Research Group Details

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Short Introduction to the lab

Our group studies the interplay between microRNAs and neurotrophic factors in reducing endoplasmic reticulum (ER) stress and promoting survival of adult dopaminergic neurons in rodent models of Parkinson's disease (PD). This work aims at the identification of neuroprotective microRNAs in adult dopaminergic neurons that may have potential clinical application in the treatment of PD. We utilize tissue-specific inducible CreERT2/LoxP system, virus-mediated transgenesis, and CRISPR-Cas9 to introduce specific genetic modifications to rodent dopaminergic neurons in vivo and in vitro, and study the progression of mutant phenotype at defined time points. We are also establishing protocols to differentiate dopaminergic neurons from human induced pluripotent stem (iPS) cells to study their properties and microRNAs regulating their physiology.

The work is supported by research grant from the Academy of Finland, and several grant applications are pending.

Project Outline

Title

Neuroprotective efficacy of CDNF and MANF in human iPS cells-derived dopaminergic neurons

Short introduction to the research problem

Neurotrophic factors CDNF and its close homologue MANF alleviate endoplasmic reticulum (ER) stress, inhibit cell death, and protect dopaminergic (DA) neurons in animal models of Parkinson'e disease (PD); however, the precise molecular mechanisms of CDNF/MANF-induced neuroprotection of DA neurons are not yet known.

Efficient derivation of DA neurons from human iPS cells and the use of CRISPR-Cas9 system to introduce specific genetic modifications open new possibilities to study the impact of specific genes on human DA neurons physiology, and the molecular mechanisms of neuroprotection against various forms of stress.

In view of planned clinical trials it is crucially important to study the neuroprotective efficacy of MANF/CDNF in human DA neurons in conditions of stress caused either by chemical factors or PD-related mutations.

Statement of experimental aims and methods

This project aims to establish human iPS cell culture and utilize CRISP-Cas9 gene editing to introduce PD-related mutations in human iPS cells. We will study the changes in electrophysiological activity and RNA expression profiles in control and mutant human DA neurons after CDNF/MANF treatment and drug-induced ER stress. We also aim at comparing the neuroprotective efficacy of CDNF/MANF applied either as recombinant proteins or as a gene therapy using viral vectors. Finally, we

hypothesise that some individual RNAs induced by NTFs treatment can possess neuroprotective activity and may code for “druggable” proteins; their identification may thus lead to the development of novel strategies and drugs for PD therapy.
Experimental methods include iPS cells culture and differentiation, cloning plasmid constructs and lentiviral vectors production, RNA/DNA preparation, immunostainings, lentivirus and AAV-mediated transgenesis, stereotaxic injections to rodent brain.

Project time table

Estimated project schedule is 2016-2018.
The first year will be spent for establishment of iPS cells culture and DA neuronal differentiation, CRISPR-Cas9-mediated gene editing in iPS cells and selection of correctly targeted clones.
The second year will include electrophysiological characterization of iPS cells-derived control and mutant DA neurons and measurement of dopamine release in control conditions and after CDNF/MANF and ER stressors treatment, and analysis of ER stress levels and progression in control and mutant DA neurons treated with CDNF/MANF and ER stressors.
Isolation and sequencing of RNA samples from control and mutant DA neurons in control conditions and after CDNF/MANF and ER stressors treatment, bioinformatic analysis of the RNA sequencing data, and in vivo experiments will be performed during the third year of the project.

Collaborators or external secondments relevant to the project

Dr. Anne Panhelainen (Prof. Saarma’s lab, University of Helsinki)
Electrophysiogical characterization of DA neurons.
Prof. Mart Saarma, Prof. Raimo Tuominen, and Docent Mikko Airavaara (University of Helsinki)
Provision of MANF, CDNF, and respective antibodies, and the research infrastructure.
Prof. Timo Otonkoski (Biomedicum Stem Cell Centre, University of Helsinki)
Provision of human iPS cells and practical training for their culture; derivation of DA neurons from human iPS cells.
Dr. Brandon Harvey (National Institute on Drug Abuse (NIDA), Baltimore, MD, USA)
Practical training and support for implementation of CRISPR-Cas9 gene editing system.

Max 5 references relevant to the project

- 1. DOMANSKYI A, Saarma M, Airavaara M, Prospects of neurotrophic factors for Parkinson's disease: comparison of protein and gene therapy. Hum Gene Ther, 26: 550-559, 2015
- 2. DOMANSKYI A, Alter H, Vogt MA, Gass P, Vinnikov IA, Transcription factors Foxa1 and Foxa2 are required for adult dopamine neurons maintenance. Front Cell Neurosci, 8: 275, 2014
- 3. DOMANSKYI A, Geißler C, Vinnikov IA, Alter H, Schober A, Vogt MA, Gass P, Parlato R, Schütz, G, Pten ablation in adult dopaminergic neurons is neuroprotective in Parkinson's disease models. FASEB J, 25: 2898-2910, 2011
- 4. Rieker C, Engblom D, Kreiner G, DOMANSKYI A, Schober A, Stotz S, Neumann M, Yuan X, Grummt I, Schütz G, Parlato R, Nucleolar disruption in dopaminergic neurons leads to oxidative damage and parkinsonism through repression of mammalian target of rapamycin signaling. J Neurosci, 31: 453-460, 2011