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Generation of Function Pluripotent Cell-Derived Brain Endothelial Cells for in vitro Modelling of the Neurovascular Unit and Blood Brain Barrier

Highly specialized endothelial cells (ECs) in the brain endothelial cells (BECs) interact with various other cell types such as astrocytes and pericytes to form the basis of the blood brain barrier (BBB). BECs exhibit unique properties, including tight junctions, selective permeability, and specific transport systems, which distinguish them from endothelial cells in other tissues. These cells play a vital role in maintaining homeostatic brain function as well as regulating interactions between the immune and nervous systems. The development of an in vitro model of the human neurovascular unit (NVU) hinges on the use of ECs which can faithfully recapitulate multiple key organotypic functions. Human pluripotent stem cell (hPSC)-derived BMECs (iBMEC) have been widely used for this purpose; however, transcriptomic and functional characterization of their cellular identity has revealed that these cells are epithelial barrier-forming cells (Epi-iBFC) rather than BMECs. Here, we describe the development of a transcription factor-mediated strategy to generate ECs from hPSCs and their use for generation of 3D NVU models. We report that constitutive overexpression of two EC transcription factors, SOX7 and ERG, converts Epi-iBFC into adult vascular ECs (SE-rEC) that express an EC gene repertoire and respond to inflammatory cues. Moreover, co-culture of SE-rECs with astrocytes and pericytes in 2D and 3D induces a BBB-specific transcriptional profile in SE-rECs. Functionally, co-culture of SE-rECs with primary brain pericytes and astrocytes in a 3D microfluidics system reduces significantly EC permeability to biocytin and 70 KDa dextran compared to ECs cultured alone, primarily due to induction of the tight junction protein Claudin-5 and acquisition of BBB-inducing gene expression. We aim to use these reprogrammed SE-rECs to develop more faithful human BBB system in vitro to understand disease mechanisms and develop methods for drug delivery to the brain.