P0277 / #4388

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

LONG-TERM NEUROLOGICAL CONSEQUENCES OF SARS-COV-2 INFECTION USING BRAIN ORGANOIDS

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SARS-CoV-2 virus has been reported neuroinvasive, neurotropic and potentially neurovirulent. Moreover, in the last months a new concern has arisen in relation to long-lasting symptoms of the infection. Interestingly, clinical studies report that SARS-CoV-2 infection could trigger neurological complications compatible with the appearance of neurodegeneration. Nevertheless, it is still not known whether these long-term neurological manifestations are caused by direct viral brain invasion, its replication, a systemic reaction to widespread inflammation, or their combination. Here, we use 6-month-old brain organoids developed from human embryonic stem cells to explore long-term consequences of COVID-19 in the brain. Our results suggest that SARS-CoV-2 virus can infect neurons, astrocytes, and choroid plexus cells. Viral infection triggers apoptosis in neurons regardless of their infection status. Signs of reactive astrogliosis have also been detected weeks after acute infection in the brain organoids. Altogether, our results describe a post-infection phenotype in the brain that could explain long-term sequelae caused by SARS-CoV-2 infection.

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

DESIGNING MAGNETICALLY RESPONSIVE NATURAL HYDROGELS FOR NEURAL REPAIR

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Neural damage from traumatic aethiology imposes a massive human, public health, and economic burden worldwide. The major limiting factor in restoring lost neural functions is the inability of central axons to regenerate, which is further aggravated by Wallerian degeneration. Therefore, there is an urgent need for alternative treatments with tailored physical, chemical, electrical, and biological properties selectively triggered by external stimuli. One such class of strategy consists of advanced materials like hydrogel nanocomposites which combine synergistically the numerous advantages of hydrogels and nanoparticles. In this work, we describe the design of novel iron oxide nanoparticles (IONPs) functionalized with chitosan (CHI) and hyaluronic acid (HA) and the use of these IONPs embedded in collagen hydrogels as potential neuro-regenerative biomaterials. IONPs were synthesized by the co-precipitation method and lyophilization of collagen solutions containing either IONP-CHI or IONP-HA was used for the synthesis of highly porous hydrogels. For analyzing neural responses *in vitro*, immunofluorescence techniques were used. Primary neural cell assays showed a tight interaction between neural cell membranes and coated-IONPs, both in suspension and embedded in the hydrogel. Cell viability, neuronal differentiation, and neurites interconnectivity were preserved up to 0.1 mg Fe/mL of IONPs concentration. This was also the case under the application of an alternating magnetic field (281 kHz, 21 mT). Encouraged by these positive outcomes, first *in vivo* trials are now being carried out in an SCI experimental model consisting of a cervical right hemisection at C6 in rats. In conclusion, coated-IONPs, both in suspension and loaded into collagen hydrogels, sustain neural viability, integrity, and functionality, thus opening their exploration for the design of novel neuro-regenerative biomaterial-based therapeutics. Further functionalization of these nanomaterials with bioactive molecules is also envisioned.

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

STUDYING THE CELLULAR AND MOLECULAR EVOLUTION OF FAMILIAR ALZHEIMER'S DISEASE USING HUMAN CEREBRAL ORGANOIDS

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The leading cause of dementia in the elderly is Alzheimer's disease (AD), and its increase is expected in the coming years. The histopathological hallmarks of AD are associated with the presence in brain of neurofibrillary tangles, due to the increase in hyperphosphorylated Tau protein, as well as amyloid plaques, due to the increase in amyloid peptide. There is no cure for AD and the treatments effective in slowing neurodegeneration. This lack of cure/treatments may be due to the lack of good study models. Until now, *in vivo* or *in vitro* monolayer cellular models have been used that do not allow recapitulating the complexity of the human brain, as well as the histopathology of AD in early stages of its development. For all these reasons, in this work we consider using the technology of three-dimensional cultures: human cerebral organoids (hCOs). In this work, using the protocol developed in our laboratory, we present the generation and characterisation of hCOs with mutations associated with familial AD (fAD) as compared with control hCOs. For this purpose, we used human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) as controls. As fAD models, we used hiPSCs with mutations in PSEN1 and APP duplication. Using immunohistochemistry and RT-qPCR we

have analysed these hCOs for markers of neural precursors, brain cell types and synaptic markers, as well as the progression of AD phenotype (amyloid plaques). We have also performed electron microscopy studies to observe differences in the ultrastructure of the hCOs. We present the differences found that show the hCOs generated from hiPSCs with AD variants are experimental *in vitro* models that will allow further study of pathology.

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P0280 / #3711

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

LEIGH SYNDROME DRUG DISCOVERY WITH INDUCED NEURONS AND MIDBRAIN ORGANOIDS UNVEILS A REPOSITIONABLE COMPOUND

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Mutations in mitochondrial complex IV assembly factor *SURF1* causes Leigh syndrome (LS), a rare incurable neurodevelopmental disorder typically affecting midbrain and basal ganglia structures. SURF1-deficient animals fail to recapitulate the patient neuronal pathology, thereby hindering the discovery of treatments. We previously generated a model of LS using patient-derived induced pluripotent stem cells (iPSCs) carrying *SURF1* mutations and identified impaired neuronal branching as a key pathogenetic mechanism. Here, we aimed to discover treatment strategies ameliorating the branching capacity of *SURF1*-mutant neuronal cells. We used single-cell transcriptomic datasets from *SURF1*-mutant cerebral organoids to feed a machine-learning algorithm that identified 28 druggable molecular targets affected by *SURF1* mutations. We prioritized nine compounds and tested them using isogenic iPSC lines engineered with CRISPR/Cas9 to carry *SURF1* mutations in either a control or patient background. We overexpressed the pan-neuronal transcription factor *Neurogenin2* (NGN2) to obtain pure neurons and evaluated the effect of the nine substances on branching outgrowth using high-content imaging. We identified one FDA-approved compound capable of rescuing neuronal branching in a dose-dependent manner in *SURF1*-mutant induced neurons. Next, we developed a three-dimensional model system of LS based on midbrain organoids. SURF1-mutant midbrain organoids showed slower growth rate, increased lactate release, and lack of synchronous activity measured by calcium imaging. Whole-organoid clearing of SURF1-mutant midbrain organoids revealed defective neuronal branching organization in both axonal and dendritic compartments. Current experiments are ongoing to assess the efficacy of our identified FDA-approved compound in reverting these defects in SURF1-mutant midbrain organoids. Our work demonstrates the advantages of using machine-learning drug predictions coupled with iPSC-based screening platforms and organoid technologies to identify effective drugs to be repositioned for the treatment of incurable neurodevelopmental diseases like Leigh syndrome.

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

DOSE- AND AND TIME-DEPENDENT EFFECTS OF TREATMENT WITH HUMAN BONE MARROW-DERIVED STROMAL CELLS IN A RAT MODEL OF SPINAL CORD INJURY

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One of the most promising therapeutic strategies of spinal cord injury (SCI) consists in the implantation of stem cells to reduce inflammation and promote neural regeneration. In the present study we tested the dose-dependent effect of human bone marrow-derived stromal cells (bmSC) as a therapy in the post-acute phase after SCI. Spinal cord contusion injury was induced in adult male rats at thoracic level T9/T10 using the *Infinite Horizon impactor*. One week after lesion, animals were treated bmSC prepared from the iliac crest of healthy volunteers, using a process of negative selection without expansion *in vitro*. One week after SCI (7 dpo) and within 48 hrs after preparation, cells were injected into the *cisterna magna*. No immune suppression was used. Doses of 0.5 x 10^6 and 2.5 x 10^6 bmSC, suspended in 150 µL saline, were compared with the following control treatments: 150 µL saline; 2.5 x 10^6 bmSC injected at 1-2 hrs after SCI; 5.0 x 10^6 frozen/reconstituted bmSC, injected at 7dpo; 2.5 x 10^6 rat-bmSC injected at 7dpo. The recovery of motor functions assessed during a surveillance period of 6 weeks (open field: BBB-scale, RotaRod, kinematic analysis), neuropathic pain (von Frey, Hargreaves) at 5 weeks. After six weeks, the animals were perfused, and the spinal cord tissue was investigated histologically. Rats did not reject the human implants and showed no sign of sickness behavior. Compared to the control groups (saline, rat-bmSC), animals injected with the high dose of fresh bmSC at 7dpo showed significantly better recovery of motor function and a lower degree of neuropathic pain. Treatment at 7dpo was more effective than treatment immediately after SCI. Bone marrow-derived stromal cells, prepared by negative selection without expansion in culture (NeuroCells$^{(R)}$/Neuroplast BV) and injected in the post-acute stage support recovery of motor function in a rat model of SCI.

Declaration of Interest Statement: Boris W Kramer is an employee of Neuroplast BV, Maastricht, the company holding the patent of the preparation of bmSC employed in this study.

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