Referee's comments for PhD thesis "Molecular mechanisms of tamoxifen resistance in breast cancer"

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Presented thesis explores mechanisms underlaying tamoxifen resistance in breast cancer cells. Tamoxifen is widely used adjuvant therapy in patients with ER+ breast cancer. While patients usually respond well to initial tamoxifen treatment, resistance development over time is frequent and well documented and eventually leads to relapse. Numerous attempts have been made to decipher mechanisms underlaying resistance development and this work tries to contribute a new piece into that jigsaw puzzle. Thesis is based on two published papers (one first author one) and number of yet unpublished data, which will likely transform int additional paper(s) in the future. In this regard, the thesis follows "traditional" long form, but with a nice twist. After concise general introduction and description of used methodology, each of the four aims has its own section covering literature overview, results and discussion. I appreciated this format a lot, since it allowed to keep focus on each of the diverse topics. Formal aspects are well under control, with well prepared figures and very good standard of English scientific writing. Undoubtedly, there would be some imperfections, but I managed to spot only one: no reference is given in the supplementary table 1 to the CD44 antibody used in Figure 4.

From the scientific standpoint, it defines four major aims: (1) establish model of MCF7 and T47D tamoxifen resistant cell models, (2) analyse mitochondrial function in these cells, (3) assess iron metabolism perturbations, and (4) study expression of ABC transporters. Second aim follows the first author paper and unsurprisingly is the most polished one. Authors clearly demonstrate dissociation of respiratory chain supercomplexes and fragmentation of mitochondrial reticulum in Tam resistant cell lines. Associated decrease in mitochondrial respiration is connected metabolic rewiring towards glycolysis and (a bit surprisingly to me) increased levels of oxidative stress. In the third part, selective perturbation of individual arms governing cellular iron homeostasis in Tam resistant cell lines is well documented. To me, most interesting observations were, that Tam resistant cells turn to non-transferrin bound iron uptake pathways and also that mitochondrial Fe uptake seems to be upregulated, possibly to compensate for defective RC supercomplex assembly. Ultimately, the last part explores levels of ABC proteins both at transcript and protein levels in Tam resistant cells. This part represents thorough analysis of all human ABC transporters with the aim to define changes driving Tam resistance. There is surprising disconnection between transcript and protein levels for individual transporters and also considerable variability between the two Tam resistant models. Nevertheless, three of them: ABCC5, ABCG1 (upregulated), and ABCF2 (downregulated) show uniform response to Tam and deserve further research.

Overall, results are clearly presented and very well discussed, but naturally some questions/comments spring into mind. I would like to hear some discussion about the following points during the defence:

- Analysis of respiratory chain complexes/supercomplexes by native electrophoretic techniques depends to a high extent on the detergent concentration used. For your experiments, you used digitonin in the concentration 10 g/g protein – did you perform rigorous detergent titration in your cells before deciding on the particular concentration for experiments?
- Respiratory complex I (particularly in T47D Tam cells) seems to be completely gone, rather than just dissociated from respirasome, which contrasts with complexes III and IV. Did you

look into this any further? Do you have any potential explanation for selective CI degradation in Tam resistant cells?

- You mention, that there was difference in mitochondrial membrane potential (MMP) between Ctrl and Tam cell lines (p. 54). This has to be considered, when measuring ROS production with MitoSox, which has MMP dependent accumulation in mitochondria. Did you perform any background checks to support your data regarding superoxide production?
- Respiratory chain supercomplexes (SCs)should allow better channelling of electrons from NADH, but this is not the case for $FADH_2$. To the contrary, CII dependent substrates may benefit from SCs dissociation. Did you study respiration with other substrates than glucose? E.g. fatty acids?
- Last nit-picking note: on p. 37, you mention, that CII is closely associated with the inner mitochondrial membrane, which is obviously not true. Subunits SDHC and SDHD protrude the membrane and CII is in this regard not different from other respiratory chain complexes.

Presented thesis fulfils all requirements and I fully recommend it for defence.

Signature:

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