BEC BIOENERGETICS COMMUNICATIONS
Bioenerg Commun 2022.11
https://doi.org/10.26124/bec:2022-0011.r1
Open peer review
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Bioenergetics in human tongue pre-cancerous dysplastic oral keratinocytes and squamous cancer cells

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Manuscript submitted 2022-08-05, 2022-10-06 (revision)

Manuscript accepted 2022-10-07

https://doi.org/10.26124/bec:2022-0011

Reviewer 1

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Manuscript reviewed 2022-08-31

https://doi.org/10.26124/bec:2022-0011.r1

*Only major points from review and responses included.

Reviewer 1

I have some questions on ROUTINE experiment in Fig. 2 for DOK cells. Comparing respiration on succinate + digitonine +CCCP (around 160-230) with pyruvate + oligo +CCCP (80.7 or around), I would say that Cplx II (SDH) or the transport of succinate is the rate limiting step. But in this conditions, with cytochrome c (reduced and in large amount I guess) the rate should be greater than with succinate and in principle could reach the value with pyruvate+CCCP.

Authors

Our main objective here was a comparison of the bioenergetic profile of DOK with SCC4 cells. Your point seems to focus on whether succinate is "rate-limiting" within DOK cells. The values you quote from Figure 2, I don't quite see. In Figure 2 I see that in intact DOK cells, given pyruvate + oligomycin + CCCP, the rate is about 160-230. In permeabilized cells the succinate + digitonin + CCCP rate is 75-120. So if I have it correct, your argument would be that that latter rate with succinate + digitonin + CCCP (+cytochrome c) should be the same or greater than the former rate pyruvate + oligomycin + CCCP. One explanation for the lower rate in the latter, is that the conditions are very different. The former has no digitonin whereas the latter has digitonin. One could argue that with digitonin there is great substrate access to mitochondria so again as you argue, one might expect a greater rate with the latter conditions. However, digitonin could

have a myriad of effects in these cells such as effects on transporter activity or enzyme complex activity. So rather than focusing on why rates are different with and without digitonin, our focus was on comparing DOK with SCC4 cells under the same conditions.

Reviewer 1

Furthermore in my experience on muscle and heart permeabilized tissues, the rate with ascorbate +TMPD is between 3 to 5 times the rate of O2 consumption with pyruvate + malate or with succinate as substrates.

Authors

I think that greater oxygen consumption rates with ascorbate + TMPD are a common observation, due to the auto-oxidation of ascorbate. But we didn't use ascorbate + TMPD in our experiments.

Reviewer 1

In addition, always in my experience on muscle and heart permeabilized tissues (which could be different of cell culture), the SDH activity is also a good measure of amounts of mitochondria and varies like citrate synthase activity , so if it were the same in your cells, there should be an increase in SCC4 SDH and therefore an increase in the respiration of SCC4 cells on succinate as compared to the respiration on succinate of DOK cells.

Do you have an explanation for that?

Authors

From my experience and that in the literature, there are definitely a greater range of metabolic parameters that vary in cancer cells compared to primary cells. One of which is SDH. There are examples of cancers linked with mutations in SDH (and FDH) but not citrate synthase, hence for cancer cells, I give greater weight to citrate synthase. Figure 3A would suggest that there is greater capacity for succinate oxidation in DOK cells compared to SCC4 cells, which we mention. In short, I have good evidence linking difference in complex one activity with differential oxygen consumption rates of SCC4 and DOK cells, but the SDH data is less definitive and hence I don't/can't draw the same solid conclusion for SDH.

Reviewer 1

Another question concerns the volume of the cells. Are their volumes equivalent? It could have an influence in the comparison of O2 consumption expressed per cell. I note that citrate synthase activity, OCR and ECAR are expressed per mg of proteins.

Authors

Irrespective of whether we express oxygen consumption per cell number (Figure 1A) or protein concentration (Figure 2B + glucose +CCCP), there is equivalent greater oxygen consumption in intact DOK cells compared to SCC4 cells, so I would argue that expressing per cell number of mg protein are equivalent for the cells.