FACULTY OF SCIENCE
Charles University

In Prague 6th of January 2025

Opponent Review of the PhD thesis of Soňa Miklovičová.

The thesis was submitted with the title **Mitochondrial respiratory complex II and its function in cancer,** and on 74 pages it contains introductory background parts on mitochondria, complex II, and its connection to disease with special focus on cancer. The text follows with definition of the research aims, list of methods and the summary of the main results of two attached publications that represent the research outputs of the PhD study. The thesis then contains discussion, conclusion part and list of 220 references, (which is a lot).
The two papers attached are: a first author publication *Mitochondrial respiratory complex II is altered in renal carcinoma* in BBA – Molecular Basis of Disease from this year, and co-authored publication *Disordered-to-ordered transitions in assembly factors allow the complex II catalytic subunit to switch binding partners* published in Nature Communications in 2024.
The core of the thesis is the characterization of the activity and assembly of mitochondrial complex II in renal cancer carcinoma (RCC) cells, specifically in its most common type of clear cell RCC (ccRCC). This part of the work converges on the discovery that ccRCC cells have reduced mitochondrial content with reduced complex II activity. The second part demonstrates the stepwise assembly of complex II via the action of two accessory proteins, SDHAF2 and SDHAF4. In general, the presented research is of high quality published in very good journals, and without a doubt represents a significant contribution to our understanding of metabolic changes in cancer cells.

At the end of my review I have a few specific questions related to the two papers. There are 10 questions in total.

Here are my points to the thesis itself:
I find the introduction well written in very good English. It mentions all the necessary aspects of mitochondrial biology that relate to the work. On the other hand, it is doesn't extend much beyond the information presented in the two publication, which would put the obtained data into a larger context of other cellular processes, such as overall metabolic adaptations, the assembly of other complexes found in mitochondria, and metabolic signaling. The text contains some oversimplifications such as the usual introductory sentence on mitochondria: „ …..are highly specialized organelles that act as main *energy producers….*" (page 15). We should keep in my mind that energy is not produced but converted into ATP. A similar sentence is repeated on page 16.

Figures are well chosen but should contain self-explanatory legends. Some of the information can be found in the preceding text but some information is not mentioned and would be much easier to read with the accompanying legends.
Also, I found figure 3 a bit odd, with complex II buried only in half of the membrane.

Here are some specific comments on the text:
On page 22, I find the following sentence interesting: "…..This highlights the role of Drp1 as a chaperone for SDHAF2, facilitating its proper localization in mitochondria for CII assembly…". it brings a question:
Q1: How can cytosolic Drp1 come into contact with matrix-localized SDHAF2?

On page 23, frataxin is labelled as iron donor, however, it was shown some time ago that its function relates rather to sulfur metabolism, specifically to the transfer of sulfur from Nsf1 (eg. Parent et al, 2015).
The chapter on complex II assembly (page 23) starts with the description of the electron transfer through the chain of co-enzymes. This part should be placed where the function of complex II is discussed.
On page 25, in the text on reverse electron transfer, there is a reference to the anaerobic mitochondrion of a ciliate and its highly adapted mitochondrial metabolism. By reading the text one could think that hydrogen might be produced also in human mitochondria, which is not correct.

Q2: What is the difference between SDH and complex II deficiency, as both terms are used?

The summary of the results is short and clear and summarizes well the core of the papers.
The following discussion combines the obtained results with already known (and sometimes conflicting) data. It demonstrates that the author is well oriented in the field but I find some parts repetitive and not fully polished. This is unfortunate, as this part of the thesis could be the most interesting and fun to read.

For example, the text contains both terms *tumor* and *tumour*.

Page 47 "……In contrast, genes encoding the key glycolytic enzymes, such as SLC2A1 (which encodes GLUT1), HK-2, and PFKP, were significantly lower in pRCC compared to ccRCC, suggesting reduced glycolytic capacity in this RCC subtype. Furthermore, pRCC tumours did not exhibit increased GLUT1 expression, and glycolytic enzymes such as HK-2 and PFKP showed low expression….."

Page 49 "……Transcriptional regulation of ETC proteins involves both nuclear- and mitochondrial-encoded genes, which are coordinately controlled by several key nuclear factors (e.g., NRF-1, NRF-2, and ERRα) and co-activators from the PGC-1 family (e.g., PGC-1α and PRC). When PGC-1 activity is reduced, it acts as a coactivator or direct activator of several transcription factors, including NRF-1 and NRF-2, which are key regulators of mitochondrial function …."

I find some sentences confusing e.g. "…..This decrease in mitochondrial content was accompanied by a reduction in the expression of nuclear-encoded OXPHOS genes, such as those coding for CII subunits. These findings align with previous studies reporting consistent **differential** expression patterns between mitochondria and nuclear-encoded OXPHOS subunits….."

Q3: You mention that ….CII stands out among mitochondrial complexes for its relatively simple composition. Are any of the subunits evolutionary related to any other mitochondrial proteins/complexes?

General questions
Q4: Can you please comment on the function of complex II in sulfide signalling?
Q5: Is there a functional reason that SDH is the membrane embedded enzyme of TCA cycle? Why not, for instance, malate dehydrogenase?
Questions to paper I
Q6: How did you determine mitochondrial protein content?
Q: What do you mean by TFAM-mediated regulation of mtDNA and how this can contribute to decrease in mtDNA content in ccRCC tumours?
Q8: Actin levels in Fig. 5C show uneven actin expression, what does it mean? Could this affect your quantifications?
Questions to paper II:
Q9: Does SDHA alone (or as SDHA-AF2 or in complex with SDHB) show SDH activity? Could this potentially be a mechanism of disconnecting TCA from ETC?
Q10: Is SDHA-AF2 complex soluble or membrane associated?

To sum up, the thesis submitted by Soňa Miklovičová presents high-quality research and despite some critical comments, I think it deserves to be defended for the degree of PhD.

Yours sincerely, Pavel Doležal