Title: **MITOCHONDRIA AS A TARGET TO TRACE DEVELOPMENTAL STAGE SPECIFICRESPONSE OF THE HUMAN CEREBRAL ORGANOIDS TO DIFFERENT OXYGEN CONDITIONS**

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Abstract:

Mitochondria play a pivotal role in a variety of processes including calcium signaling and cell apoptosis. The role of mitochondria dynamics in neural cell fate is also well documented. Our previous experiments on different neuronal differentiation models showed that normoxic oxygen conditions (5% O_2) impact neural to glial cell fate by increasing expression of the astrocytic marker, GFAP and lowering expression of the neuronal marker, βtubIII. In this work, we used a brain organoid model from human induced pluripotent stem cells (hiPSCs) at three different stages of differentiation – 11-day neurospheres (11D-N), 44-days (44D-CO) and 4-month brain organoids (4M-CO) grown in two different oxygen conditions –5 and 21% O_2 to decipher an impact of different oxygen conditions to mitochondrial morphology and dynamics at at different stages of CO development. Our mitochondria analysis framework revealed changes in mitochondrial networks parameters throughout cerebral organoid development. Further analyses revealed that normoxic oxygen conditions (5% O_2) affected expression of neural stem cells markers (Sox2, Nestin) in 11D-N. Finally, different oxygen condition influenced neuronal (βtubIII) and glial markers (GFAP) in 44D-CO and 4M-CO. These results suggest that oxygen conditions influence neural fate by inducing changes in mitochondrial dynamics. Overall, our framework offers a powerful tool to elucidate mitochondrial fate/morphology at different stages of neuronal differentiation upon different oxygen condition in the cerebral organoid model.

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