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Bioenergetics in human tongue pre-cancerous dysplastic oral keratinocytes and squamous cancer cells

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Reviewer 2

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*Only major points from review and responses included.

Reviewer 2

The value of the article lies primarily in the presentation of methodologies that allow measuring the oxygen consumption of cell cultures.

Now some recommendations:

A section could be added to the introduction that briefly explains why it is necessary to measure mitochondrial respiration in cancer cells.

Authors

We have these sentences already in the introduction which I thought explain why it is necessary to measure mitochondrial respiration in cancer cells. We had mistakenly left out the "IL-6" in the second sentence which is now included : *We expected to see a less oxidative metabolic profile in the SCC-4 cancer cells, compared to the dysplastic cells (DOK) as would be predicted by the Warburg effect (Warburg 1956). In addition, we anticipated seeing a further decrease in SCC-4 cancer cell oxidative metabolism again commensurate with IL-6 driving a pro-cancer phenotype (Tang et al 2008; Chuang et al 2014; Karavyraki, Porter, 2022).*

Reviewer 2

The concentration of glucose is not clear in the cell culture medium. The metabolic phenotype of a cell is highly dependent on the growing environment :substrates, oxygen

concentration etc. (Gstraunthaler, G.; Seppi, T.; Pfaller, W. Impact of culture conditions, culture media volumes, and glucose content on metabolic properties of renal epithelial cell cultures. Are renal cells in tissue culture hypoxic? Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 1999, 9, 150-172, doi:10.1159/000016312: Sherr, C.J.; DePinho, R.A. Cellular senescence: mitotic clock or culture shock? Cell 2000, 102, 407-410, doi:10.1016/s0092-8674(00)00046-5; Koit, A.; Shevchuk, I.; Ounpuu, L.; Klepinin, A.; Chekulayev, V.; Timohhina, N.; Tepp, K.; Puurand, M.; Truu, L.; Heck, K., et al. Mitochondrial Respiration in Human Colorectal and Breast Cancer Clinical Material Is Regulated Differently. Oxid Med Cell Longev 2017, 2017, 1372640, doi:10.1155/2017/1372640.)

Authors

We now specify the glucose concentration of 4.5 g/L in the medium and we had already conveyed in the M&M section that the cells were grown at 37 ^{o}C in a humidified environment containing 95 % O_2 and 5 % CO_2.

Reviewer 2

Figure 2: Why CCCP added twice? Is the CCCP optimal concentration titrated earlier?

Authors

CCCP was added twice purely to confirm that mitochondria were uncoupled in the absence and presence of digitonin.

Reviewer 2

Figure 3 What could be the reason for the extensive changes in the intermediates of the TCA cycle?

Authors

Figure 3 doesn't depict intermediate/metabolite levels, but rather metabolic flux after addition of these metabolites. The data demonstrates that the there is greater capacity for Krebs Cycle enzyme activity in DOK cells compared to SCC4 cells. This observation would be consistent with greater complex I activity measured independently.

Reviewer 2

The work is rather descriptive, probably the readers would expect a little more discussion.

Authors

We have taken on board what you say and have modified the last two sentences of the manuscript: *Overall, our prediction that there would be a less oxidative metabolic profile in the SCC-4 cancer cells, compared to the dysplastic cells (DOK), is clearly evident. Furthermore, it appears that IL-6 is driving a less oxidative metabolic phenotype in these already cancerous SCC-4 cells, again as we would have predicted from our knowledge that IL-6 drives anoikis resistance (Karavyraki, Porter 2022).*