Plano de trabalhos para tese de Mestrado 2018/2019

Exploring stem cell-derived 3D models to unravel the role of microenvironment remodelling in neurological disorders

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Duração: 1 ano lectivo

Sumário:

Brain microenvironment plays an important role in neurological development, function and degeneration. Extracellular matrix (ECM) components modulate neural cell survival, migration, proliferation and neuronal function. Few studies have demonstrated a mechanistic correlation between changes in ECM and neurodegeneration. The major challenge in studying these mechanisms is the lack of human cell models in which the molecular crosstalk between different cell types are represented so that and consequent microenvironment remodelling during disease progression can be recapitulated in vitro. At Animal Cell Technology Unit, one of our research lines is focused on assessing the role of tissue microenvironment in disease progression and biopharmaceutical response, developing and employing advanced cell-based disease models. We have implemented a methodology based on perfusion bioreactors for three-dimensional (3D) neural differentiation of neural stem cells derived from induced pluripotent stem cells (hiPSC-NSC). This generates tissue-like 3D structures containing neuronal, astroglial and oligodendroglial cells than can be maintained in culture for long periods of time. Importantly, we've demonstrated in vitro recapitulation of neural cellular and extracellular developmental features along this culture time. We are now working with iPSC derived from neurodegenerative disease patients (Alzheimer's and Parkinson's disease) and lysosomal storage disorders patients (Mucopolysaccharidosis VII). These disease models are currently being developed and validated in terms of recapitulation of cellular and molecular hallmarks of the diseases.

The specific objective of this research project is to characterize the dynamic remodelling of extracellular protein signatures in human stem cell-derived neural disease models during long-term cultures, and compare them between each other and with models derived from healthy donors. Ultimately this will contribute to identify molecular players involved in early stage events of disease progression.

Project Tasks:

Task 1. Cell culture and characterization methods training

During this task the student will learn the fundamentals of animal cell culture and 3D cell culture, as well as the required characterization methods for this project (immunofluorescence, Western Blot, qPCR, etc.). Environmentally controlled stirred-tank bioreactors and perfusion strategies, which enable a tight control and monitoring of key culture parameters (pO2, pH, temperature and media composition) are applied to achieve robust and reproducible neural differentiation. Neural Stem Cells originated from patient-derived

iPSC will be applied. Differentiation and cell-type composition and functionality, namely neuronal synaptic activity, Ca2+ signalling and neurotransmitter synthesis/release, will be evaluated.

Task 2. Characterization of the ECM of disease 3D neural models

Extracellular matrix (ECM) synthesis, secretion and composition will be studied in both neural disease models and healthy donor derived models, currently ongoing in the lab. This will include untargeted proteomics approaches and targeted detection of specific components ((western blot, confocal microscopy) as well as. The aim will be to characterize the neural microenvironment and identify potential targets deregulation during neurodegeneration.

Task 3. Master thesis preparation

In this final task the student will be focused on writing and preparing his master thesis.

Main methodologies: Neural differentiation of human stem cells (hiPSC and hNSC), 3D cell culture, bioreactor culture, luminescence-based assays, immunodetection (microscopy, Western blot, ELISA), confocal microscopy, proteomics analysis.