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Appendix to the abstracts of the Joint Conference

The 13th International Symposium MOLECULAR BASIS OF PATHOLOGY AND THERAPY IN NEUROLOGICAL DISORDERS

The 4th International Conference STEM CELLS: THERAPEUTIC OUTLOOK FOR CENTRAL NERVOUS SYSTEM DISORDERS

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Lectures

Thursday, November, 17th, 2016

Methamphetamine-induced aberrant neurogenesis in the hippocampus

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Disruption of the blood-brain barrier (BBB) and the development a neurocognitive deficits have been identified as the primary events in methamphetamine (METH) abuse. It is now recognized that adult brains have progenitor cells, which differentiate into specific lineages, including neurons. A frequently overlooked fact is that these cells are in close proximity to the BBB. We hypothesize that METH-induced disruption of BBB impairs differentiation of neural progenitor cells to mature neurons, affecting neurogenesis. Physical exercise is known to promote cell survival and functional recovery after brain injuries. We also reported that preceding voluntary exercise protected against METH-induced disruption of the BBB (Toborek *et al*., Mol Neurodegener, 2013). Therefore, we assessed the impact of exercise, in a form of voluntary wheel running, on METH-induced abnormal neural differentiation.

While no effective therapy is available for the treatment of METH-induced neurotoxicity, behavioral interventions, including aerobic exercise, are being used to improve depressive symptoms and substance abuse outcomes. The present study focuses on the effect of exercise on METH-induced neurotoxicity in the hippocampal dentate gyrus (DG) in the context of the BBB pathology. Mice were administered with METH or saline (vehicle) by i.p. injections three times per day for 5 days with an escalating dose regimen in 4 h intervals, starting from 0.2 mg/kg. One set of mice was sacrificed 24 h post last injection of METH, and the remaining animals were either subjected to voluntary wheel running (exercised mice) or remained in sedentary housing (the sedentary group). METH administration resulted in decreased expression of tight junction (TJ) proteins and increased BBB permeability in the hippocampus. These changes were preserved post METH administration in sedentary mice and were associated with the development of significant aberrations of neural differentiation. Exercise protected against these effects by enhancing the protein expression of TJ proteins, stabilizing the BBB integrity, and enhancing differentiation of progenitor cells to neuronal lineage. In addition, exercise protected against METH-induced systemic increase in inflammatory cytokine levels. These results indicate for the first time that exercise protects against chronic METH-induced impaired hippocampal neurogenesis by enhancing BBB integrity and decreasing systemic production of proinflammatory cytokines, such as IL-1β and TNF-α.

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Micro and macro-scale approach of stem cell based developmental neurotoxicity testing

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Developmental neurotoxicity (DNT) in humans refers to the damage of the central nervous system (CNS) as the effect of exposure to an adverse substance during *in utero* and early postnatal development. It is widely accepted, that developing brain in children and fetuses is much more vulnerable to chemical perturbation than the adult brain. DNT often lead to the loss of cognitive skills, autism, ADHD and, what's more, may also cause silent damage manifesting itself after number of years by contributing to neurodegenerative diseases such as Parkinson's or Alzheimer's diseases. Developing reliable human based *in vitro* systems to study drug toxicity and their mode of action is a major challenge for establishing new and safe DNT therapies. However, research efforts are hampered by limited access to human tissue, especially those that represent the earliest stages of development of human embryos.

Both ethical and technical concerns regarding the use of human embryo or fetuses for derivationof neural stem cells can be circumvent by the possibility to acquire human induced pluripotent stem cells (hiPSC) that are similar to human embryonic stem cells (hESC), but can be generated from any adult tissue in the body. For this reason in our studies we have applied innovative, patient-specific approach to obtain human induced pluripotent stem cells (hiPSC) – based 3D culture of organoid spheres called "embryonic bodies" (EB), that recapitulate the earliest stages of development and can be successfully committed for neural differentiation. The composition of the obtained 3D aggregates/spheres included cells expressing typical markers of the three germ layers, which in proper differentiating conditions can acquire advanced neuronal

markers MAP-2, Doublecortin, Synapsin, TH as well as glial markers: PDGFRa, GFAP and GalC. ThehiPSC-derived EBs cultures at different stages of differentiation were investigated by our group for MeHgCl induced embryotoxicity and genotoxicity.

The advancement to the DNT screening possibilities in stem cell-based culture systems is provided by the emerging technologies, which are implicated to create high content/high throughput drug discovery *in vitro* platforms that are useful in filling the gap between animal testing and clinical trials. There are two different strategies to create such advanced research "biomimetic" *in vitro* systems. The first is to establish "microscale" environments to test cell behavior and molecular mechanisms, even at the single cell resolution. The second approach is to provide a "macroscale" structural, biomaterial based 3D template for cell differentiation and function, that allows the growth of complicated human tissues and organoids. The ability to use conditional bioengineering to manipulate biomaterials in "real time", is emerging as a powerful tool in regulating behavior of stem cells that are encapsulated in the scaffolds. Both, micro- and macroscale systems provide new tools to screen *in vitro* for chemical effects on the critical DNT events, such as proliferation, migration, neurite outgrowth or synaptogenesis. In the previous studies of our group the micro-scale engineering techniques (surface patterning: micro-contact printing and piezzoelectric spotting) were used to control cell microenvironment interactions (cell-cell, cell-ECM, and cell-soluble factor interactions) as well as cellular processes (proliferation, migration, differentiation) in the culture of human neural stem cells, that were immobilized to the bioactive surface and exposed to developmental neurotoxicant (e.g. MeHgCl).

This talk will provide the state of the art on the development of human stem cell based *in vitro* systems for DNT testing, with stem cell 3D models and micro/nano engineered drug screening platforms, used to test variety of compounds. The results of our group implementing both: "micro" and "macro"- scale approach to DNT testing will be discussed in the context of the advancement in the field.

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Endocrine disruptors and their neurotoxic effects on developing brain

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Endocrine disrupting chemicals (EDCs) are environmental organic compounds which are able to interfere with hormone receptors, hormone synthesis or hormone conversion, thus altering the hormone-dependent processes and disrupting functions of endocrine glands. In addition to EDC properties, organic compounds such as dioxins, polychlorinated biphenyls (PCBs), pesticides (e.g. DDT, DDE), brominated flame retardants, plasticizers (e.g. nonylphenol) and personal care products possess capacities of altering neural transmission and neural networks. Although EDCs are known to cross blood-brain barrier, little is known about their impact on the nervous system, especially at early developmental stages. Systematic and complex data concerning mechanisms of actions of EDCs in neuronal cells are missing. Recognition of these mechanisms is particularly important because EDCs through alteration of epigenetic status and dysregulation of apoptosis and autophagy could impair neural development and/or cause neurotoxicity and neurodegenerations. Furthermore, interactions of EDCs with steroid and xenobiotic receptor signaling pathways at early stages of neural development could cause abnormalities which might reveal in adolescent or adult nervous system. Interestingly, epidemiological data showed correlations between exposures to environmental pollutants and increased risk of neuropsychiatric disorders, including autism, attention deficit and hyperactivity disorder, learning disabilities, aggressiveness and depression. Exposalto pesticides or PCBs has been associated with neural degenerations, involving Parkinson's and Alzheimer's diseases. Recently, we have demonstrated that stimulation of aryl hydrocarbon receptor (AhR)-signaling and impairmentof G-protein coupled receptor 30 (GPR30)-signaling play important roles in the propagation of DDT-induced apoptosis in mouse neurons. We have also shown that the stimulation of retinoid X receptor (RXR)-mediated signaling is important for DDE-induced apoptosis and neurotoxicity that is accompanied by global DNA hypomethylation. Moreover, we provided evidence on key involvement of RXR/pregnane X receptor (PXR)/constitutive androstane receptor (CAR) signaling pathways in the apoptotic and neurotoxic actions of nonylphenol. These new data give prospects for understanding the neurodevelopmental pathomechanisms of actions of EDCs. Targeting xenobiotic nuclear receptors could be asset in searching for effective neuroprotective

strategies against EDCs and their controlled use, especially during the early stages of neural development.

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Triggering role of Ca^{2+} imbalance in the induction of acute oxidative stress and cytotoxicity in primary cultures of rat cerebellar granule cells challenged with TBBPA

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The aim of this study, performed using primary cultures of rat cerebellar granule cells (CGC), was to determine the role of increases in intracellular calcium concentration ($[Ca^{2+}]_i$) in inducing oxidative stress and cytotoxicity in neurons acutely treated with brominated flame retardant tetrabromobisphenol A (TBBPA). Neuronal cultures were exposed for 30 minutes to 10 or 25 μM TBBPA. Changes in $[Ca^{2+}]_i$, in the production of reactive oxygen species (ROS), and in the potential of mitochondria ($\Delta\Psi m$) were measured fluorometrically in CGC during exposure to TBBPA, the intracellular level of glutathione (GSH) and catalase activity were determined immediately after incubation, and cell viability was evaluated 24 h later. Application of TBBPA concentration-dependently increased $[Ca^{2+}]_i$ and ROS production; it reduced also GSH content, catalase activity, $\Delta\Psi m$ and neuronal viability. Antagonists of NMDA and ryanodine receptors which, when applied in combination, completely inhibited rises in $[Ca^{2+}]_i$ evoked by both concentrations of TBBPA, only partially reduced neuronal death. They entirely prevented oxidative stress and drop in $\Delta\Psi m$ induced by 10 μM TBBPA, while these effects of 25 μM TBBPA were only partially reduced. Cyclosporin A did not prevent TBBPA-evoked drop in $\Delta\Psi m$ and ROS production, but was partially cytoprotective, exclusively at high concentrations against toxicity of 10 μM TBBPA. Free radical scavengers significantly reduced indices of oxidative stress in CGC treated with TBBPA and improved their viability, but did not interfere with rises in $[Ca^{2+}]_i$ and drop in $\Delta\Psi m$, while their co-administration with NMDA and ryanodine receptor antagonists almost completely protected the cells. In conclusion, both, Ca^{2+} imbalance and oxidative stress mediate acute toxicity of TBBPA in CGC. TBBPA-induced increase in $[Ca^{2+}]_i$ is a primary and major event which triggers oxidative stress and depolarization of mitochondria in CGC. At high TBBPA concentration Ca^{2+}-independent portion of oxidative stress and cytotoxicity was revealed.

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Glutaminase, HIV-associated neurocognitive disorders and beyond

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Glutamate serves as a crucial excitatory neurotransmitter that is essential for the proper functioning of the brain. However, the excess level of glutamate proves to be neurotoxic and contributes to the pathogenesis of neurodegenerative dissease, indlucing HIV-1 associated neurocognitive disorders (HAND). HIV-1-infected and/or immune-activated microglia and macrophages are pivotal in the pathogenesis of HAND. Glutaminase, a metabolic enzyme that facilitates glutamate generation, is upregulated and may play a pathogenic role in HAND. Our previous studies have demonstrated that glutaminase is released to the extracellular fluid during HIV-1 infection and neuroinflammation. However, key molecular mechanisms that regulate glutaminase release remain unknown. Recent advances in understanding intercellular trafficking have identified microvesicles (MVs) as a novel means of shedding cellular contents. We posit that during HIV-1 infection and immune activation, microvesicles may mediate glutaminase release, generating excessive and neurotoxic levels of glutamate. MVs isolated through differential centrifugation from cell-free supernatants of monocyte-derived macrophages (MDM) and BV2 microglia cell lines were first confirmed in electron microscopy and immunoblotting. As expected, we found elevated number of MVs, glutaminase immunoreactivities, as well as glutaminase enzyme activity in the supernatants of HIV-1 infected MDM and lipopolysaccharide (LPS)-activated microglia when compared with controls. The elevated glutaminase was blocked by GW4869, a neutral sphingomyelinase inhibitor known to inhibit MVs release, suggesting a critical role of MVs in mediating glutaminase release. More importantly, MVs from HIV-1-infected MDM and LPS-activated microglia induced significant neuronal injury in rat cortical neuron cultures. The MV neurotoxicity was blocked by a glutaminase inhibitor or GW4869, suggesting that the neurotoxic potential of HIV-1-infected MDM and LPS-activated microglia is dependent on the

glutaminase-containing MVs. These findings support MVs as a potential pathway/mechanism of excessive glutamate generation and neurotoxicity in HAND and therefore MVs may serve as a novel therapeutic target.

Glutaminase, ammonia and hepatic encephalopathy: an opportunity for therapy

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Hepatic encephalopathy (HE) is a major complication of liver cirrhosis, and is classified into three types: Type A (acute) HE is due to with acute liver failure (ALF); Type B (by-pass) HE is due to portal-systemic shunting without intrinsic liver disease; and Type C (cirrhosis) HE occurs in patients with underlying cirrhosis. However, the appearance of hepatic encephalopathy in patients with acute-on-chronic liver failure was not included in this classification. HE manifests as a spectrum ranging from minimal disturbances in mental function that impacts on attention, cognition and quality of life to coma. Hepatic encephalopathy is a complex neuropsychiatric syndrome in patients with liver dysfunction or porto-systemic shunts. Stages of HE have been defined by West-Haven criteria: Stage 0 means no abnormality detected. Stage 1 trivial lack of awareness with shortened attention span, euphoria and anxiety and inability to do easy calculations. Stage 2 is characterized by lethargy, disorientation for time, changes in personality, inappropriate behaviour. Stage 3 was defined by somnolence and semi stupor, keeping response to stimuli with confusion, gross disorientation for time and space and bizarre behaviour. Stage 4 was defined by coma.

HE in patients with cirrhosis decompensation without criteria for ACLF has been strongly related to previous episodes of hepatic encephalopathy and the abuse of diuretics, but not with hyponatremia, infections or alcohol binge. Interestingly, GI bleeding seems to protect against HE instead to promote it. Improvement in the management of variceal bleeding avoiding infections and controlling bleeding could explain, at least in part, this result. On the other hand, in patients with ACLF, HE was also associated with previous bouts of overt HE but not with diuretics abuse, GI bleeding, alcohol binge or infections. These precipitant factors were equally distributed in patients with and without HE. The strong association between previous bouts of overt HE and HE support the hypothesis of the impact of gene alteration on the risk of developing HE. A microsatellite in the promoter region of gutaminase type K gene has been associated with increased risk of HE (form long-long of the microsatellite). However, other genes could be implicated on HE and a GWAS analysis is warranted to define the genetic profile associated with risk of overt HE in cirrhotics. Diuretics-induced renal insufficiency seems to be a major cause of HE in cirrhotics with acute decompensation, highlighting the role of kidneys on HE. Brain impairment appeared as consequence of hyperammonemia in the brain, oxidative stress, activation of microglia, hyponatremia and benzodiazepine-like substances able to promote an astrocyte-neuron dysfunction, neurological basis for HE.

In the management of patients with HE and liver dysfunction is mandatory to exclude other causes of neurological or psychiatric disorders and keep in mind other types of encephalopathy like sepsis or hyponatremia. Mental status should be explored using Glasgow scale. Nutritional assessment should also be included. Biochemical analysis include: full blood count, liver and kidney function, electrolytes, ammonia, thyroid function, inflammatory reactant, glycaemia, vitamin B_{12} and urine analysis. Patients with HE and ACLF should be admitted in the intensive care unit. The first step is removing any precipitant factor or treating it (infections by antibiotics; diuretics abuse: volume expansion; alcohol binge: thiamine and in cases of malnutrition nutritional support). If no precipitant factor was detected with have to focus on modulation of inflammation plus ammonia lowering drugs. In patients without response and preserved liver function, large porto-systemic shunts should be ruled out and embolised if present. Lastly, liver transplantation remained as the therapeutic option in patients with HE without response to all mentioned measures.

Several ammonia-lowering drugs are also able to avoid glutamine accumulation (that could serve as substrate for glutaminase transforming it into glutamate and ammonia – Trojan Horse hypothesis) excreting by urine it in form of phenylacetyl-glutamine. Ornithine-phenylacetate and glycerol or sodium phenylacetate belonged to this type of drugs. CB-839 a glutaminase inhibitor demonstrated in portacaval shunted rats its ability as ammonia lowering drug. The role of these drugs in management of overt HE requires future studies.

Conflict of interest

Manuel Romero-Gómez was inventor of THDP-17, a glutaminase inhibitor, which was licensed by Janus Development, S.L. He has ongoing research collaboration with Umecrine, S.A., Sweden. He has also received speaker fees from Bama-Geve, Merz and Norgine, S.A.

Brain glutaminase in psychiatric disease: from mouse models to schizophrenia

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Glutaminase (GLS1) is the rate-limiting step in the recycling of neuronal glutamate. GLS1 gene expression is highest in the hippocampus and cortex in wild-type mice. Mice heterozygous for a null-STOP mutation in GLS1 ($GLS1^{+/-}$ mice) display reduced GLS1 expression in hippocampus and prefrontal cortex, and reduced glutamate levels in these regions. Furthermore, fMRI imaging of $GLS1^{+/-}$ mice points to a focal reduction in hippocampal activity, mainly in the CA1 subfield. This finding contrasts with recent studies that demonstrate CA1 hyperactivity in patients with schizophrenia, prodromal patients and mouse models of increased glutamate transmission. $GLS1^{+/-}$ mice also demonstrate an attenuated behavioral and neurochemical response to the psychotomimetic drug amphetamine, and behavioral alterations in hippocampus-dependent and independent tasks. Interestingly, changes in NMDA receptor gene expression patterns in $GLS1^{+/-}$ mice emerge in adulthood but not in adolescence. Taken together, these findings support the centrality of GLS1 to normal hippocampal function, and indicate that GLS1 inhibition in adulthood may be a therapeutic target in treating schizophrenia-related abnormalities.

Glutamine addiction in brain cancer

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Cancer cells develop and succeed by shifting to different metabolic programs compared with their normal cell counterparts. One of the classical hallmarks of cancer cells is their high glycolytic fluxes even in the presence of abundant O_2 and heightened levels of lactate produced (Warburg effect). Another common metabolic feature of cancer cells is a high rate of glutamine (Gln) consumption normally exceeding their biosynthetic and energetic needs. The term Gln addiction is now widely used to reflect the strong dependence shown by most cancer cells for this essential nitrogen substrate after metabolic reprogramming. A Gln/Glu cycle occurs between host tissues and the tumor in order to maximize its growth and proliferation rates. Support for the existence of this cycle *in vivo* has come also from studies on enzymatic activities of glutamine synthetase and glutaminase in host tissues during tumor development. In this presentation, we review glutaminolysis in tumor cells by focusing on glutaminase proteins and with special emphasis on brain cancer. The mechanistic basis for this altered metabolic phenotype and how these changes are connected to oncogenic and tumor suppressor pathways are becoming increasingly understood. Based on these advances, new avenues of research have been initiated to find novel therapeutic targets and to explore strategies that interfere with glutamine metabolism as anticancer therapies.

Friday, November, 18th, 2016

Engineered human neural stem cells for treating spinal cord gliomas: a neurobiology-based approach

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There are currently no experimental models showing autonomic dysfunction for intramedullary spinal cord gliomas (ISCG), a lethal disease with no effective treatment. We have developed a rat model of ISCG and determined whether genetically engineered human neural stem cells (hNSC) could be developed into potent therapies for ISCG. ISCG rats received injection of hNSC.CD-TK, hNSC.CD or hNSC.CD-TK debris adjacent to the tumor epicenter 7 days after glioma cell implantation, followed with daily prodrug administration (5-FC and GCV; i.p. throughout the study). Post-tumor survival was assessed by time lasted before loss of body weight-bearing stepping in the hindlimb. Also evaluated were autonomic functions and tumor growth rate *in vivo*. ISCG rats with hNSC.CD-TK treatment showed significantly improved survival than controls that received hNSC.CD or hNSC.CD-TK debris ($P < 0.05$, median rank test), with better maintained autonomic function and reduced tumor growth rate. hNSC.CD-TK cells migrated diffusively into ISCG clusters to mediate targeted oncolytic effect in manners that spared spinal cord projection pathways. Through impeding glioma growth and preserving spinal cord neurobiology, dual gene-engineered hNSC regimen significantly prolonged survival in

a rat model that emulated sensorimotor and autonomic dysfunctions of human cervical ISCG. Our findings may provide a stem cell-based multimodal approach to treating ISCG and help formulate a recovery neurobiology-based therapeutic strategy for gliomas.

Hematopoietic stem cell-based therapy in neurodegenerative disorders – cellular and humoral mechanisms

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Advance of research on pathophysiology of stem cells (SCs) gives a chance on elucidation of the mechanisms regulating both normal human development as well as pathologic processes. Recently, a growing body of evidence suggests that SCs might be used in the adjuvant therapy of severe neurodegenerative disorders. Although there is currently a lack of compelling evidence for the effectiveness of cell-replacement therapies, the main benefit that is accessible from transplanted cells would be paracrine secretory activity, which is theoretically more convincing and fits nicely into the concept of adjuvant/supportive roles for SC-based therapy. Neurotrophic factors regulate survival, development, and function of nervous tissue. Illumination of their physiological role in the maintenance of central nervous system homeostasis as well as regeneration of damaged tissue have ignited expectations to heal neurodegenerative diseases, including amyotrophic lateral sclerosis. It has been demonstrated that SCs that are genetically modified with viral vectors (e.g., MSCs transduced to express NT-4) are capable of long-term survival after transplantation when NTs (e.g., NT-4 or BDNF) are continuously delivered and that this survival results in significant improvements in functional parameters that are observed with objective methods. On the other hand, there are evidences for the presence of neurotrophins and their receptors in distinct hematopoietic cell populations, showing that these cells express NTs and NT receptors at both the mRNA and protein levels. Of note, NT expression is greater under stress-related conditions. Furthermore, bone marrow-derived Lin- SCs administeredvia a lumbar puncturenoticeable modulated expression of both NFs as well as angiopoetic and proinflammatory factors in the cerebrospinal fluid. Overall, the advances in experimental studies suggest that SC-based therapy might represent a novel treatment modality for the repair and regeneration of injured neural tissue. However, further extensive studies are definitely required to understand the mechanisms of SC actions, particularly their paracrine activities, and to present SCs as a new treatment option for clinical approaches.

Stem cell therapy for Parkinson's disease

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Studies in animal models of Parkinson's disease (PD) have shown that transplanted dopamine neuroblasts can restore dopaminergic neurotransmission in the grafted striatum and reverse PD-like motor impairments. Open-label clinical trials in patients with PD have shown that dopamine neuroblasts obtained from fetal human midbrain tissue can survive and function over many years in the brain of PD patients, restore striatal dopamine release, and provide sustained and long-lasting improvements in motor behavior. The ethical and practical problems associated with the use of fetal tissue isa serious obstacle to further developments of this approach. Further progress, therefore, is critically dependent on the development of transplantable dopamine neurons from stem cells. The most promising results so far have been obtained using pluripotent stem cells, ESCs or iPSCs, as starting material. Recently developed and optimized protocols allow efficient generation of midbrain dopamine neurons from human ES cells that survive well following transplantation to the striatum, in the absence of any contaminating tumor-forming cells, and differentiate into genuine midbrain dopamine neurons of both A9 and A10 subtypes. In recent experiments performed in immunosuppressed and immunodeficient rats we have shown that the hESC-derived neurons grow to form an extensive axonal terminal networks in appropriate striatal, limbic and and cortical targets and reverse PD-like motor impairments. The results indicate that transplantable and fully functional midbrain dopamine neurons can be generated from human ES cells, ready to be used in patients.

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New strategies of stem cell therapy in retinal disease

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In the retina the damage of retinal ganglion cells, retinal pigment epithelial cells and photoreceptors cells cause potentially blinding diseases as glaucoma, age-related macular degeneration, retinitis pigmentosa and others. Cell therapy has raised new hopes for the management of these diseases. *In vivo* studies and phase I-II clinical trials have shown promising results. However, several factors as cells source, route of administration, integration and function of cells into the retina as well as safety are still under close evaluation. New non-invasive techniques of molecular imaging of the retina might accelerate the process of clinical application of stem cells.

Challenging tasks and future perspectives of cell therapies in retinal diseases will also be discussed.

Stem cells for ALS: an overview of possible therapeutic approaches

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Over the past 25 years, stem cell technologies have become an increasingly attractive option to investigate and treat neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). The pathogenesis of ALS remains unclear, multiple factors are thought to contribute to the progression of ALS, such as network interactions between genes, environmental exposure and impaired molecular pathways.

The neuroprotective properties of neural stem cells (NSCs) and the paracrine signaling of mesenchymal stem cells (MSCs) have been examined in multiple pre-clinical trials of ALS with promising results. The data from these initial trials indicate a reduction in the rate of disease progression. The mechanism through which stems cells achieve this reduction is of major interest. Here, we review up to-date pre-clinical and clinical therapeutic approaches employing stem cells, and discuss the most promising ones.

Stem cells and neurorepair – from bench to clinic and back

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Central nervous system is one of the least well-known structures in our body. This is due to its complicated structure, and the difficulties to study the system both *in vitro* and *in vivo*.

The consequence of brain damage is a motor disability of the patient as well as neurological degeneration. The cause of encephalopathy can be, among others, mechanical trauma, metabolic problems, infection or pregnancy poisoning. Current encephalopathy treatments e.g. pharmacotherapy and hypothermia followed by motor and neurological rehabilitation do not bring the expected results. This is at least partially due to a lack of understanding of the biological aspects of the regeneration of the damaged brain tissue and the possibility of intervention in that process, which could lead to improved health status of patients.

The results of our recent clinical study, conducted by a multidisciplinary team of translational researchers from Department of Transplantation UJCM and clinicians from Department of Neurosurgery UJCM demonstrated that autologous mesenchymal stem cells (MSC) transplantation in children with encephalopathy leads to an improvement of the clinical picture of patients, including increase motor skills and overall neurological recovery.

To determine the mechanism of MSC action on the central nervous system cells research has been conducted with the use of induced pluripotent stem cells differentiated into GABAergic neurons. Preliminary results indicate that factors MSC increased activity of GABAergic precursors and their neuronal differentiation potential.

The project was supported by the research grant from the National Science Centre UMO-2015/17/B/NZ5/00294.

Modulation of microglial activation by CD200R activation in models of neurodegeneration

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The main role of microglia, which are the primary immune cells of the brain, is protective, and it is generally considered that acutely-activated microglia, that retain their ability to return to the resting state, are responsible for protection. On the contrary chronic activation of microglia is probably the primary cause of the neuroinflammatory changes are a feature of many, if not all neurodegenerative diseases. Therefore a significant challenge is to understand the factors that drive microglial activation and to identify strategies that can manipulate microglial activation. It is known that interaction of microglia with other cells plays a part in maintaining microglia in a quiescent state and this is achieved by ligand-receptor interactions that result in neuroimmune modulation. One of these ligand-receptor pairs is CD200-CD200R. Here evidence will be presented which support the view that this interaction is important in neuroprotection in a number of neurodegenerative conditions and in endothelin-1-induced ischaemia. The possibility that this interaction can be exploited to optimize the beneficial effects of mesenchymal stem cells in a model of stroke will be considered.

Pre-transplantation optimization of MSC function for enhancing their regenerative potential

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Nowadays, when the regenerative medicine enters the clinic, there is a need for establishing precise protocols for stem/progenitor cell preparation. The results of published trials are diverse and depend on the source of stem cells, as well as the method of cell isolation and propagation.

To optimize MSC isolation and propagation techniques that could increase their neurogenic potential, neuroprotective properties, extend their survival and slow down their aging we have compared: (i) properties of freshly isolated vs neuraly commited MSC, (ii) cells isolated via mechanical vs enzymatic method and (iii) MSC cultured in normoxic vs physioxic* oxygen conditions.

Our experiments showed that the strongest ability for neuroprotection was provided by freshly isolated cells and the first cohort of migrating MSC cells (passage O). Along further passaging the cells phenotype changed substantially and cell neuroprotective effect declined together with modification of paracrine capabilities of WJ-MSC-secreted cytokines. These results will be challenged with our previous data gathered in preclinical and clinical experimentations showing that undifferentiated, SRTF** expressing MSC, capable to time-locked proliferation, migration and ultimately to neural differentiation are the most effective in various therapeutic transplantation models.

Our results show significant differences between MSC populations obtained with two methods (mechanical vs. enzymatic) of isolation. Despite comparable level expression of typical mesenchymal markers (CD73, CD90, CD105, CD166, Vimentin, Collagen, Fibronectin), mechanically isolated cells were more stable in culture, with shorter Population Doubling Time, higher ability to CFU-F formation and lower number of the senescent cells. Moreover a significantly higher expression of neural/neuronal markers: Nestin, β Tubulin III, GFAP, NF-200 and primitive marker α-SMA was observed. The preferable method seems to be the mechanical one. The method of cell isolation may substantially affect cell properties, determining their neural differentiation ability and presumably the neuroprotective properties. Therefore the efficiency cannot be the main determinant to choose the method of isolation.

We have focused also on the mechanism of adult type stem cells phenotypic plasticity evoked by culturing MSC in physioxic O_2 conditions. Results strongly suggest that induction of the less differentiated, SRTF-expressing, pluripotent-like state of MSCs significantly increase they proliferation, epigenetic stability, survival, and capability of cells to differentiation into neural as well as endothelial directions.

To guarantee the high quality of the obtained cells, we should go beyond the framework of criteria developed by ISCT and focus on a number of other extremely important features which could help to select this particular cell population.

**Normoxic conditions – 21% oxygen concentration; physioxic conditions – 5% oxygen concentration*

***SRTF – Stemness-Related-Transcription-Factors (Oct4A, Nanog, Rex1, Sox2)*

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