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Project title

Neuronal disease modeling using induced pluripotent stem cells (iPSC) for future drug development

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Research area:

Genomics and Genetics, iPSC disease models

Brief description

Neurodevelopmental and neurodegenerative disorders comprise a heterogeneous group of conditions for which treatment options are limited. Our group develops cell models of central nervous system disorders using induced pluripotent stem cells (iPSC). The long-term objective is to identify disease biomarkers that can be used for rescue-screens in search for candidate compounds and drug development.

Aim

The master projects (several offered) aim to characterize cell models of neurodevelopmental and neurodegenerative disorders *in vitro* using iPSC derived cells (neural stem cells, neurons, neural crest cells, organoids). The projects are focused on methodologies to identify cellular and molecular abnormalities associated with distinct disease phenotypes. Disease-associated abnormalities are evaluated for future rescue screening and drug development. *A specific master project will be selected according to the student's interest and within one of the four project plans below.*

Background

A limiting factor for the analysis of disease mechanisms in disorders of the central nervous system has been access to appropriate model systems to recapitulate human brain pathophysiology. The introduction of induced pluripotent stem cell (iPSC) technology provides the possibility to overcome this limitation (Takahashi et al. Cell, 2007). iPSC has the ability to differentiate into mature neurons that recapitulate functions in vivo. Our group have developed neural iPSC models of different central nervous system disorders with a known genetic cause (e.g. for Down syndrome, Alzheimer disease, Dravet syndrome, etc). The iPSC models are derived from affected individuals, or established after genome editing (CRISPR/Cas9) of specific genes, followed by differentiation into neuronal subtypes. Various methods are used to identify disease specific cellular and molecular perturbations, such as imaging, high through-put RNA sequencing, mass spectrometry and different functional assays.

Project plan

Down Syndrome (DS)

iPSC lines from DS patients and trisomy for chromosome 21 show dysregulated genes in various brain related models (e.g., organoids, neural stem cells, neurons, neural crest cells). We observed downregulation of the DNA-methyl transferasease DNMT3B in patient derived cells. The reduced expression of

DNMT3B is strong candidate contributing to abnormal brain development in DS is.
In this project, we aim to rescue the phenotype by overexpressing DNMT3B in neural DS lines. Furthermore, we will mimic the DS phenotype in neural control lines using a specific DNMT3B inhibitor.
Methods: Generate stable iPSC lines, verify DNMT3B vector integration and overexpression in transfected lines, neural differentiation, analyze selected genes by qRT/PCR and immunostaining, perform DNA methylation analysis.

Incontinentia pigmenti – NEMO deficiency
NEMO (or IKBkG) deficiency causes Incontinentia Pigmenti (IP), a disorder of the neural crest lineage. We have generated an iPSC line depleted of NEMO (using CRISPR/Cas9) to model the disease. Following differentiation into the neural crest lineage and RNAseq, we recently identified dysregulated genes and cellular phenotypes.
In the project, we aim to confirm our findings by overexpressing NEMO in our K.O. line (rescue) and by downregulation of NEMO in the control line by RNAi to mimic the IP phenotype.
Methods: Rescue NEMO deficiency in iPSC K.O. line. Transfect and express a NEMO transgene, verify integration and overexpression. Mimic NEMO deficiency in W.T. cells after transgene expression of shRNA against *NEMO* mRNA. Transfection, crest cell differentiation, analysis of cells for expression of selected genes by qRT/PCR and by immunostaining.

Dravet Syndrome (DD) – genetic epilepsy
DD is a drug-resistant epilepsy caused by mutations in the Na-channel SCN1A. Using patient derived iPSC lines and ATAC-seq, we have identified impaired chromatin remodeling in patient derived cortical GABAergic interneurons.
In the project we aim at defining the genomic regions containing chromatin changes associated with DD.
Methods: Immunostaining to characterize neuronal cell populations, DNA methylation profiling, qRT/PCR of selected genes.

Cell phenotype and drug screening platform – optimization
In collaboration with the Uppsala node of CBSC we are setting up a pipeline for screening of disease associated cell phenotypes in iPSC lines. The method is based on "Cell Painting" (i.e., visualizing cell morphology by staining intracellular organelles and structures).
Methods: Optimizing cell differentiation protocols for screening (cell densities etc.) and staining. qRT/PCR to characterize different cell populations. Interaction with the CBCS platform for optimization of cell paint methodology and through-put.

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