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OPONENTSKÝ POSUDEK DISERTAČNÍ PRÁCE

Student: Mgr. Martin Ondrej

Název disertační práce: Radiosensitization of cancer cells by modulation of autophagy: Phosphoproteomic analysis

Posudek zpracoval: prof. Ing. Lenka Hernychová, Ph.D.

The presented dissertation is prepared in English as an annotated summary of five published articles (the sum of IF is high 18,617) in journal quartiles Q1 and Q2. The dissertation deals with a very current topic interested in the occurrence of radioresistance in cancer treatment. The assignment of the dissertation is focused on radiosensitization of the tumor cell line H1299 derived from non-small cell lung cancer, modulation of autophagy and phosphoproteomic analysis. The content of the thesis corresponds to the approved topic. All goals set in the dissertation were met and the original results were published in peer-reviewed scientific journals. The chosen methodological approaches were adequate to solve the goals of the work. These were advanced techniques in the field of proteomics (phosphoproteome, SILAC quantification) which required necessary optimization involving pre-experiments and bioinformatics (bioinformatic tools for proteomic data interpretation - gene ontology, STRING pathway interaction network) that follow the latest methodological trends mentioned in the commented part of the work (Chapter 6 - Optimization of the proteomic workflow).

The added values of the dissertation and thus the student's work were clearly summarized in Chapter 5 (Summary of the key results I), Chapter 7 (Summary of the key results II), and Chapter 8 (Conclusion). I consider the involvement of the Lys05 autophagy inhibitor to confirm the radiosensitization of H1299 cells as one of the main benefits as well as the discussion of the associated mechanism at a late stage after cell treatment, and finally the down-regulation of AMPK-ULK1_ATG13 pathway. The obtained results suggest possible solutions to the problem of radioresistance in the treatment of cancer patients.

This thesis meets all the requirements established in Part Three of the Measure of the Rector of the University of Defense in Brno No. 3/2018 of the Final Thesis. Considering all the above circumstances, I recommend the work for defense of the thesis.

Opponent's questions to the author of the dissertation

1) How would you explain the increase in autophagy after cell treatment and then its decrease (as was described in the page 21)? Are the molecular mechanisms covering this phenomenon known?

2) You tested the molecular mechanisms of action of Lys05 *in vitro*. Are its effects known *in vivo* in animal models and in clinical trials? Will it be suitable for radiosensitization in the treatment of cancer patients?

3) You used a reference protein database (human SwissProt database) to evaluate proteomic data, but it does not contain information about protein mutations that occur in cancer in the patient's proteome. You may have lost a lot of interesting information. Would you know how to solve this issue (for example Next-Generation Sequencing)?

Datum: 3. 9. 2020 Podpis oponenta: ...