therefore able to manage independent scientific work. The issues and goals of the work are clearly formulated, the goals of the work were met and enhanced the knowledge in the field of not only military radiobiology. Publication of the thesis data in impacted journals (first and second quartiles) already showed the quality of this work. In my opinion the dissertation fulfils the criteria to be defended.

In view of the above, I **recommend** accepting the thesis for defence.