Master's Thesis – 30/60 hp

Development of an immune-responsive protocol for generation of Brain endothelial-like cells.

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• Background

Brain is arguably the most complex organ in the human body, it performs a plethora of involuntary and voluntary functions. Hence, it is crucial that the brain is protected from blood-borne pathogens and any agents that can cause harm. The blood-brain barrier (BBB) serves that function. The cells that line the vessels in the brain are specialized endothelial cells that are different from the vasculature of the rest of the body. More specifically, brain endothelial cells *in vivo* have a higher transendothelial resistance and, hence, restrict entry to pathogens and large molecules while allowing the diffusion of small polar or hydrophilic molecules.

Primary cells or cell lines have been the primary source of brain endothelial cells, but these options are hampered by low transendothelial electrical resistance (TEER <30 Ωxcm2). These "leaky" models are not optimal for *in vitro* studies e.g. drug transport studies. The use of hiPSCs have solved that issue; by generating brain endothelial-like cells from hiPSCs, researchers can now generate a barrier that is close to the in vivo conditions (TEER in frogs and rats in vivo has been reported to be >2000 Ωxcm2).

We are interested in the immunological response of these cells. Preliminary data from our lab suggest that these cells are not immune-competent. A slightly altered differentiation strategy results in cells that elicit upregulation of inflammatory markers. However, we have experienced that that strategy is highly variable and cell line-specific, which is also verified by other researchers in the field.

• Project Focus

The focus of the project is to develop a robust differentiation protocol that generates immune-competent brain endothelial-like cells.

The student will:

1. Evaluate the inflammatory responses of the default differentiation protocol we use in the lab with different inflammatory stimuli.
2. Explore slightly altered differentiation strategies and evaluate a) the resistance (TEER) and b) inflammatory responses to stimulants
3. If time permits, evaluate the inflammatory responses of brain endothelial-like cells in co-culture with iPS-derived astrocytes or iPS-derived pericytes.

- ## Technical and knowledge objectives

During the project the master student will learn the following techniques:

General

- Sterile culturing techniques

Cell Cultures

- Human primary culturing
- hiPSC Culturing
- Transwell culture

Evaluation techniques

- Immunocytochemistry (ICC)
- TEER measurements
- RNA isolation and cDNA synthesis
- Polymerase Chain Reaction (PCR)
- RealTime-quantitative PCR (RT-qPCR)
- Imaging (Wide field)

The student will acquire a basic understanding of:

Basic Knowledge of the embryonic development of the brain

- Cell types of the neurovascular unit (with a focus on brain endothelial cells)
- Signaling pathways that influence cell fate and growth

Differentiation

- Use of induced pluripotent stem cells in research
- Brain endothelial differentiation

- ## Requirements

- Mammalian cell culture is not obligatory, but recommendable.
- Motivation and sense of responsibility
- Attention to details and positive disposition

- ## References

1. **Brain endothelial like-protocol used in our lab:** A Simplified, Fully Defined Differentiation Scheme for Producing Blood-Brain Barrier Endothelial Cells from Human iPSCs (Neal et al. 2019).
2. **Immune-responsive BBB differentiation.** Directed differentiation of human pluripotent stem cells to blood-brain barrier endothelial cells (Qian et al. 2017).