PhD Thesis Review

Name of the opponent: Prof. RNDr. Jan Brábek, Ph.D.

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Author. Sara Escudeiro Lopes, MSc

Title: **Characterization of LACTB-induced tumor suppressor pathway**

The main aim of the dissertation of Sara Escudeiro Lopes was to further elucidate the biology of tumor suppressor Serine beta-lactamase-like protein (LACTB).

The Thesis is presented in "standard" (long) form including all (requisite) chapters, i.e. Abstract, Introduction, Aims, Materials and Methods, Results and Discussion, Conclusions and References. The structure and content of the Thesis conforms to the rules and requests for the PhD Thesis. These mainly include the choice of the research topic and formulation of the aims and objectives, and the adequacy of the student's own research contribution. The length of the Thesis is appropriate with respect to the ambitious objectives. It is evident that the author studied the extensive number (378 references!) of bibliography sources that were properly cited.

The thesis is divided into prescribed parts. The Abstract in English and Czech is followed by a well written Literature Review with many comprehensive Figures. The Review begins with chapters on Tumor Suppressor Genes, concentrating on their mode of regulation, haploinsufficient tumor suppressors, and tumor suppressors in mitochondria. The second part of the Literature Review concentrates on tumor suppressor LACTB, including its evolutionary origin, modes of action as a tumor suppressor, modes of regulation and structure.

The main aim of the work - to elucidate the functional role of tumor suppressor LACTB is developed into several sub-objectives: i) uncovering the mechanism of LACTB regulation in cancer cells, ii) Identification of LACTB binding partners and substrates, iii) characterization of LACTB filament formation. Materials and Methods section is comprehensive and contains appropriate description of methods used within this thesis. In the Results chapter, the results related to the different objectives of the thesis are described in great detail in 5 sections with 21 chapters! in total.

Sara and coworkers have shown that LACTB possesses autoproteolytic activity which, in cancer cells, is enhanced by the Mitochondrial Ribosomal Protein S34 protein thus leading to post-translational downregulation of LACTB. This process can be partially reverted by "A4" chemical compound that has the ability to reactivate LACTB. They further elucidate the biology of LACTB through uncovering binding partners (MRPS34, Drebrin, SND1, COQ6 and HIGD1A), and additional substrate candidates of LACTB (SND1 and PC) and uncovered the requirements for salts (particularly calcium chloride) and ions in LACTB's filament formation and enzymatic activity. Furthermore, Sara and coworkers unveiled the mechanistic circuitries of LACTB-induced cell death, revealing that LACTB can induce caspase-independent cell death, mainly through increasing reactive oxygen species in cancer cells. LACTB was also identified as a tumor suppressor in ovarian cancer setting, where its expression leads to inhibition of Slug transcription factor and consequently the inhibition of the process of epithelial-to-mesenchymal transition.

The 21-page Discussion is truly outstanding, evaluating most of the results and relating them to recent work in the field, presenting open questions and future perspectives. The Discussion is followed by a brief overall Summary of Results, a review of the literature used and a list of the author's publications.

The stated goals of the dissertation were clearly met. In her work Sara used a large number of methods of molecular and cell biology. She demonstrated the ability to formulate hypotheses, perform complex experiments, critically evaluate them and put the results into a broader context. She has also demonstrated the ability to prepare manuscripts of scientific papers for publication in international impacted journals. Directly in the thesis, there are included three original articles and one manuscript - in two of them, Sara is the first author. During her PhD, Sara also contributed to another 2 publications in one of which she is also the first author. All these articles are in high quality journals.

I fully recommend this outstanding dissertation thesis for defense, and after successful defense to confer the title "doctor" (PhD) on the candidate Sara Escudeiro Lopes, MSc.

Formally, I have no objections to the thesis except for rare typos, the thesis is written in excellent English, the visual documentation (46 Figures and 7 Tables!) and the overall layout is of a high standard.

I have the following questions related to this thesis:

1) Could the candidate summarize known mechanisms, through which LACTB could inhibit cancer cell invasion and metastasis (developing on what is written in the Introduction)?

2) Could the candidate comment on (rare) published evidence related to tumor and metastasis-promoting function of LACTB in some cancers?

3) Could the candidate suggest how drebrin antagonist BTP2 could be used in analysis of the potential role of drebrin/LACTB in regulation of cancer cell migration/invasiveness?

4) Could the candidate speculate on the potential role of novel LACTB interactions in regulation of cancer cell metabolism?

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