Culturing iPSC and Characterization of Human Brain Organoids by Immunohistochemistry

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Abstract

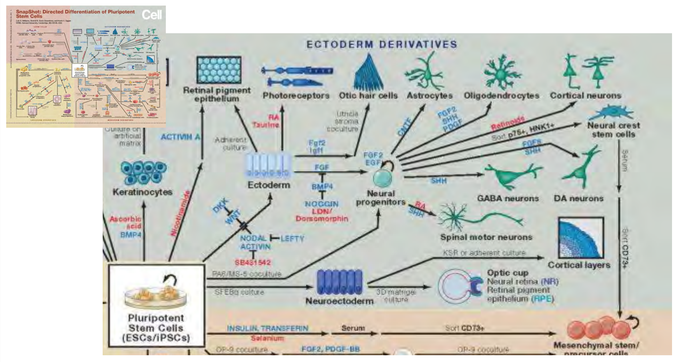
Induced pluripotent stem cells (iPSCs), are skin or blood cells that have been reprogrammed back into an embryonic-like pluripotent state. These cells can then be differentiate into most types of cells in the human body.

A brain organoid is a three dimensional tissue derived from iPSCs that is able to simulate the architecture and functionality of a human brain. Bran organoids could serve as a model to study brain development, understand cell-cell interactions inside of the brain, and explore the potential neurodevelopmental origins of neurodegenerative disorders.

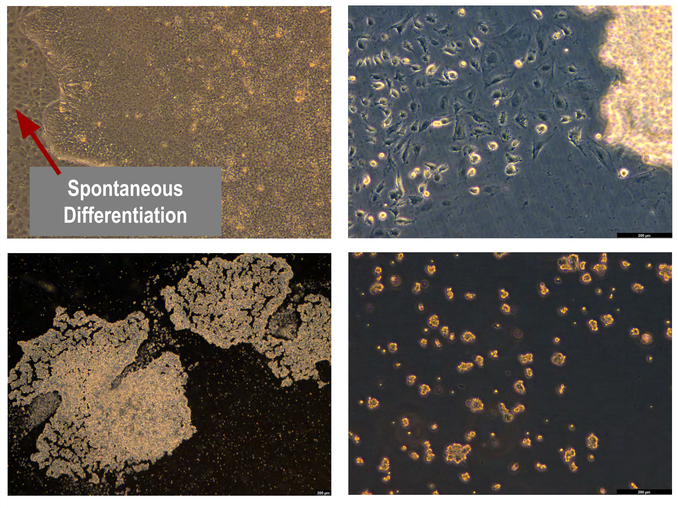
In this project I hoped to learn how to:

1. Culture iSPCs
2. Generate brain organoids
3. Section fixed tissue
4. Perform immunofluorescence staining
5. Capture and analyze images with microscopy

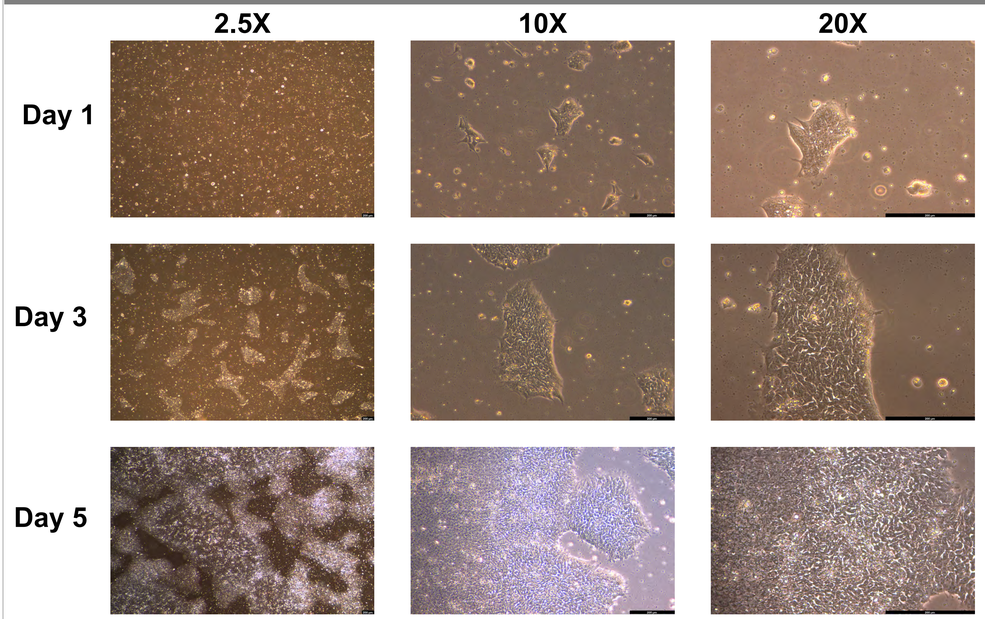
Protocols to Differentiate iPSC



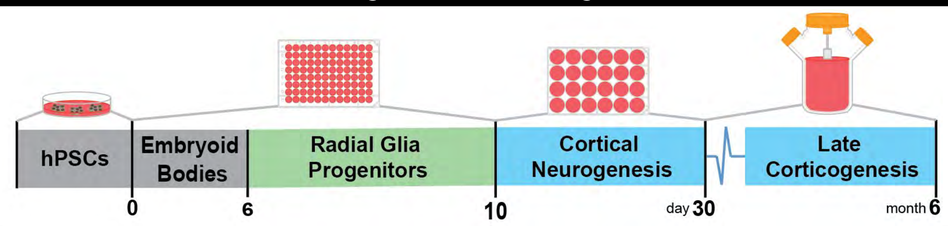
Stem Cell Passaging - ReLeSR treatment



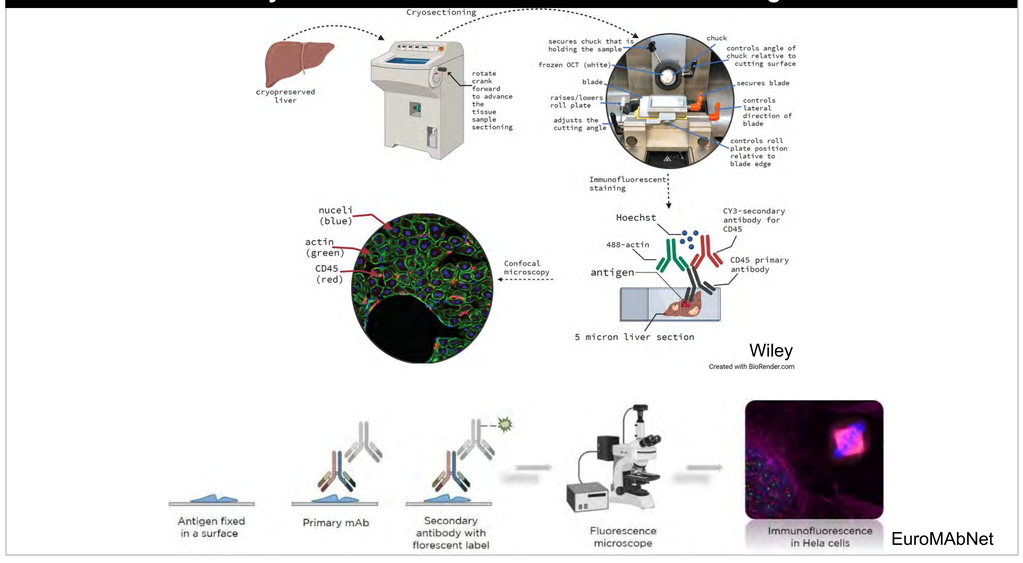
Culturing Pluripotent Stem Cells



Culturing Human Brain Organoids

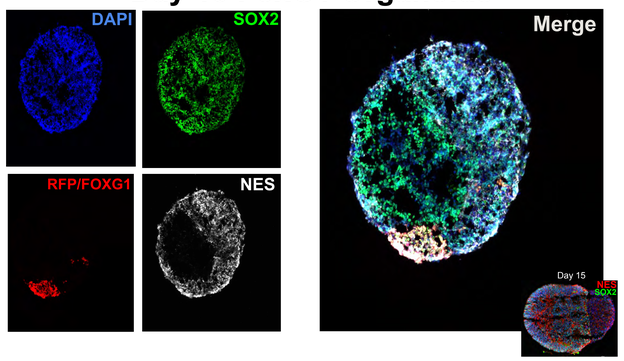


Cryosection and Immunofluorescent Staining

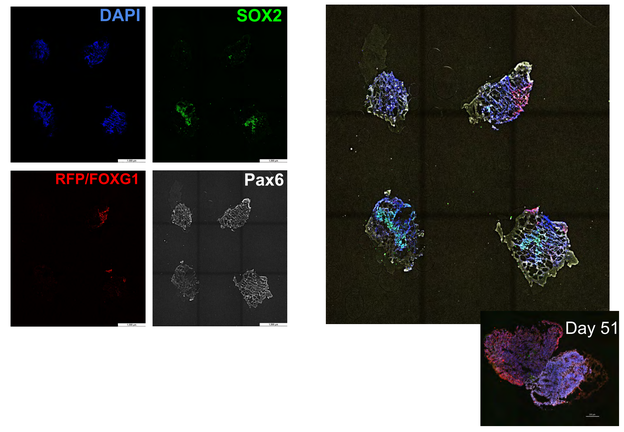


Immuno-staining of Brain Organoids

Day 18 FoxG1 Organoids



Day 40 FoxG1 Organoids



Summary

Accomplished:

1. Cultured the iPSC line 11a for three weeks and passaged the culture once.
2. Did not generate brain organoids but learned in detail the process and steps involved.
3. Used the cryosection machine to section FoxG1 organoids.
4. Created a washing and antibody solution to wash the slides and apply the primary and secondary antibody.
5. Used epifluorescent microscope to capture images.
   a. Day 18 organoids showed some formation of rosette structures but not robust as prior differentiation (see insert)
   b. Day 40 organoids showed some dorsal forebrain markers.

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