

P0270 / #3157

Topic: AS03 Stem Cells, Organoids, Neural Injury Neurotoxicity and Repair

HIGH-THROUGHPUT KNOCKDOWN SCREENING FOR MODIFIERS OF NEURONAL MORPHOLOGY IN LEIGH SYNDROME PATIENT-DERIVED NEURONS

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Leigh syndrome (LS) is a rare incurable mitochondrial disease leading to intellectual disabilities and movement defects in children. LS is caused by mutations in genes encoding for components of the mitochondrial respiratory chain that can be encoded by either the nuclear or the mitochondrial DNA (mtDNA). A hotspot for genetic defects is the mtDNA gene *MT-ATP6*. mtDNA defects are particularly challenging to study given the paucity of cellular and animal models available. We previously used LS patient-derived induced pluripotent stem cells (iPSCs) to generate neurons and brain organoids to model the disease, and discovered impaired neuronal outgrowth capacity. We therefore developed a high-content analysis approach for automatic quantification of neuronal branching. We next introduced a genetic knockdown (KD) step into our pipeline. For this, we established a novel transfection protocol that allows short interfering (si)RNA-based KD in human neurons with very high efficiency. We employed control iPSC lines and LS iPSC lines carrying mutant *MT-ATP6*, and engineered them to express the transcription factor Neurogenin 2. This enabled us to rapidly obtain pure inducible neurons (iNs) upon doxycycline exposure. We first performed a proof-of-concept siRNA KD screen in control iNs to confirm that our approach is capable of identifying known modulators of neuronal morphology. Next, we will carry out a large siRNA KD screen in LS iNs based on the ‘druggable genome’: ~800 genes that are known targets of FDA-approved medical drugs. For candidate genes whose siRNA knockdown could improve the branching capacity of LS iNs, we plan to test the corresponding FDA-approved compounds using LS brain organoids, which we know exhibit defects in cellular organization and function. In conclusion, our innovative KD screening approach has the potential to uncover novel modifiers of neuronal branching and respective drugs that may be considered to be repositioned to treat children affected by LS.

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NUCLEAR LOCALIZATION OF HDAC4 AND HDAC5 PROMOTES SURVIVAL OF PRIMARY NEURONS.

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Strokes of the optic nerve and diseases such as ischemic optic neuropathy cause permanent blindness due to RGC axon damage and retrograde cell death. In our research we proposed hypothesis that class IIa HDAC-mediated repression of MEF2A promotes neurosurvival and neuroprotection in stroke providing new insight into the mechanisms underlying the pathophysiology of ischemic injury. Class IIa HDACs when localized to the nucleus bind MEF2 transcription factors on chromatin and mediate gene repression. Using primary cultures of rat e18 hippocampal neurons in starvation condition we tested the role of MEF2A and class IIa HDACs in neuronal survival and axon growth as a model for axon regeneration. According to our hypothesis, nuclear localization of HDAC4 and HDAC5 via inhibition of MEF2A-dependent gene expression, promoted neuronal survival and axon outgrowth in cultured neurons. Silenced MEF2a, overexpression of HDAC4 and HDAC5 wild-type and constitutively cytosolic mutants did not show any changes or even shortening of neurites. The impact on neuronal survival was assessed with the live cell marker calcein, the dead cell marker sytox. Constitutively nuclear HDAC mutants promote survival and neurite extension *in vitro* in the absence of growth promoting stimuli such as KCl. In addition, expression of apoptosis-related genes and sumoylation of MEF2A was changed for HDAC4/5 mutants. Obtained results could form a basis for novel gene therapy with use of HDAC4/5 mutants for retinal ischemic disease and white matter stroke. Supported by National Science Center grant no. 2019/33/B/NZ4/00587

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EFFECTS OF POLYSTYRENE NANOPLASTICS ON THE BIOLOGY OF HUMAN NEURAL STEM CELLS AND HUMAN CEREBRAL ORGANIDS.

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Global plastic production has increased exponentially in recent decades and a significant proportion persists in the environment,

where it is degraded by mechanical and physical processes giving rise to micro (< 5mm) and nanoplastics (< 1000 nm; NP) and can reach humans through ingestion, inhalation and the dermal route. There is growing concern about the effects that NPs may cause on human health, in particular there are few studies that assess the effect of NPs on the developing brain, although they have been shown to be able to cross the blood-brain barrier and the placenta. In this study, we evaluate the effects of 30 nm polystyrene NPs (PSNPs) on human neural stem cells (hNSCs) and human cerebral organoids (hCOs). In these models we perform studies on cell death, cell proliferation and phenotypic differentiation. As initial results, it can be said that the NPs penetrate cells and organoids, observing an increase of apoptotic markers at higher concentrations, indicative of cell death and alterations in cell proliferation and phenotypic differentiation. Overall, our study suggests the vulnerability of human stem cells to PSNPs exposure, resulting in functional disturbance that might lead to neurodevelopmental disorders.

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SINGLE-CELL SEQUENCING WORKFLOW TO STUDY CELLULAR COMPOSITION AND CELL TYPE SPECIFIC EXPRESSION PROFILES OF HUMAN CEREBRAL ORGANIDS

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Human cerebral organoid culture is a technology with immense potential in the areas of developmental neurobiology and neurodegeneration for example to study cell types, mechanisms involved, to discover of new biomarkers, to propose specific therapeutic strategies or to study the effects of compound-induced toxicity. Single-cell RNA sequencing (scRNA-seq) is a promising technology that will help to define the identity of the cerebral organoids and to understand cellular composition and cell type specific expression profiles. Standardization of workflows to do the scRNA-seq analysis is an important means to improve the use of this technology. We present the workflow and results of the scRNA-seq performed for cerebral organoids generated from the AND-2 cell line of human embryonic stem cells (hESCs). Dissociated cerebral organoid samples were loaded on the 10X Chromium and single cell libraries were prepared according to 10X Genomics standard procedures and sequenced on the Novaseq sequencer (Illumina). The data were checked and aligned to the GRCh38 human reference genome with Cell Ranger v6.0.2 and analyzed with Seurat v4.0. After quality filtering and data normalization with the SCTransform function, we performed Principal component analysis (PCA) using the highly variable genes, built a Shared Nearest Neighbor (SNN) graph using the Louvain method. To visualize data, Uniform Manifold Approximation and Projection

(UMAP) dimensional reduction was performed. The identities of the cell clusters were assigned using the expression of genes specific of each cell type. We annotate in the AND2 cerebral organoids clusters for intermediate progenitor cells, astrocytes, oligodendrocyte precursor cells, excitatory neurons, inhibitory neurons, and mesodermal cells. We find also some cells in these organoids with expression of endothelial and microglial gene markers. Enrichment analysis of the highly variable differentially expressed genes (DEGs) was utilized to characterize the assigned cell types with Gene Ontology (GO), PanglaoDB and Cellmarker databases.

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MULTICILIATED EPENDYMA RECOVERY THROUGH A SEQUENTIAL CELL THERAPY IN POSTHEMORRHAGIC HYDROCEPHALUS

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Posthemorrhagic hydrocephalus (PHH) is a significant cause for premature children's morbidity, mortality, and peri/postnatal neurodevelopmental impairment. PHH is mainly triggered by germinal matrix hemorrhages (GMH) and causes germinal matrix and ependyma dysfunction. Ependyma constitutes a relevant tissue barrier with roles in cerebrospinal fluid homeostasis, circulation, and neurogenesis, hence situating ependyma as a main target when treating PHH. Clinical treatments are directed to eliminate immediate inflammatory condition triggered by the bleeding, to drain excess of CSF if needed, but not to treat or recover ependyma structure. Ependymal progenitors were obtained from P0 mice. Cells were cultured under specific conditions to enhance either ependymal proliferation or differentiation status. Different GMH/IVH neuroinflammatory conditions were mimed in the ependyma cultures, different stem cell therapies tested and effect on the ependymal differentiation measured. Additionally, ventricular wall explants from mice with induced PHH were obtained and cultured as ex-vivo system of PHH. A combination of stem cells was applied on the tissue to probe its regenerative capabilities on the multiciliated ependyma. All samples were analyzed through immunofluorescence and laser confocal microscopy and quantified. Results show that (i) ependymal progenitors' maturation is hindered under neuroinflammatory conditions, showing no multiciliated ependyma and (ii) the tested stem cell combination promotes ependymal progenitors' survival albeit does not alter the differentiation of the selfsame. In summary, it can be stated that the final differentiation of the ependyma is disrupted by the molecular conditions triggered by GMH/IVH, which our proposed cell therapy is able to counteract through increased survival and differentiation in a murine model of experimental