Modeling Physioxic Niche in Brain Cortical Organoids to Study Mitochondria-Dependent Neuroprotection in Developing Human Brain

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Self-organizing capacity of pluripotent stem cells to buildup 3D tissue architecture displaying unique layers of neural and glial cells at different developmental stages emerge brain organoids as the only existing *in vitro* model of prenatal brain development. The low oxygen condition (5% O2), often referred to as "physioxia," is typical for endogenous niches of neural stem cells, moreover, Physioxia is critical for faithfully recapitulating the natural microenvironment of brain development. However, the majority of in vitro studies utilizing brain organoids are performed under atmospheric oxygen levels of 21%.

In human dorsal forebrain organoids (hDFO), physioxic conditions suppress neuronal apoptosis and increase SOX2+ progenitors. They also moderately decrease markers of Tbr2+ intermediate progenitors and Hopx+ outer radial glia, as well as deep-layer cortical neurons (Tbr1+, Ctip2). Unexpectedly, this coincided with upregulation of Syp+ synaptic puncta in the cortical layer of DFO, suggesting a shift in developmental timing. Additionally, physioxic conditions reduce mitochondrial number and network complexity as well as decrease mitophagy (MitoKeima, Parkin), which is accompanied by suppression of MT-OXPHOS (MT-CO2) in the cortical layer of hDFO.

In hDFOs, short-term hypoxia (1% O_2) induced neuronal loss (CTIP2, TBR2, HOPX), mitochondrial network damage, and activation of the apoptosis and unfolded protein response (UPR) programs. Preconditioning hDFOs at 5% O_2 partially restored hypoxia-induced neuronal loss and mitochondrial alterations and attenuated hypoxia-induced reductions in mitochondrial network complexity. Transcriptomic analysis of 44-day hCOs showed metabolic reprogramming toward glycolysis and activation of neuroprotective and proteostatic programs. Alternative splicing analysis further revealed changes in mitochondrial gene splicing, enabling identification of potential therapeutic targets.

These insights reveal importance of optimizing oxygen levels *in vitro* to improve the fidelity of brain organoid models for research purposes and new therapeutic strategies.

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