UNIVERSITY OF HELSINKI

PhD Thesis Review

Candidate: Sara Escudeiro Lopes, MSc
Opponent: Ove Eriksson-Rosenberg, PhD, adj. prof.
Date: 24.5.2024

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

This thesis deals with a relatively unknown mitochondrial protein called LACTB. The gene encoding LACTB was described in 2001 and the endogenous LACTB protein was isolated and characterized in 2009. LACTB is conserved from bacteria to human and based on sequence alignments and comparisons, it has serine protease activity. In addition, LACTB can form filamentous homopolymers, the structure of which was recently resolved by cryo-electronmicroscopy. LACTB has been shown to function as a tumor suppressor in several model systems, and low expression level of LACTB correlates with an unfavorable clinical outcome in many tumor forms.

Despite that many properties of LACTB have been elucidated, it has proven extremely challenging to identify its physiological substrate(s) and to define the cell biological function of the polymerization and its regulation. Also, the molecular and biochemical basis for its tumor suppressive effect remains to be clarified. Therefore, a deeper understanding of LACTB's biochemical and cell biological function is urgently needed. The topic of this thesis is hence timely, and the approach taken by the candidate is based on an inclusive up-to-date knowledge of the field.

In the thesis work, a broad front approach has been taken to address open questions on LACTB's function, and the candidate has successfully been able to provide answers to many of the posed questions. To achieve this, the candidate has been using a vast array of various methodologies ranging from protein chemistry to cytogenetic approaches and chemical library screening. Taken together, the results presented in this thesis represent a significant step forward in the understanding of LACTB's function.

I anticipate that several of the findings presented will open up novel avenues of research on LACTB, and in this respect, the following results are particularly noteworthy: (i) identification of LACTB's autoproteolytic activity and the mechanistic separation of its activity on external substrates (ii) the interaction and reciprocal regulation of LACTB with MRPS34, (iii) identification of a small molecular inducer of LACTB expression, and (iv) the regulation of LACTB's polymerization state by calcium.

The methodology chosen is appropriate and the conclusions are usually based on several complementary assays, and the candidate is clearly aware of the limitations in extrapolating the results obtained under simplified experimental conditions into a bigger physiological setting.

The format of the thesis follows a traditional layout, and the candidate demonstrates a high capability of writing in clear and concise scientific English. The thesis contains a number of illustrations that are all of high quality and serve to explain and clarify the concepts discussed. Perhaps some parts of the text could have been condensed somewhat without loss of clarity, as certain things are reiterated many times, but this is more a matter of stylistic preference.

In this work, the candidate has demonstrated excellent skills in formulating working hypotheses, in experimentally testing these hypotheses and in drawing pertinent conclusions of the results, revealing a profound dedication to a high scientific standard.

I have no objections regarding the way in which this study has been performed and presented.

I recommend this outstanding thesis for defence, and after successful defence to confer the doctoral title to the candidate Sara Escudeiro Lopes, MSc.

I have a few questions of mainly general character:

1. LACTB has evolved from a class of proteins involved in the synthesis of peptidoglycan in bacteria. The question hence arises as to how and when during the evolution LACTB was co-opted for a role as a tumor suppressor? Could the candidate elaborate on possible scenarios?

2. LACTB has previously been shown to act by preventing the conversion of phosphatidylserine to phosphatidylethanolamine in certain tumor cells. This is an elegant mechanism that would explain a general cell growth inhibition. Could the candidate discuss this mechanism in the light of her own findings on LACTB?

3. One of the key findings of this study is the interaction between MRPS34 and LACTB. Given that MRPS34 and LACTB are located on different sides of the inner mitochondrial membrane (as shown in figure 24), how does the candidate envisage the mechanism of this interaction at molecular level?

4. A number of studies have been published in which different solid tumors have been analyzed by various omics techniques in order to identify mutations causative for the tumor type in question. Can the candidate comment on whether LACTB has been identified in any such study and if so in which tumor type(s)?

5. If the candidate were to continue the work on LACTB, what would be the next direction to take?

Yours Faithfully,

Ove Eriksson-Rosenberg, PhD, adj. prof.
Faculty of Medicine
University of Helsinki
Finland