Regenerative Pharmacology (session 4/4)

Date: Tuesday, October 13th
Time: 04:00 pm – 05:00 pm (CEST)
Location: Zoom Video Conferencing
Registration: Required
Organizing partners: NVF & UIPS

Program:

04:00 pm – 04:30 pm

Human stem cell models for targeting mitochondria and inflammation in neurodegeneration

Prof. Amalia Dolga, Associate Professor at University of Groningen

Abstract:

Mitochondrial dysfunction and inflammation are major contributors to progressive cell death, a characteristic of many neurodegenerative conditions, including ischemia, Alzheimer's and Parkinson's disease. Therefore, targets of these pathways might represent future potential therapeutic approaches for neurodegenerative diseases. Small conductance calcium-activated potassium (SK) channel modulation is an emerging therapeutic approach for treatment of neurodegenerative diseases. Our previous studies showed that activation of SK channels in microglia reduced LPS-mediated inflammatory processes. Further, we revealed that SK channel activation attenuated mitochondrial fission, prevented the release of pro-apoptotic mitochondrial proteins, and reduced cell death in a models of glutamate toxicity and cerebral ischemia. However, to translate these studies performed in murine models to human conditions, we need reliable models to study the human brain degeneration. The differentiation of human neurons and microglia was achieved from human induced pluripotent stem cell (iPSC) lines. We obtained 85% of pure microglial progenitors positive for CD14 and CX3CR1 and demonstrated the maturity of microglia by immunofluorescence of CD11b, TMEM119, and Iba-1 markers. The function of the iPSC-derived microglia was tested by stimulating differentiated microglia with LPS and alpha-synuclein. Using xCELLigence real-time cell impedance system, we could demonstrate morphological alterations, indicative of inflammatory processes in the LPS-treated microglia. The phagocytosis activity was also increased by the LPS presence, as revealed with the IncuCyte system.

In addition to the inflammatory pathways studied in human microglia, we investigated mitochondrial function in iPSC-differentiated neurons. Neuronal differentiation led to changes in metabolic profile of the cells carrying mutations linked to Alzheimer's disease. These stem cell-derived models represent essential steps towards a more reliable and predictive approach to mimicking the human neurodegenerative condition that could be used to find novel targets of mitochondrial function and inflammation.

04:30 pm – 05:00 pm

Validation of vessel-on-chip model using cells from HTNB patients

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Introduction

Human vascular studies currently focus on in vivo measurements and measurements in isolated human tissue. While both these methods help to gain insights into vascular mechanisms, neither of them allows in depth studying of vasculature of specific patients. An upcoming new technique focusses on creating vessels-on-chips, in which patient-specific cells can be used to culture 3- dimensional blood vessels. While the vessel-on-chip technology is increasing, it is for a number of characteristics still uncertain how the vessels-on-chip relate to intact human blood vessels and what their predictive value is. In the current project, we develop an iPSC-derived vessel-on-chip culture from patients diagnosed with autosomal-dominant hypertension with type E brachydactyly (HTNB).

These patients have a gain of function mutation in phosphodiesterase 3A (PDE3A), resulting in increased peripheral resistance and severe hypertension. As these patients have a very clear phenotype with known origin, they form an ideal group to validate our vessel-on-chip model.

Methods

Blood samples were obtained from three HTNB patients and a related family member. Erythroid progenitors that are present in the blood were reprogrammed to iPSCs. Next, iPSCs are differentiated into endothelial cells and vascular smooth muscle cells. The vessel is cultured in a round channel in a PDMS chip, and consists of vascular smooth muscle cells embedded in a collagen matrix and endothelial cells covering the luminal surface. The chip is perfused to mimic in vivo blood flow.

Results and expectations

DNA sequencing confirmed the presence of the PDE3A mutation in the cells of HTNB patients. When comparing our cultured vessel derived from HTNB patients to a related healthy control, we expect to see the upregulation in PDE3A, resulting in decreased intracellular cAMP levels. Moreover, we expect to see an altered response of HTNB vessels to calcium, or certain vasoconstrictors and vasodilators, measured in a Mulvany myograph system. The PDE3A inhibitor cilostazol should be able to reverse the pathology seen in HTNB vessels.

Conclusion

If the HTNB phenotype is indeed observed in the vessel-on-chip, this model could possibly be used to unravel other disease mechanisms with unknown pathology, and/or to study the effect of pharmacological interventions.

Organizing Partners:

NVF Dutch Pharmacological Society

UIPS Utrecht Institute for Pharmaceutical Sciences