Organoids as a tool for personalized medicine

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In our laboratory, we developed an in vitro system composed by human brain organoids (minibrains) and Blood Brain Barrier (BBB) by co-culturing brain capillary endothelial cells and astrocytes. We demonstrated that BBB is essential to optimize minibrains' differentiation due to the presence of BDNF, which is mainly secreted by the endothelial component. Recently, BDNF has been proposed as a potential diagnostic biomarker and therapeutic molecule for the Alzheimer Disease (AD). Low concentrations of BDNF were measured in serum AD-patients, and its levels decreased 3-4 fold in brain from AD-patients at autopsy. Interestingly, a reduction of BDNF levels was detected in mice with apolipoprotein (APO)E4 variant. The presence of APOE4 isoform strongly increases the risk of sporadic AD. Minibrains expressing APOE4 show a robust increase of Amyloid-β 42 (Aβ42) and phosphorylated tau (p-tau). On these bases, we propose to use the novel BBB-minibrains model to test the potential beneficial effects of BBB on AD-minibrains (BBB-AD-minibrains). The AD-minibrains will be generated from human induced Pluripotent Stem cells (iPS) expressing APOE4 edited through CRISPR/Cas9 technology. The following experimental points will be analysed:

1) BBB-minibrains, using minibrains generated from iPS from healthy donor;
2) AD-minibrains and BBB-AD-minibrains.

The study will be performed as follows:

i) evaluation of the cortical organization of minibrains by immunofluorescence using the cortical markers (i.e. CTIP2 and TRB2);
ii) quantification of BDNF and other neurotrophic factors (i.e NGF, GDNF) by ELISA;
iii) amount of Aβ42, p-tau and Amyloid Precursor Protein (APP) by western blot and immunofluorescence;
iv) quantification of β-site APP cleaving enzyme 1 (BACE1) activity by ELISA. This pilot study will enlighten the importance of the cross-talk between BBB and brain and unveil the role of BDNF.