Review of the PhD thesis by Katarina Smolkova

This is a strong PhD thesis composed of an extensive introduction, succinct description of used methods and obtained results, a clear cut discussion and an impressive number (9) of attached papers. The sum impact factor of them is about 36, which ranks it at the top of PhD theses I had the opportunity to review in the Czech Republic. Thematically the papers fall in the thesis, with the sole and thus unimportant exception of the paper in Anesthesiology. Since all attached papers have either been published or are under review in respected journals, below I will only deal with the non-published parts of the submitted thesis.

The text deals with a complex subject yet it reads very well and is written in excellent English. I have several questions for the defense, and only minor additions or corrections, less important issues regarding the language are listed below.

Major questions:

In respect to complexities of targeting of CIDE proteins (f.e. page 16), do you know what is eclipsed localization? Can’t some of the described (dual?) localization fall in this category?

Since you did not find much support for the famous Warburg effect I wonder what is your opinion about it as a whole? Is it just a rare modification of cancer cell metabolism or is it still considered to be a general phenomenon, yet perhaps masked by other changes? Do you still believe that mutations in SDH or other mt proteins are the cause or consequence of cancer?

In your opinion, the mutation in subunits of ATPase (page 19) are primary or secondary defects? What about the decreased levels of mtDNA? The referenced papers are several years old. Are there newer reports confirming and perhaps elaborating on these proposals?

You refer to the Lum et al. 2007 paper in respect to elevated mt membrane potential? Is this indeed the most appropriate reference (I could not check it
myself). How frequent is this alteration for cancer cells? How would you measure different membrane potentials?

There is a very important statement in the beginning of the 2.4.2. paragraph on UCPs. Could you sum up why is the field so super-controversial?

Based on just two papers you say that specific antibodies against individual UCPs can’t be produced. Is there more evidence for such a strong call? Availability of specific antibodies would help to address number of controversial issues in the field.

Could you provide more experimental details on how you followed mt network morphology, as mentioned on page 76?

You said “Few studies have performed in-depth analysis of mt bioenergetics in cancer cells”. What are in your opinion the main reasons behind this surprising lack of so potentially important data?

I am intrigued by your claim that CIDEa is in mitochondrion in hiding position. Could you corroborate on the redistribution issue? Is there any follow-up of the Valouskova et al., (2008) study?

Minor questions:
1. Could you describe (the methodology and results of) the repopulation of “empty” petite mutant mitochondria with mtDNA nucleoids?
2. Where does the number 1500 proteins (page 10) comes from? As this is an important and highly controversial number, a reference would be in place.
3. Could you describe common features of export of signaling proteins, as mentioned on page 13?
4. How difficult is the ATP measurement (3.1.6.)? How many cells do you need? How reproducible are the measurements?

Minor issues or typos:
1. You use “hence” too often, see f.e. pages 5-7
2. Plural incorrectly used in the last sentence of 1st paragraph, page 6
3. …..survive otherwise… is correct (page 6)
4. The paper by Mannella 2006 is not recent anymore
5. Improve English in “Next to the mitochondrial....” (page 13)
6. “Biological” function is correct (page 15)
7. “...with UCP1 in mitochondria” is correct (page 16)
8. I’d use “the” for Crabtree effect (page 20 and elsewhere)
9. fumarate hydratase is correct (page 26)
10. ...some cancer cells... is correct (page 28)
11. ...it has been reported... is correct (page 29)
12. ...but were able to do so upon addition... is correct (page 32)
13. ...confirmed by another study....is correct (page 34)
14. ...has increased glutamine metabolism....is correct (page 35)
15. ...Isolation of mitochondria ...is correct (page 64)
16. Note that glutamine content.....is correct (page 67); We hypothesized that
    ....is correct (page 88)
17. ...inhibition by oligomycin...is correct (page 70)
18. ...fragmentation... is correct (page 86)
19. ...proteins content did not change ....is correct (page 91)

When stating that mtDNA is 16.5 kb long, it should be also mentioned that this
applies to humans, while in other organisms, this organellar DNA is much
bigger and more complex in numerous ways.

In respect to references:
1. sometimes abbreviated name of the journal is used (e.g. Essop 2007),
   elsewhere full name (e.g. DeBerardinis et al., 2007)
2. Big letters after the comma in Diaz-Ruiz et al., 2008 clearly show the title
   was copy pasted from JBC, which has this weird twist. Should be
   avoided.
3. Wrong symbols appeared in Spanish names in the Finocchietto et al.
   (2008) paper
4. All papers with 1st authors commencing with “Ch” shall be transferred to
   “C”
5. Sometimes all words in the titles start with big letters, sometimes only the
   1st one. (e.g. Koong et al. 1994 vs Koong et al., 2000)

I would like to state that the presented dissertation thesis fulfils according
to my opinion all postulations and I recommend it to be accepted as a partial
fulfillment of the requirements for the degree of Doctor of Philosophy at the 1st
Faculty of Medicine of the Charles University in Prague. In fact it fulfils the
requirements, apart from teaching, for receiving a docent degree.
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