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Evaluation of the dissertation of Christos Chinopoulos entitled MITOCHONDRIAL SUBSTRATE-LEVEL PHOSPHORYLATION: FROM SALVAGING HYPOXIC CELLS TO A PROMISING CANCER TARGET

The work of Dr. Chinopulos assesses different aspects of mitochondrial substrate level phosphorylation and wittily explains and assesses the interconnections of mitochondrial membrane potential, transmembrane nucleotide transport and the events of oxidative phosphorylation. I must say here that I enjoyed the topic and was left with lessons learned on mitochondrial function and experimental setups.

The doctoral dissertation has a bottom-to-top buildup, beginning with the simplest mitochondrial studies and explaining the basic considerations, closing with studies on immunometabolism, knockout mouse models of the enzymes of succinate metabolism and human brain specimens. The length of the doctoral work is 292 pages long of which 216 pages make up the scientific content that is definitely very long. The length and depth of the doctoral work becomes evident if one looks at the author. According to the MTMT database, Dr. Chinopoulos has 84 papers of which he is a first author of 29 and the senior author of 30. This body of work represent a plethora of different state-of-the-art methodologies and its contents largely exceed the usual amount that largely exceeds the expectations. I must emphasize the well-controlled, meticulous experimentation and the plethora of the model systems and critical evaluation of the results.

I was tasked with a critical evaluation of the dissertation meaning that, although, I highly value the scientific content, I need give a critical evaluation.

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Given the breadth of the contents, it would have been better to structure the text more and be less verbose. On multiple occasions the content that fits better as introduction or discussion was presented among the results making it difficult to comprehend the description of the results.

The discussion is scientifically correct and the applicability of the findings is unquestionable. Nevertheless, in my opinion the links to neoplasia, more precisely glioblastoma multiforme, were not evident from the introduction and the discussion of the results. The strict sense discussion of the results can be found at the same spot as the description of the results.

Furthermore, there are many abbreviations that are not resolved, ambiguous or were not listed on the list of abbreviations. For example, what is the difference between mSLP and SLP or is it the same (i.e. substrate level phosphorylation)?

Along the same lines, I have not identified a list of aims, neither a list of new scientific results. That latter is truly missing, I would like to ask Dr. Chinopoulos to introduce a slide with that content at the viva voce.

I would like to ask the following questions regarding the scientific content from Dr. Chinopoulos.

1. What was the rationale of selecting the metabolites for energizing mitochondria, as these were different between experimental setups and when mixtures were used not all individual components were assessed individually. As examples please see the upcoming two questions below.

2. On Figure 7.3 the mitochondria from the double heterozygous mice treated with malate+beta-hydroxybutyrate and glutamate+malate+beta-hydroxybutyrate is not displayed. What is the reason for that? In line with that, the representation of the lipidomics results often lacks the display of data from the double heterozygous mice.

3. In chapter 7.4 mutliple anaplerotic substrates are supplied to mitochondria. What is the reason for not assessing all substrates alone (e.g. glutamate was not assessed alone)? Given the potentiating effects, to me it remains an open question whether glutamate alone would be able to modulate mitochondrial oxidation.

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4. p.66. “Assuming 1 microliter of matrix volume for every mg mitochondrial protein” what builds a case for this estimation?

5. Fig.6.2E There seems to be a MW difference of SUCLA2 between brain synapses and the three other preps. Is it possible that there is a difference in the posttranslational modifications of SUCLA2 among these source of mitochondria?

6. p89. The hypothesis that other compartments may transfer phosphorylation potential is interesting. I have two questions on that issue. First, what does phosphorylation potential as a term refer to? Second, what compartments may be involved? Is the endoplasmic reticulum a potential compartment?

7. Chapter 8.2. is entitled “Identifying mitochondria as extramitochondrial ATP consumers during anoxia”; this title and the subsequent discussion seems to be contradiction in terms to me. How can mitochondria be extramitochondrial?

8. In terms of itaconate metabolism, figure 11.4 implicate that itaconate may reverse the succinyl-CoA → succinate reaction through succinate-CoA ligase. Is this possible or is it a wrong though based on the figure?

9. Apparently, SUCLA2 protein expression is specific for neurons, however, SUCLA2 mRNA expression appears in S100 positive glial cells. What is the reason for that? Can there be an issue of the SUCLA2 protein detection limit (i.e. SUCLA2 levels are below the detection limit in glial cells)? If so, it would be better to discuss a large difference in protein expression between glial cells and neurons.

10. With regard to the expression of OGDHL in neuros, it appears to me that the protein preferentially localizes to mitochondria close to the nucleus and the expression decreases in mitochondria distant to the nucleus – at least based on the representative images in COS7 cells (Figure 16.4). Did the authors observe that, can this observation be generalized?

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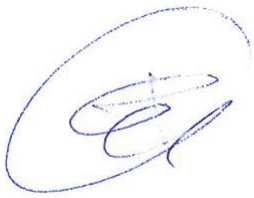
As I have already stated previously, the doctoral work fulfills and largely exceeds the criteria set, therefore, **I suggest for the committee accepting the present doctoral work**. My questions aim to facilitate the further discussion of the results.

Debrecen, 8th February 2023.



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