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**Ischemic Stroke Induces Delayed Mesencephalic Astrocyte-Derived Neurotrophic Factor (MANF) Protein Expression in Brain Inflammatory Cells**

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Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an endoplasmic reticulum (ER)-resident protein important for ER homeostasis and protects neurons from various injuries. MANF is neuroprotective in ischemic injury, and post-stroke MANF treatment promotes recovery. In the normal brain MANF protein expression is neuronal, but expression after ischemia is poorly characterized. Utilizing immunohistochemistry, we studied how endogenous cerebral MANF expression evolves after focal cerebral ischemia in rats (post-stroke day (psd) 2, 7, 14, 28, 56, and 112), mice (psd14), and humans (psd1–2). Cortical infarct was induced with transient (90 min) distal middle cerebral artery occlusion (dMCAo) in rats and permanent dMCAo in mice. Gene-modified Nestin\(^{Cre/+:}\)::Manf\(^{fl/fl}\) mice were used to investigate post-stroke MANF expression after neuronal and astroglial MANF deletion. Specificity of the anti-MANF antibody was confirmed with pre-adsorption controls and MANF knockout tissue. In comparison to the contralateral hemisphere, MANF expression was markedly decreased in the infarct core at psd2 in rats and humans. However, MANF was strongly upregulated in the infarct core at psd7 in rats simultaneously with the phagocytic marker CD68. MANF was also upregulated in areas of secondary damage, i.e. the striatum and thalamus, starting from psd14, coinciding again with CD68 upregulation. Colocalization of MANF and CD68 was verified using confocal microscopy. In the Nestin\(^{Cre/+:}\)::Manf\(^{fl/fl}\) mice MANF expression was also induced in the infarct core, peri-infarct region, and the ipsilateral striatum and thalamus at psd14, verifying that the post-ischemic MANF upregulation was not neuronal nor astroglial. In conclusion, we are the first to show how endogenous MANF expression is temporally altered after cerebral ischemia and demonstrate that MANF is evidently expressed in phagocytic microglia/macrophages at later time points. We also provide the first human data on post-stroke MANF expression. Our findings provide important insight into how endogenous MANF may contribute to post-stroke recovery and the regenerative role of phagocytes, supporting further investigation into MANF-based therapeutic applications.

**Development and Characterization of hiPSC Cortical Neurons and their Application to Drug Evaluation in CNS Disease Models**

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The differentiation of functional cortical neurons from human induced pluripotent stem cells in vitro easily lends itself to a serum-free, drug-delivery platform advantageous for testing novel chemicals for safety and efficacy in disease treatment. Initially, cortical neuron cultures were characterized morphologically by phase microscopy and immunocytochemistry and functionally by patch-clamp electrophysiology. Specifically, the expression of neuronal markers and neuronal activity increased throughout maturation. On day 0 of maturation, 50% of the culture expressed layer V cortical neuron marker ctip2 and neuronal marker beta-III tubulin and displayed spontaneous and repetitive firing through whole-cell patch clamp. By day 28 of maturation, 90% of the culture expressed the aforementioned markers and displayed electrical activity. Subsequently, neurons were cultured on multi-electrode arrays (MEAs) to determine the effects of chemicals on neural circuit physiology for modeling brain disease phenotypes. In this system, we tested GABA\(_A\) receptor antagonists and agonists as chemical convulsants or anti-convulsants, respectively. GABA\(_A\) receptor antagonist administration enhanced...
spontaneous activity mimicking an epileptic phenotype, while GABA$_A$ receptor agonist administration quieted spontaneous activity. The versatility of this model lies in its ability to present an array of brain diseases characterized by functional brain deficits. Chemicals affecting receptor binding can be added to manipulate neuronal activity. This serum-free, hiPSC cortical neuron model establishes a platform for the evaluation of neuron activity as well as a platform for drug testing in vitro.

**Progerin Induced Aging of the Rat Nigrostriatal System**

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Aging is the greatest risk factor for development of Parkinson’s disease (PD). Yet, interrogating the biological intersection between aging and PD in rodents is challenging as these animals do not exhibit PD-like neurodegeneration even at advanced ages. It has been speculated that this may be attributable, in part, to their limited lifespan. We asked the question “if aging is accelerated in the nigrostriatal system of young adult rats, will PD-like degeneration of this system ensue?” To test this, we ectopically over-expressed progerin in the midbrain of rats using recombinant adeno-associated viral vectors (rAAV). Progerin is the protein responsible for the human genetic premature aging disorder Hutchinson–Gilford syndrome and is a mutant version of the filament protein Lamin A, a major component of the nuclear lamina. Expression of progerin induces many phenotypes, including abnormal nuclear shape, loss of heterochromatin, and increased DNA damage, leading to cellular senescence. In a proof-of-concept experiment we delivered rAAV-progerin or rAAV-mCherry (control vector) into the substantia nigra of young adult (3-month-old) rats. Ten weeks later, tissue was collected and processed for histological analysis. Immunostaining for progerin confirmed successful viral transduction and proper nuclear localization. Immunostaining for tyrosine hydroxylase revealed severe loss of midbrain dopamine neurons. Ongoing studies are aimed at further characterizing this model, including a progerin dose response, the analysis of nuclear morphology, alterations in epigenetic markers, quantification of DNA damage, the temporal course of neural degeneration, and selective vulnerability of neuronal populations. Our findings indicate that progerin overexpression can produce local acceleration of the aging process leading to neurodegeneration in young rats. Progerin expression may serve as a useful tool to manipulate aging in models of aging-related neurodegeneration in short-lived species.

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**Addressing Concerns of Neurological Chimeras in Human–Animal Blastocyst Complementation**

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Blastocyst complementation is an emerging methodology in which human stem cells are transferred into genetically engineered preimplantation animal embryos. The microinjected embryos are transferred to a surrogate eventually giving rise to fully developed human tissues and organs within the animal host for use in regenerative medicine. The ethical, legal, and social implications surrounding this method have caused the National Institutes of Health to issue a moratorium on funding that involves the injection of human pluripotent stem cells into preimplantation animal embryos. A noted concern over blastocyst complementation is the “creation of human–animal beings with partly or substantially human brains” and whether such chimeras possess “humanized” characteristics. To address this concern, we performed a review of the neural transplantation literature in which human cells have been transplanted into a nonhuman animal. Our aim was to determine how the integration of human cells into the nonhuman neural circuitry has altered the cytoarchitecture and/or behavior of the host. Despite reports of widespread integration of human cell transplants, our review of 150 transplantation studies found no evidence suggestive of humanization of the animal host. We conclude that, at present, concerns over humanization should not prevent research on blastocyst complementation to continue. Caveats of our study include the species and age of the animal in which human cells were transplanted. Of note for regenerative cell therapies for neurological disorders, neural precursors derived from the fetal brain are the most suitable for transplantation, therefore addressing the concerns of live-born chimeras that may express human behaviors. We suggest proceeding in a controlled and transparent manner, and have included recommendations for future research with careful consideration for how human cells may contribute to the animal host nervous system.

**Serotonergic Hyperinnervation in Non-Motor Circuits in the Parkinsonian Rat**

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Support: Gibby and Friends vs Parky fund at Michigan State University.
It is becoming increasingly clear that non-motor symptoms (NMS) in Parkinson’s disease (PD) comprise a significant portion of debilitating features of the disease, and such symptoms are often more debilitating than the motor symptoms themselves. Despite its initial effectiveness, chronic L-DOPA administration leads to motor side effects such as L-DOPA-induced dyskinesias (LID) in up to 90% of patients and NMS in as many as 60% of patients. Reports have revealed extensive modifications to serotonergic (5-HT) innervation of the striatum as dopamine (DA) cells are lost, and that these 5-HT terminals are crucial in the formation of LID. However, it is unclear what changes occur in other areas of the brain. To address this we utilized an AAV reporter virus to label the dorsal raphe (DR) 5-HT innervation in the hemiparkinsonian rat. 5-HT innervation from the DR was dramatically enhanced in areas such as the amygdala and prefrontal cortex, suggesting that changes in 5-HT circuitry may be responsible for NMS. In order to better understand the role of this circuitry in NMS we generated a projection-specific viral vector approach whereby specific DR 5-HT terminals are targeted using novel AAV vectors with retrograde transport capacity, and paired with viral vectors expressing regulatory elements specifically in the 5-HT soma. However, in order to achieve single circuitry precision we need to better understand individual projections. Thus, in ongoing experimentation we are using Multiplexed Analysis of Projections by Sequencing (MAPseq) in combination with AAV, DNA barcoding, and Next Generation Sequencing in order to map each individual 5-HT projection from the dorsal raphe. This approach will systematically determine how neuroadaptation in 5-HT innervation and function modulate DA neurotransmission in the parkinsonian brain, facilitating therapeutic targeting of such maladaptive changes to optimize NMF treatment and therefore quality of life for PD patients.

**Evaluation of DNA-Binding Domains to Selectively Reduce the Expression of Mutant Huntingtin in Patient-Derived Cells and Transgenic Mice**

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Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder characterized by the presence of a misfolded mutant Huntingtin (muHTT) protein. Reduction of muHTT is an attractive therapeutic approach; however, one must take into consideration the role of the normal, non-expanded version of Huntingtin. An ideal therapeutic approach would be able to selectively silence only the expanded allele, affect a large population of striatal neurons, and have a durable effect. We have previously shown allele-specific silencing of the muHTT transcript in patient-derived fibroblasts via transcription activator-like effectors (TALE) by targeting a single nucleotide polymorphisms (SNP) that is highly associated with the mutant allele. In this study we examine the use of an adeno-associated virus (AAV) as putative delivery vehicle for our therapeutic TALE transgene. AAV9-TALE was directly injected into the striatum of YAC128 at an early stage (9 months of age) of HD-like symptoms. Mice were then tested on a motor coordination task to evaluate functional recovery every 2 weeks until they were 12 months of age, at which point brains were analyzed via immunohistochemistry (IHC) for expression of the TALE and for molecular assessment for reduction of muHTT at the RNA and protein level. Presently, our data demonstrate a functional sparing, attenuation of striatal atrophy, and reduction of muHTT following injection of AAV9-TALE into the YAC128 transgenic HD mouse. Additionally, we have expanded this work to include a novel Cas9 variant (xCas9.3.7) that has broad PAM specificity. This novel construct was cloned into our iCas9 backbone to allow for the evaluation of various effector domains in both an independent and in combinational fashion. dxiCas9 was fused with either KRAB, DMNT3a, or both and guide RNAs were designed for previously identified SNPs in our patient cell lines (Fibroblasts GM02151, GM04281b, GM04287 and iPSC NN003930 and NN000003). Following nucleofection of both guide RNA and dxiCas9 constructs, significant reduction of huntingtin was observed. The ability of TALE or dxiCas9 to selectively reduce expression of mutant huntingtin carries significant therapeutic value.

**Temporal Activation of Paternal Ube3a in Angelman Syndrome Reporter Mouse Following Transplantation of Zinc Finger Secreted Mesenchymal Stem Cells**

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Angelman syndrome (AS) is a genetically inherited neurodevelopmental disorder characterized by impaired cognitive development, lack of speech, seizures, and motor ataxia. The genetic cause for AS is usually due to a de novo deletion of the maternal ubiquitin protein ligase E3a (UBE3a) gene in the chromosome 15q11-q13 region. Additionally, brain-specific postnatal imprinting of the intact paternal UBE3a gene results in complete loss of UBE3a in mature neurons due to the presence of a long antisense transcript driven by the neighboring small nuclear ribonucleoprotein polypeptide N (SNURF/SNRPN) promoter. Our group has previously shown re-activation of the paternally silent Ube3a gene in the brains of the E6-AP adult AS mouse following intraperitoneal (i.p.) injection of a Kruppel-associated box (KRAB)-fused Zinc Finger (referred to as S1 K) protein targeted toward the Snurf/Snrpn promoter—effectively silencing expression of the antisense transcript. As an alternative delivery method, we have engineered activating transcription factor (ATF)-secreting bone-derived mesenchymal stem cells following lentiviral reprogramming.

Presently, we have engineered mouse bone marrow-derived mesenchymal stem cells (BM-MSCs) to secrete S1 K as confirmed by uptake into Neuro2a cells with MSC-S1 K conditioned media via fluorescent microscopy. Incubation of MSC-S1 K conditioned media with E15.5 E6-AP: YFP AS mouse primary neurons demonstrated significant re-activation of YFP fused Ube3a as compared with a scramble zinc finger MSC (MSC-SR6) and non-transduced MSC (MSC-N) 48-hrs post treatment. We then bilaterally transplanted 250,000 MSCs from each treatment type into their respective treatment arms (MSC-S1 K, MSC-SR6, MSC-N) into 8-week old E6-AP: YFP mice. We observed significant re-activation of silent Ube3a via immunohistochemistry (IHC) and western blotting for YFP expression in the hippocampus, cerebellum, and cortex as compared to MSC-SR6 and MSC-N treatment groups 3 and 6 weeks post-transplantation. Currently, we report the first-in-its-kind use of MSCs as a delivery platform for genetic modifiers in disease.

**Conditioned Medium Derived from Endothelial Progenitor Cells Exerts Neuroprotection on Cultured Cortical and Midbrain Neuronal Stem Cells**

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There is compelling evidence that stem and progenitor cells secrete a wide array of trophic factors that could be exploited for repairing injured tissues. We have previously reported that the conditioned medium (CM) obtained from endothelial progenitor cell (EPC) cultures promotes angiogenesis and protects the striatal GABA immunoreactive (-ir) cell viability against 3-NP toxicity. In the present study, we tested the hypothesis that EPC-CM may support cortical and midbrain neuronal cell function and/or survival. EPCs were isolated from the peripheral blood of healthy human donors and cultured in hypoxic conditions (1.5% O$_2$) to stimulate the secretion of growth factors. Primary cultures from fetal rat embryonic (E14) cortex (CX) and ventral mesencephalon (VM) were treated with EPC-CM and challenged by glucose and serum deprivation (GSD) or MPP$^+$, respectively. First we found that EPC-CM treatment resulted in a significantly increased number of TH-ir cells in the VM as well as of GABA-ir cells in the CX cultures. EPC-CM administration exerted neuroprotection against GSD, an effect seen either when EPC-CM was administered prior or subsequent GSD incubation. Similarly, EPC-CM protected the dopaminergic neurons in the in vitro model of Parkinson’s disease. Our findings identified EPC-CM as a powerful tool to promote viability and/or differentiation of cultured neuronal cells. In conclusion our results suggest that EPC-CM might be useful to expand the repertoire of available tools to tackle neuronal degeneration.

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show a trend for 50 μg/day adropin decreasing aneurysm formation in female mice (adropin: 33% (2 of 6) vs. vehicle: 86% (6 of 7), p=0.10), with no effect on rupture rate (adropin: 100% (2 of 2) vs. vehicle: 66.7% (4 of 6), p=1.00). 5 μg/day had no effect on formation in either female (adropin: 40% (4 of 10) vs. vehicle: 77.7% (7 of 9), p=0.17) or male mice (adropin: 42.8% (3 of 7) vs. vehicle: 77.7% (7 of 9), p=0.30), and also had no effect on rupture rate in females (adropin: 100% (4 of 4) vs. vehicle: 85.7% (6 of 7), p=1.00) or males (adropin: 100% (3 of 3) vs. vehicle: 71.4% (5 of 7), p=1.00). More investigation is needed on various dosing regimens and the mechanisms through which adropin exerts its protective effects.

**Sural Nerve Grafting as a Cell-based Disease-modifying Therapy for Parkinson’s Disease**

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Parkinson’s disease (PD) is the second most common neurodegenerative disorder, with an annual incidence of 60,000 and costs of $25 billion in US. There is no current treatment that can slow or reverse PD. We investigate a new cell-based therapy to enhance the survival of the dying brain dopaminergic cells using Schwann cells (SC), which reside within sural nerve grafts. Combined research shows that SCs can transdifferentiate into neural repair cells mainly through c-Jun signaling. Our current clinical trials (NCT01833364 and NCT02369003) involve the implantation of the conditioned sural nerve tissue to the substantia nigra in combination with deep brain stimulation (DBS) for the treatment of advanced PD. The SC grafts are harvested from the sural nerve of participants undergoing DBS surgery. RNA sequencing of the conditioned grafts showed transcriptome changes consistent with the SC repair phenotype. Currently, 21 participants have completed the two-year follow-up and demonstrated a significant motor improvement using the MDS-UPDRS scoring system. Postmortem studies of one participant showed evidence for preservation of the dopaminergic cells in the substantia nigra in response to the graft. To further study the neurobiology of the SC grafts and how they may affect the host brain tissue, we grafted human sural nerve tissue into the brains of athymic nude rats, which we call “Neuro-Avatars.” Each animal received a unilateral graft into the dorsal striatum with a contralateral sham insertion. The brains were processed 2 weeks and 6 months post implantation. Immunostaining against human nuclear antigen showed a substantial survival of the grafted cells in addition to host responses to the grafts. Our future experiments will study the use of similar nerve grafts in a 6-hydroxydopamine rat model of PD. In conclusion, regenerative changes in the peripheral nerve tissue may hold therapeutic potential for the treatment of neurodegenerative diseases like PD.

**FKN Acts as a Double-Edged Sword**

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Inflammation is known to contribute to neurodegeneration in a number of neurological disorders including Alzheimer’s disease (AD), and represents a realistic and valuable therapeutic target. Neuroinflammation can be modulated by neuron–glial signaling through various soluble factors, such as CD200, CD22, CD47 and fractalkine (FKN, CX3CL1). For instance, loss of FKN signaling has been shown to contribute to increased neurodegeneration in Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS) and AD. We have also demonstrated that increasing the expression of a soluble FKN (sFKN) is neuroprotective in both PD and a tauopathy mouse model of neurodegeneration, which is consistent with our and others’ findings that increasing neuroinflammation in mouse models of PD and tau deposition can exacerbate pathology. However, a debate continues in the literature as to whether increased sFKN is a benefit to tau pathology, thus confounding the use of FKN as a therapeutic target for neurodegeneration. We present data that shed light on the above divergence of the use of FKN. We demonstrate that the commonly used chemokine domain-only peptide of FKN (ckFKN) has a 10 fold decrease in receptor binding affinity compared with sFKN, which is consistent with differences seen in calcium signaling between ckFKN and sFKN. More intriguing is that we have observed opposing effects with FKN concentration, where at low concentrations (1 nM) FKN reduces the microglial proinflammatory response, but at higher concentrations (>30 nM) FKN can begin to exacerbate the proinflammatory response. Inhibitor studies show that this proinflammatory signaling is likely through a noncanonical receptor not currently identified or reported for FKN. This is contrary to the current belief that FKN is an anti-inflammatory molecule that signals through its sole receptor CX3CR1. These data may clarify conflicts in the literature and demonstrate that care must be taken with respect to in vitro and in vivo studies using FKN, in turn emphasizing the underlying need for better understanding of the biology of FKN and its function.
**In Vivo Mapping of the Spatio-Temporal Invasion of Immune Cells into Extracellular Matrix Hydrogel in a Rat Model of Stroke Using 19F Magnetic Resonance Imaging**

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Hydrogels formed from extracellular matrix (ECM) have the potential to promote tissue restoration after a stroke. Infiltration of immune cells, especially macrophages, is a pivotal event to drive biodegradation that leads to the invasion of neural cells. However, spatio-temporal dynamics of the infiltration of immune cells into peri-infarct tissue and the ECM hydrogel remain poorly understood. The tagging of immune cells using perfluorocarbon (PFC) nanoemulsions affords their in vivo visualization using 19F magnetic resonance imaging (MRI). Rats with middle cerebral artery occlusion (MCAo), a model of ischemic stroke, were implanted with ECM hydrogel 14 days after infarction using MRI guidance to define the site and volume of injection. One day prior to the ECM implantation, animals were injected with PFC through the tail vein and baseline 19F MR image was acquired to verify the injection and potential passive influx to the stroke-damaged brain. However, no PFC influx to the brain was evident at this time point. “Time lapse” imaging over 24 hours indicated that no immune cells invaded the brain for 6 hours after ECM implantation, although a major infiltration in the scalp incision wound was evident within 1 hour after surgery. Infiltration of immune cells was first evident in the peri-infarct area at 9 hours post-implantation with subsequent invasion into the ECM hydrogel. By 24 hours a major invasion of immune cells into the ECM hydrogel was evident, as verified by immunohistochemistry in post-mortem tissue. Histological analyses revealed that almost all invading macrophages were labeled with PFC, indicating that these were peripheral macrophages rather than brain-derived microglia. ECM hydrogel implantation therefore induces a delayed invasion of macrophages into the stroke-damaged brain through the peri-infarct area that is governed by peripheral macrophages effecting the initial biodegeneration events that lead to brain tissue restoration.

**Potential Benefits of Environmental Enrichment in a Rodent Model of Tauopathy**

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Parkinson’s disease (PD) is a devastating neurodegenerative disease with progressive degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta. There are over 10 million people afflicted with PD and the yearly mortality rate is more than 100,000 worldwide. Unfortunately none of the available treatment options have the potential to restore the damaged nigrostriatal pathway. Cell-based therapies have shown considerable promise because they can achieve significant biochemical and clinical improvements for several years in some patients. We have demonstrated in preclinical PD models that transplantation of human parthenogenetic derived neural stem cells (ISC-hpNSC) promotes behavioral recovery and increases DA levels, as well as DA neuron innervation and number. Intra-nigrostriatal administration of clinical grade ISC-hpNSC is safe, well tolerated, reduces inflammation, and provides neurotrophic support and neuroregeneration to the nigrostriatal pathway. We are conducting a First-In-Human study to evaluate the safety and functional activity of ISC-hpNSC, making it the world’s first pluripotent stem cell-based therapy for PD (ClinicalTrials.gov: NCT02452723). This is a single-arm, open-label, Phase 1 study evaluating three dose regimens of 30, 50 and 70 million ISC-hpNSC. There are 12 patients in this study divided into 3 cohorts of 4 patients each. Patients receive immunosuppression and stereotactic bilateral injections of 7 cell deposits per hemisphere into the caudate nucleus, putamen, and substantia nigra. Patients are evaluated for 12 months with a 5-year long-term follow-up. The primary endpoint of the study is to assess the incidence of treatment-emergent adverse events. Secondary endpoints evaluate efficacy by measuring the change from baseline in 18F-dopa PET, UPDRS, PDQ-39, BDI, CGI, QUIP-RS, AIMS and MOCA. Eleven patients have been successfully transplanted with 30, 50 and 70 million ISC-hpNSC, respectively. Delivery of ISC-hpNSC to the striatum and substantia nigra went according to plan without intraoperative complications. No serious adverse events (SAE) associated with ISC-hpNSC or the immunosuppression regimen has been reported. No graft-induced dyskinesia or evidence of tumors, inflammation or infection has been reported. Six month analysis of 18F-dopa PET scans and neurological scores from the first and second cohort will be presented. In summary, interim data shows that administration of ISC-hpNSC is safe and has the potential to repair the nigrostriatal pathway.

**Phase I Clinical Trial Update: Human Neural Stem Cell Treatment for Parkinson’s Disease**

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The primary endpoint of the study is to assess the incidence of treatment-emergent adverse events. Secondary endpoints evaluate efficacy by measuring the change from baseline in 18F-dopa PET, UPDRS, PDQ-39, BDI, CGI, QUIP-RS, AIMS and MOCA. Eleven patients have been successfully transplanted with 30, 50 and 70 million ISC-hpNSC, respectively. Delivery of ISC-hpNSC to the striatum and substantia nigra went according to plan without intraoperative complications. No serious adverse events (SAE) associated with ISC-hpNSC or the immunosuppression regimen has been reported. No graft-induced dyskinesia or evidence of tumors, inflammation or infection has been reported. Six month analysis of 18F-dopa PET scans and neurological scores from the first and second cohort will be presented. In summary, interim data shows that administration of ISC-hpNSC is safe and has the potential to repair the nigrostriatal pathway.
An astounding 1 in 3 senior citizens die with Alzheimer’s disease (AD) and Alzheimer’s-related dementias, yet no reliable treatments for these disorders exist. AD in humans has multiple underlying molecular pathologies including beta-amyloid plaque formation and hyper-phosphorylation of microtubule-associated protein tau. Research in rats and humans suggests environmental enrichment (EE) preserves cognitive function during aging, but the molecular mechanisms are largely unknown. Results from our laboratory demonstrate that 1 month of EE results in an activity-dependent increase in phosphorylated p70S6 kinase (p-p70S6 K) for 30 minutes following synaptic stimulation in the hippocampus. This improves learning and memory in young and aged rats. p70S6 K is involved in protein synthesis important to learning, memory, and synaptic plasticity. Curiously, human AD brains show constitutive phosphorylation of p70S6 K, suggesting that p70S6 K is dysregulated in AD and may result in adverse effects on cognitive function. We predict EE will have a protective effect by regulating phosphorylation levels of p70S6 K in a rodent model of tauopathy. Here, we delivered wild-type human tau using adeno-associated virus (AAV-Tau4 R) to the hippocampus of adult rats. We found a 1.3-fold increase in hippocampal tau levels 15 days following viral vector injection and a 3.4-fold increase in basal levels of p70S6 K. To test the effects of EE in alleviating the p-p70S6 K dysregulation in this model, we will house animals in EE or standard conditions (SC) for 1 month prior to AAV injection. Fifteen days post-injection, we will perform behavioral testing, and quantify basal and synaptic activity-dependent p-p70S6 K levels. We hypothesize that EE AAV-Tau4R-injected animals will show normal basal and increased activity-dependent p-p70S6 K levels, and improved learning compared to SC injected animals. The results from this study will provide knowledge about the therapeutic value of EE in regulating molecular pathways contributing to age-related disorders.

Exosomal Biomarkers in Brain Injury and Disease

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Individuals with Down syndrome (DS) exhibit Alzheimer’s disease (AD) neuropathology and dementia early in life, with almost complete penetrance. Reliable blood biomarkers are lacking and could improve early diagnosis and confirm effects of experimental therapeutics. Extracellular vesicles (EVs) including exosomes are nanosized particles that are secreted by all cell types and are present in all bodily fluids. Exosomes contain bioactive molecules including proteins, mRNA, miRNA, and lipids that can be used for signaling and transportation across the neuraxis and between individuals. We have examined the presence of AD-related biomarkers in the cargo of exosomes derived from neurons obtained from blood samples of people with DS at different ages, and compared with age-matched controls and patients with AD. Similar biomarkers have also been examined in exosomes from athletes with one or several concussions, to determine whether repeated mild traumatic brain injuries (mTBIs) lead to elevated brain injury biomarkers long term or at different intervals post-concussion. Further, we have demonstrated that exosomes derived from patients with DS and AD can develop tangles containing hyperphosphorylated Tau (p-Tau) when injected into the hippocampus of wild-type mice. These findings suggest that neuron-derived exosomes can be developed into a novel and reliable biomarker method, both for mTBI and AD, and that AD pathology can spread from exosomes into the brain between individuals following injection. Thus, the novel exosome methodology can provide new answers as to how AD pathology can spread between adjacent brain regions.

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Reparative Effects of Stem Cell Factor and Granulocyte Colony-Stimulating Factor in Aged APP/PS1 Mice

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Alzheimer’s disease (AD) is the most common cause of dementia among the elderly. Increased amyloid-beta (Aβ) aggregation and neuroinflammation are crucially involved in the pathogenesis of AD. Microglia, the primary innate immune cells of the brain, play a key role in Aβ clearance and neuroinflammation. Our previous studies have demonstrated that administration of two hematopoietic growth factors, stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF), in 9-month-old male amyloid precursor protein/presenilin 1 (APP/PS1) mice leads to reductions of cerebral Aβ aggregation. To determine the
The therapeutic efficacy of SCF+G-CSF in aged APP/PS1 mice, in this study, 12-day injections of SCF+G-CSF were given to 25-month-old male APP/PS1 mice. Our data revealed that the percentage area, the number, and the size of Aβ plaques in both the cortex and hippocampus were significantly reduced by SCF+G-CSF treatment. The capillaries with Aβ deposits were also significantly decreased in the cortex and hippocampus after SCF+G-CSF treatment. In addition, the association of triggering receptor expressed on myeloid cells 2/ionized calcium-binding adaptor molecule 1 (TREM2+/Iba-1+) microglia with Aβ plaques was significantly increased by SCF+G-CSF treatment. Importantly, increased P2RY12 positive microglia were seen in both the cortex and hippocampus of the SCF+G-CSF-treated APP/PS1 mice. SCF+G-CSF treatment also led to significant increases of the branches in the purinergic receptor P2Y, G-protein coupled, 12 (P2RY12)+ microglia. The decreased pro-inflammatory molecule nitric oxide synthase 2 (NOS2) and increased anti-inflammatory molecule interleukin 4 (IL4) were observed in the SCF+G-CSF-treated APP/PS1 mice. Moreover, SCF+G-CSF treatment prevented the loss of microtubule-associated protein 2 (MAP2)+ dendrites in the cortex and hippocampus of the aged APP/PS1 mice. These findings suggest that SCF+G-CSF treatment in aged APP/PS1 mice increases Aβ clearance, reduces neuroinflammation and prevents dendritic degeneration. The beneficial effects of SCF+G-CSF in aged APP/PS1 mice are associated with the modulation of microglial activation. This study sheds new light on the therapeutic potential of SCF+G-CSF in AD.

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The Contribution of Stem Cell Factor and Granulocyte Colony-Stimulating Factor in Reducing Neurodegeneration and Promoting Neural Network Reorganization after Traumatic Brain Injury

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Traumatic brain injury (TBI) is a major cause of death and disability in young adults worldwide. TBI-induced long-term cognitive deficits represent a growing clinical problem. The aim of this study was to determine the effects of stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF) on long-term neurological outcomes, neurodegeneration, and neural network reorganization after TBI. The controlled cortical impact (CCI) model of TBI, which severely damages the motor cortex, was used in this study. TBI was performed in 8-week-old male C57BL mice. The combination treatment of SCF and G-CSF (SCF+G-CSF) was initiated 3 weeks after induction of TBI. Neurobehavioral tests were performed for examination of neurological deficits before treatment as well as 2 and 6 weeks after treatment. Spatial learning and memory was evaluated through the Morris Water Maze test, and the Rotarod test was used for examining motor function. Hematoxylin and eosin (H&E) staining was utilized to calculate the tissue loss volume. Neurodegeneration was determined by Fluoro-Jade C Staining. Immunohistochemistry of SMI-312 was performed to assess the axons. We found that SCF+G-CSF treatment significantly reduced latency to platform (escape latency) in Morris Water Maze at 6 weeks post-treatment. Two weeks after treatment, TBI-vehicle-treated mice showed significant increases of escape latency as compared with sham controls, while the escape latency of SCF+G-CSF-treated TBI mice did not show differences from the sham controls. Before treatment, there were no differences in neurobehavioral tests between the two groups of TBI mice that would be treated with/without SCF+G-CSF. SCF+G-CSF treatment did not change the performance of Rotarod and the tissue loss volume after TBI. However, TBI-induced neurodegeneration in the contralateral cortex was significantly reduced by SCF+G-CSF. In addition, TBI-induced abnormal increases of SMI-312 positive axons in the ipsilateral cortex adjacent to the TBI cavity were prevented by SCF+G-CSF treatment. These data suggest that SCF+G-CSF treatment in the late subacute phase of TBI improves cognitive function, reduces neurodegeneration, and prevents post-TBI overgrowth of cortical axons.

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(-)-Phenserine Ameliorates Contusion Volume Loss, Neuroinflammation, and Behavioral Impairments Induced by Traumatic Brain Injury in Mouse

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Traumatic brain injury (TBI) is one of the major causes of death and disability and affects an estimated 10 million
people worldwide. Currently, there is no approved drug for ameliorating the pathological processes of TBI. Previous studies have shown that (-)-phenserine (phen), an acetylcholinesterase inhibitor originally designed as a candidate drug for Alzheimer’s disease (AD), was tested in clinical Phase II studies and showed no adverse effects. Besides its anti-amyloid activity in AD, our previous data also showed phen can prevent several neurodegenerative mechanisms as well as reduce the cognitive impairments induced by mild TBI using a weight-drop mouse model. In this study, we used a mouse model of moderate to severe TBI induced by controlled cortical impact to assess the effects of phenserine on somatosensory functions. Animals were treated with phen (2.5 mg/kg, BID) by intraperitoneal injection for 5 days started from injury day at a clinically translatable dose and the effects were evaluated by behavioral and histological examinations at 1 and 2 weeks post-injury. Phen significantly attenuated TBI-induced contusion volume, enlargement of the lateral ventricle, and behavioral impairments in sensorimotor functions. The morphology of microglia was shifted to an active form from a resting form after TBI, and phen dramatically mitigated the population ratio of activated to resting microglia, suggesting that phen also mitigates neuroinflammation following TBI. Taken together, these results show that post-injury treatment with phen over 5 days significantly reduced the lesion volume and the enlargement of the lateral ventricle caused by TBI. In addition, phen effectively reduced sensory and motor deficits at 7–14 days post-injury. These data suggest a potential development of this compound for clinical use in TBI therapy.

Direct Conversion of Astrocytes to Neurons Enhances Neuronal Repair and Functional Recovery after Ischemic Stroke

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Regenerative therapies, including cell transplantation, have been extensively investigated in recent years for the treatment of central nervous system (CNS) injuries, such as stroke. In the present investigation, we explored an innovative stroke treatment of reprogramming endogenous astrocytes into induced neurons (iNeurons) for neuronal repair after focal ischemic stroke. A mCherry-tagged NeuroD1 lentivirus was generated under a mouse glial fibrillary acidic protein (GFAP) promoter for in vitro and in vivo infections. Cultured astrocytes expressing mCherry/NeuroD1 adopted neuronal morphology and expressed neuronal markers TuJ1, NeuN, and synaptic proteins 2–4 weeks after infection. Proliferation was drastically reduced compared with control cultures. Converted cells were found to have developed significantly longer processes (>20–100 mM) when inspected at 6 weeks after infection. A focal ischemic stroke of the right sensorimotor cortex was induced in adult GFAP-Cre x Rosa-yellow fluorescent protein (YFP) mice. Astrocytes from this mouse remained YFP positive regardless of cell phenotype. The lentivirus containing mCherry-NeuroD1 and the GFAP promoter was injected into the peri-infection region 3 days after stroke, which transduced ~10% of reactive astrocytes accumulated in the peri-infarct region. Six weeks later, converted cells were identified by the colabeling of YFP (the astrocyte origin marker), mCherry (the marker for NeuroD1 transduction), and NeuN (mature neuron marker). Around ~60% of converted cells expressed NeuN and showed neuronal morphology. Western blot assay of the peri-infarcted region detected significantly higher levels of brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 10 (FGF10) in NeuroD1-transfected mice compared with animals that received empty vectors. Meanwhile, the area of gliosis noticeably decreased. Stroke mice that received NeuroD1 transfection performed significantly better in the rotarod test and corner test, and a preventive effect was observed with regard to chronically developed depressive behavior 4 months after stroke. Thus, astrocyte-to-neuron reprogramming enforces parenchymal neurogenesis and helps to improve functional recovery after stroke.

Active Immunization with Tau Epitope in a Mouse Model of Tauopathy Induced Strong Antibody Response Together with Improvement in Short Memory and Tau Pathology

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Abnormal tau hyperphosphorylation and its accumulation into neurofibrillary tangles are a hallmark of tauopathies, which are neurodegenerative disorders that include Alzheimer’s disease (AD). Tau immunotherapy has therefore been proposed as a new therapeutic approach to AD. The aim of this study was to test if active immunotherapy with a highly immunogenic tau epitope in a mouse model of tau deposition was capable of reducing levels of tau pathology in the brain and improving cognition. Tg4510r mice, carrying the human four-repeat tau with the P301 L mutation (4R0 N tauP301 L) and the CamK-II tetracycline-controlled transactivator protein were used. Male and female transgenic rTg4510 mice (3 months old; n=36) were subdivided into 3 groups (n=12 per group) and received intramuscular injections of tau vaccine,
Abeta vaccine, or adjuvant only. Non-transgenic and tet only littermates (tetracycline-controlled transactivator protein expressing mice) were used as control groups for behavioral testing and anatomy comparisons. All groups received three injections in alternating weeks and were boosted an additional three times (4 weeks apart) for a total of 7 injections. Mice were subjected to behavioral testing including open field, Y maze, radial arm water maze and novel object recognition by an observer blind to the treatment/genotype of the mice in order to evaluate learning, memory, and general activity. Active immunization induced strong humoral immune responses in both non-transgenic and transgenic mice. Mice vaccinated with the tau epitope displayed an improvement in short-term memory when compared with adjuvant- and Abeta-treated mice during novel object recognition test. Hyperphosphorylated tau but not total tau was also reduced in the cortex of tau-vaccinated mice compared with Abeta-treated mice. Altogether, these data indicate that active immunotherapy with tau epitope was effective in improving cognition and reducing pathology in a mouse model of tau deposition.

**Human Embryonic Retinal Pigment Epithelial Cell (hRPEC) Grafts Provide Immunomodulation of the Host Microenvironment through Secreted Cytokines**

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Human embryonic retinal pigment epithelial cell (hRPEC) xenotransplantation in animal models of Parkinson’s disease (PD) and high-dose cocaine self-administration have been shown to produce behavioral improvements without the need for immunosuppression. In these experiments, hRPEC xenografts did not evoke an inflammatory response that is seen with other central nervous system (CNS) xenografting paradigms. hRPEC graft survival was excellent without any inflammatory responses in the dorsal striatum or nucleus accumbens. To understand the mechanism through which the hRPEC xenografts provide immunomodulation and avoid deleterious inflammatory responses, we tested the effects of secreted cytokines from hRPECs on activated microglia and T lymphocytes using hRPEC-conditioned medium (hRPEC-CM). Murine microglial cells prepared from postnatal day 0–2 rats and cultured in vitro were activated using lipopolysaccharide (LPS)/interferon-γ (IFN-γ), and were exposed to hRPEC-CM. IFNγ/LPS activation of microglial cells incubated in fresh media resulted in a robust increase in expression of all the three cytokines tested, when compared with the cells, which were not activated (p<0.001). Expression of mRNA for interleukin-1β (IL-1β) was increased 5.3 fold, tumor necrosis factor-α (TNFα) 2.5 fold and IL-6 6.3 fold as a result of IFNγ/LPS activation. In the IFNγ/LPS activated cells, expression of all three cytokines were significantly reduced after incubation with hRPEC-CM when compared with the cells incubated with fresh media. IL-1β expression was reduced 2.8 fold (p<0.019), while interleukin-6 (IL-6) expression was decreased 2.6 fold (p<0.001) and TNF-α expression was reduced 1.6 fold (p<0.001). Immunomodulatory effects of hRPEC-CM and hRPEC monolayer on activated human T-cell proliferation were also assessed in vitro using a tritiated thymidine incorporation assay, which showed substantial suppression of T-cell proliferation by 30% with conditioned medium, and when incubated with RPE monolayer the proliferation was suppressed by 80%. These results show that hRPEC xenografts make the graft microenvironment more receptive, allowing for its effective use without the need for continuous life-long immunosuppression.

**Unilateral Optogenetic or Chemogenetic Inhibition of the Nigrostriatal Pathway Causes Reversible Hemiparkinsonism that is not Associated with Spontaneous or Levodopa-Induced Dyskinesias**

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Levodopa (L-dopa) provides effective relief in Parkinson’s disease (PD) but in the long term causes levodopa-induced dyskinesias (LID). The loss of continuous dopaminergic stimulation (CDS) hypothesis would predict that hemiparkinsonian (HP) patients and HP animals would develop unilateral LID. However, our studies show that HP rats and monkeys with preserved interhemispheric nigrostriatal fibers do not develop LID. This suggests that loss of interhemispheric nigrostriatal connections may be critical for the genesis of LID. We tested the hypothesis that unilateral nigrostriatal will lead to reversible HP that will not be associated with dyskinesias. We used recombinant viral vectors to conditionally express eNpHR3.0 or hM4Di to cause unilateral inhibition of the left nigrostriatal pathway. In model #1, Sprague Dawley rats and in model #2 transgenic TH-Cre rats were used. In both models, recombinant virus AAV5-Ef1-a-DIO-eNpHR3.0-EYFP or AAV8-hSyn-DIO-hM4Di-mCherry was injected into the left substantia nigra pars compacta (SNpc). A cre-
mediated switch, AAV2-Ef1a-mCherry-IREs-WGA-Cre, was injected unilaterally into the left striatum of model #1 animals. Animals were repeatedly tested for onset and reversal of right HP (RHP) state. The eNpHR3.0-treated animals served as controls for the hM4Di-treated animals and vice versa. In the chemogenetic cohorts, animals were tested using both Clozapine-n-oxide (CNO) and a novel high-affinity ligand JH37160. With both compounds, animals showed a mean 75% reduction of the vibrissae-evoked forelimb placement score (VEFP, p<0.05). Additionally, the optogenetic cohort also showed a similar 75% reduction in their VEFP score. Other parkinsonian behavioral tests were also similarly effective to document reversible HP states. In both models, RHP was completely reversible with no residual parkinsonism or adverse events and histological verification of unilateral gene expression. Repeated induction of RHP or hRHP with L-dopa treatments failed to elicit dyskinesias. These findings support the notion that preservation of interhemispheric nigrostriatal fibers and dopaminergic synapses will mitigate/prevent LID in PD.

**Intravenously Administered Human Neural Stem Cell-Derived Extracellular Vesicles Ameliorate Cranial Radiation-Induced Brain Injury**


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Cognitive deficits following clinical radiation therapy for central nervous system tumors are progressive and debilitating to surviving cancer patients, causing a deterioration of quality of life. Using an immunocompromised rat model, we previously demonstrated that cranial engraftment of human neural stem cell (hNSC)-derived extracellular vesicles (EV) ameliorated radiation-induced cognitive dysfunction, neuroinflammation, and neuron structure damage. Building on those observations, we showed that those same beneficial effects of EV treatment are conferred to cranially irradiated (IRR) immunocompetent rodents without the need for immunosuppression or risk of teratoma formation. Furthermore, toward advancement to a clinically translatable application, we demonstrated that injection of EV into the retro-orbital venous sinus (IV injection) represents an effective and significantly less invasive EV delivery strategy as compared with the previously used intra-hippocampal route of injection. Behavioral analyses of these IV injected animals, 1 and 6 months later, demonstrated that the EV were effective in the rescue of IRR-induced cognitive deficits. We also observed significantly reduced microglial activation and synaptic protein loss. In addition, RNA-seq data from the mouse hippocampi uncovered specific neuronal signaling, synaptic and microglial genes, and pathways altered in the irradiated group and rescued in the treatment groups. The miRNA microarray of hNSC-derived EV was integrated with the RNA-seq data to suggest a role for miR-124 in the mechanism of EV-mediated remediation of the irradiated brain. Collectively, these studies represent a key advancement in developing an evidence-based clinical treatment strategy that will ease the neurocognitive side effects of cranial IRR.

**Gutting the Parkinson’s Brain of Inflammation: Human Umbilical Cord Blood Stem Cells and Plasma Target Gut–Brain Axis**

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Current therapies for Parkinson’s disease (PD), including both L-3,4-dihydroxyphenylalanine (L-DOPA), and clinical trials investigating dopaminergic cell transplants, have generated mixed results with the eventual induction of dyskinetic side effects. Although human umbilical cord blood (hUCB) stem/progenitor cells present no or minimal capacity of differentiation into mature dopaminergic neurons, their transplantation significantly attenuates parkinsonian symptoms likely via bystander effects, specifically stem cell graft-mediated secretion of growth factors, anti-inflammatory cytokines, or synaptic function, altogether promoting brain repair. Recognizing this non-cell replacement mechanism, we examined here the effects of intravenously transplanting a combination of hUCB and plasma (from the same cord blood unit) into the 6-hydroxydopamine (6-OHDA)-induced rat model of PD. Animals received repeated dosing of either 4×10⁶ hUCB cells with plasma or vehicle at 3, 5, and 10 days after stereotactic 6-OHDA lesion, then behaviorally and immunohistochemically evaluated over 56 days post-lesion. Compared with vehicle treatment, transplantation with hUCB and plasma significantly improved motor function, gut motility, and survival of dopaminergic neurons in the substantia nigra pars compacta (SNpc), which coincided with reduced pro-inflammatory cytokines in both the SNpc and the intestinal mucosa and dampened inflammation-associated gut microbiota. These novel data directly implicate a key pathological crosstalk between the gut and brain, ushering a new avenue of therapeutically targeting the gut microbiome with hUCB-derived stem cells and plasma for PD.
Disclosure: This study was supported by Saneron CCEL Therapeutics Inc. J.E. is the Director of Research and Development for Saneron. C.V.B. is a consultant for, and P.R.S is a cofounder of Saneron. P.R.S. is also the Co-Editor-Chief for Cell Transplantation. Neither P.R.S. nor any of the other authors took part in the editorial processing of this Abstract.

**Structural and Functional MRI as Biomarkers for Repair Processes Following Stem Cells Therapy of White Matter Stroke**

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Subcortical white matter stroke (WMS) constitutes up to 30% of all stroke subtypes and consists of a range of pathophysiological events, from small infarcts to more diffuse areas of damage. There is currently no specific therapy for WMS, either to prevent disease progression, or to improve the brain’s ability to recover from this insult. The use of stem cells is an emerging therapy for neural repair in WMS. Skin fibroblast-derived induced pluripotent stem cells (iPSCs) can be differentiated toward glial enriched progenitors (GEPs) and may be used to enhance endogenous repair mechanisms. iPSC-GEPs are suited for brain repair because they differentiate into immature astrocytes, the most affected neuroglial cell population after WMS. Previous pre-clinical studies have shown that iPSC-GEPs both replace cells lost in WMS and induce surviving cells to repair damaged axons. Stem cell therapy in WMS is ideally suited for the development of a non-invasive biomarker of tissue repair. WMS regions are currently imaged as hyperintensity on T2 magnetic resonance imaging (MRI), or altered diffusivity using diffusion weighted MRI. However, there are currently no in vivo imaging biomarkers of the brain repair process. Therefore, in this study, we used a recently developed mouse model of WMS to establish a quantitative measure of WM structure in vivo using diffusion tensor imaging metrics (FA, AD, RD and MD) throughout the repair processes that is produced by iPSC-GEPs therapy. Additionally, we explored the use of resting state functional MRI as a biomarker to provide a readout of functional enhancement in the cortex during the brain repair process. The development of a biomarker of iPSC-GEPs repair in WMS will substantially accelerate clinical application of this iPS therapy, by enabling clinical trials to be conducted with smaller sample sizes and shorter durations compared with current standards that use cognitive outcome measures.

**Human iPS-Derived Interneurons Enhance Functional Recovery after Cortical Stroke**

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Stroke is the leading cause of adult disability. There are no therapies that promote recovery from this disorder. Cell transplantation in cortical stroke has utilized neural progenitors in early differentiation stages; however, these have often shown poor survival, migration, and differentiation after transplant. The present studies develop a different paradigm of cell transplantation in cortical stroke by using induced pluripotent stem cell-derived (iPS)-interneurons. Interneuron transplantation for diseases other than stroke shows that these cells survive in hostile brain environments, migrate widely within the adult central nervous system (CNS), and integrate into the adult brain circuitry. iPS-interneurons were produced using small molecule inhibitors of Wnt, transforming growth factor-β (TGF-β), and bone morphogenetic protein (BMP), and are therefore termed iPS-3i. The iPS-3i cells progress through a molecular expression pattern resembling median ganglionic eminence (MGE) cells. iPS-interneurons were transplanted into the stroke cavity with a hyaluronan (HA) hydrogel containing clustered vascular endothelial growth factor (VEGF) immobilized on heparin nanocapsules to reduce transplant stress from the direct transplantation into the stroke cavity. Transplants were done at either the subacute or chronic stage after cortical stroke (7 days vs. 1 month after stroke). In order to assess motor deficit and recovery after stroke, gridwalking and pasta-handling tasks were performed. We report significant behavioral recovery in mice suffering from motor cortex stroke when the HA-hydrogel+ VEGF nanoparticles, and iPS-3i were injected simultaneously into the stroke core. Confocal images of ipsilateral tissue show concurrent structural changes while electrophysiological recordings demonstrate integration of iPS-3i into pre-existing circuitry. Notably, these results are seen when treatment is administered in either the subacute or chronic stage of stroke. Overall findings provide promising evidence of a stem cell treatment for stroke unique to the subtype iPS-3i.

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Neural Stem Cell Function During Aging: Critical Periods, Regulation, Therapeutic Opportunities

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Understanding neural stem cell (NSC) biology in the context of aging has significant implications toward developing therapeutics for age-related neurodegenerative disorders. As aging progresses, it is known that the regeneration of NSCs in the two major neurogenic niches—the subventricular zone (SVZ) in the forebrain and the subgranular zone (SGZ) of the hippocampus—undergoes a notable decline. Our recent work indicates that while NSC function, in both the SVZ and the SGZ, continuously decreases with advancing age, there is a critical time period during middle-age (13–15 mos) when a striking reduction in NSC survival and regeneration occurs (Corenblum et al, 2016; Ray et al, 2018). These studies also determined the reduced expression of the redox transcription factor, nuclear factor (erythroid derived 2)-like 2 (Nrf2), as key in mediating this phenomenon. Therefore, we investigated whether increasing Nrf2 expression could potentially mitigate the decline in NSC regeneration across the identified critical period. Specifically, recombinant adeno-associated viral (rAAV2/1) vectors encoding Nrf2 were administered into the SVZs of aging rats, at timepoints either before or after the critical period. Results indicate that animals treated with rAAV2/1-Nrf2, before the critical middle-age period (at 12 mos), showed greater NSC proliferation, neurogenesis, and migration, and associated olfactory discrimination function, compared with animals receiving control rAAV2/1-eGFP viruses. On the other hand, Nrf2 overexpression after the critical period (at 21 mos) did not significantly alter NSC activity at either cellular or behavioral levels. Similarly, enrichment of the hippocampus via the transplantation of rAAV2/1-Nrf2 overexpressing NSCs before the critical period alleviated the age-related decline in SGZ NSC regeneration and improved cognitive function (pattern separation abilities). Overall, these data highlight the importance of redox mechanisms in controlling NSC regeneration and support targeting the Nrf2 pathway as a potential approach to advantageously modulate NSC activity with age.

Brain Dysfunction in a Model of Gulf War Illness Continues into Middle Age with Elevated Oxidative Stress and Waned Mitochondrial Activity

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Gulf War Illness (GWI) affects ~40% of military personnel who served in the first Gulf War (GW). Multiple studies have suggested that concomitant exposure to GW-related chemicals and stress underlie this illness. The chemicals include the nerve gas prophylactic drug pyridostigmine bromide (PB), insect repellent DEET, and the insecticide permethrin (PM). Our previous studies have suggested that cognitive and mood impairments in GWI rats were associated with increased oxidative stress and mitochondrial hyperactivity in the brain. Here, we examined the progression of brain dysfunction into middle age in GWI rats. Male Sprague-Dawley rats were exposed daily to GW-related chemicals, PB (2 mg/kg), DEET (60 mg/kg) and PM (0.2 mg/kg), and 15 minutes of restraint stress for 28 days. Five months later, a series of behavioral tests revealed cognitive and mood dysfunction in GWI rats. The brain tissue demonstrated elevated levels of oxidative stress markers in the cerebral cortex and reduced concentration of antioxidants. Moreover, the hippocampus displayed increased expression of genes encoding proteins linked to mitochondrial respiration, with increased levels of mitochondrial complex proteins, which implied the presence of hyperactive mitochondria. Analyses of GWI rats 12 months after exposure revealed the persistence of cognitive and mood dysfunction in GWI rats. The brain tissue demonstrated elevated levels of oxidative stress markers in the cerebral cortex and reduced concentration of antioxidants. Moreover, the hippocampus displayed increased expression of genes encoding proteins related to mitochondrial respiration and reduced activity of mitochondrial complex I. Thus, cognitive and mood impairments in GWI continue into middle age with persistently increased oxidative stress and hypoactive mitochondria. It appears that mitochondria initially react to elevated oxidative stress by enhancing their activity. However, such a compensatory response leads to the production of higher levels of reactive oxygen species (ROS), which likely damages the mitochondrial respiratory chain and leads to hypoactivity.
Transplantation of Mesenchymal Stem Cells Genetically Engineered to Overexpress Interleukin-10 Induces Autophagy, Mitophagy, Molecular Chaperone Response, and Protected Neuronal Damage in a Rat Model of Traumatic Brain Injury

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Autophagy plays vital role in neuronal death and survival in traumatic brain injury (TBI). Transplantation of bone marrow-derived mesenchymal stem cells (BM-MSCs) has been shown to increase autophagy and provide neuroprotection in animal models of neurological diseases by increasing anti-inflammatory cytokines, such as interleukin-10 (IL-10). However, due to heterogeneity of MSCs, it is difficult to measure the levels of IL-10 released by MSCs. We developed genetically engineered MSCs to overexpress IL-10 in order to deliver a greater amount of IL-10 to the injured brain area. The present study investigated the mechanisms of neuronal death and survival, along with autophagy and mitophagy markers using a rat model of TBI after transplantation of BM-MSCs-IL-10. Adult male Sprague-Dawley rats were divided into four groups: Sham + Hank’s balanced salt solution (HBSS), TBI + HBSS, TBI + MSC-GFP (n=9/group). Sham + HBSS, and TBI + HBSS received HBSS, whereas TBI + MSC-GFP-IL-10 and TBI + MSC-GFP were transplanted with MSC-IL-10 or MSC-GFP, respectively, 36 h after TBI. We investigated the neuronal changes, using Cresyl violet, Fluoro jade B, and TUNEL staining. In addition, macroautophagy, mitophagy, and chaperone-mediated autophagy (CMA) markers, along with levels of molecular chaperones were analyzed after 3 weeks of TBI and transplantation. Furthermore, cell survival markers, synaptic markers, and neuroinflammatory markers (glial fibrillary acidic protein (GFAP), ionized calcium-binding adapter molecule-1 (Iba-1)) were also investigated. We observed a significantly increased number of pyknotic, degenerated, and TUNEL-positive cells in TBI rats in the cortex, and in the CA1 and CA3 subfields of the hippocampus in comparison to sham controls. Whereas transplantation of BM-MSCs-IL-10 significantly reduced cell death in all of these brain areas, along with increased autophagy, mitophagy, CMA markers, and molecular chaperones levels. Furthermore, cell survival and synaptic markers were restored and neuroinflammatory markers were decreased by BM-MSC-IL-10 transplantation. Overall, our data suggest that induction of the autophagy mechanism using BM-MSC-IL-10 may be used to protect against TBI-induced cell death.

Association of Hyperacute Blood Pressure Parameters with Baseline Infarct Volume and Collateral Status of Patients with Anterior Circulation Large Vessel Occlusion undergoing Endovascular Therapy

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Blood pressure (BP) mean and variability (BPV) have been previously implicated in clinical outcomes of large vessel occlusion (LVO) stroke due to unclear mechanisms. Here we tested whether hyperacute BP parameters are associated with baseline ischemic core volume and collateral status as stroke outcomes.

In a single-center retrospective analysis of 94 patients with middle cerebral artery (MCA) or intracranial internal carotid artery (ICA) occlusion treated with endovascular treatment (ET) within 24 h of stroke onset, systolic (SBP), diastolic (DBP), standard deviation (SD), coefficient of variation (CV), and successive variation (SV) were measured from door to recanalization time. Associations between BP parameters with baseline infarct core volume (RAPID software) and CTA collateral status (Miteff’s score) were tested in univariate and multivariate analysis adjusting for age and National Institutes of Health Stroke Scale (NIHSS).

The mean age of patients was 71 ± 15, median NIHSS was 17 (IQR, 13–21), 60% were female and the mean ischemic core volume was 18.6 ± 28.8 ml. The median number of pre-recanalization BP measurements per patient was 17 (IQR, 13–24). Among 74 patients with collateral assessment 15% had excellent collaterals. Mean SBP (156 ± 23 mmHg) and DBP (79 ± 13 mmHg) were not significantly correlated with baseline core volume. Mean SBP CV
(9.7 ± 5.9) had modest correlation with ischemic core volume (p = 0.215; P = 0.037), which was not statistically significant in multivariable analysis. Patients with good collaterals had higher mean SBP (168 ± 24 vs. 154 ± 22; P = 0.039), DBP (84 ± 9 vs. 77 ± 12; P = 0.025) and minimum SBP (147 ± 8 vs. 128 ± 3; P = 0.012). Mean SBP (OR 1.04, 95% CI 1.00–1.07; P = 0.047) and minimum SBP (OR 1.05, 95% CI 1.00–1.07; P = 0.01) were independently associated with better collaterals. BPV measures were not significantly correlated with collateral status.

Increased BP levels are associated with better baseline collateral status, but not ischemic core volumes. No significant association was found between hyperacute BPV and baseline ischemic core or collateral status. Permissive hypertension may increase collateral capacity in LVO stroke patients.

**T-Cell Phenotype in Murine Osteopontin-Mediated Aneurysm Healing**

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Cerebral aneurysms (CA), or balloon-like weaknesses in blood vessels supplying the brain, occur in up to 16 million individuals or 5% of the US population. Though safer than open surgery and clipping, treatment by endovascular coiling carries the limitation of up to 44% incomplete treatment. Our premise is that CA recurrence, often attributed to recainalization, can be mitigated by investigating the immune-mediated process of endovascular aneurysm healing. Our previous studies showed that local delivery of osteopontin, a cytokine expressed by myeloid cells such as macrophages, improves murine aneurysm healing. We hypothesized that increased osteopontin improves healing due to downstream cytokine and cellular mediators. In our established mouse aneurysm coiling model, we eluted osteopontin into the aneurysm lumen using a polymer-coated coil to promote aneurysm ingrowth. Osteopontin-eluting and vehicle coiled aneurysm tissue lysate were compared by cytokine array for mechanistic downstream mediators at early timepoints. Coiled murine and human aneurysms were also sectioned and immunostained for CD3+ T cells. With osteopontin-eluting coil placement in our CA model, we observed a significant increase compared with vehicle control in the protein expression of CD25 (5.8 fc; p = 0.005) at 1d, ST2/IL1RL1 (3.5 fc; p = 0.006) at 3d and interleukin-(IL)4, IL5, and IL9 (>1.5 fc, respectively; p < 0.05) at 7d after coil placement. Immunostaining in coiled murine and human aneurysms validated the presence of T cells in aneurysm healing.

With the local delivery of osteopontin into the coiled aneurysm, an increase in CD25, ST2, and type 2 interleukins indicated a potential role for T lymphocytes in osteopontin-mediated aneurysm healing. Ongoing cytometric studies will clarify the role of T lymphocytes in this pathway.

**The AAV Alpha-Synuclein Model of Parkinson’s Disease—Optimization of Genetic Constructs**

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Animal models based on α-synuclein overexpression that replicate a range of disease pathology arguably represent a more clinically relevant alternative to toxins-based Parkinson’s disease (PD) models. However, the inability to faithfully reproduce the temporal and neurochemical patterns of pathology and behavior of published work on these models hinders its wide applicability in the research laboratory. To generate a replicable, “standardized” adeno-associated virus (AAV) vector-mediated α-synuclein model, we comparatively analyzed commonly used AAV serotypes coupled with various DNA promoter and post-transcriptional regulatory elements. We designed various AAV genomes: ssAAV-CBA-αSyn-WPRE-bGHpA, ssAAV-Syn-αSyn-WPRE-lateSV40pA, scAAV-CBh-αSyn-WPRE3-enSV40pA, scAAV-Syn-αSyn-WPRE3-enSV40pA, scAAV-CMV/Syn-αSyn-WPRE3-enSV40pA, and scAAV-PGK-αSyn-WPRE3-enSV40pA. All constructs were packaged into AAV5, while scAAV-CBh-αSyn-WPRE3-enSV40pA was also packaged into AAV2 and 6. Vector titers were determined using digital droplet PCR in order to ensure future reproducibility. Titer-matched vectors were unilaterally injected into the rat substantia nigra and brains were harvested 4 weeks post-injection. Using tyrosine hydroxylase (TH) immunoreactivity in conjunction with a neural network/machine learning paradigm we quantitatively assessed the extent of nigral neurodegeneration. Quantitative near-infrared imaging was performed to assess transgene expression. We found significant differences in both α-syn expression and nigral neuron death between the different AAV constructs. In ongoing experimentation we are assessing the efficacy of selected constructs in long-term experiments where behavioral deficits are assessed over time.

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Regenerative Medicine for Spinal Cord Injury Using iPSC Cells—from Bench to Bedside

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We have reported numerous research results with the aim of establishing regenerative medicine for spinal cord injury (SCI). Regarding cell transplantation, we analyzed both the efficacy and safety using human induced pluripotent stem cell (iPSC)-derived neural progenitor cells (iPSC-NPCs). With respect to safety in particular, we focused on γ-secretase inhibitor, which inhibits Notch signaling that is necessary for maintaining the undifferentiated status of stem cells. We administered this drug to determine the tumorigenicity of iPSC-NPCs in vitro. Upon transplantation of iPSC-NPCs into animal models of SCI, tumor formation was prevented and motor function was restored. Furthermore, since the tumorigenicity differs depending on the iPSC lines, we performed genomic / epigenomic analysis which revealed that expression of tumor suppressor genes and genes for stabilizing reprogramming was decreased in highly oncogenic cell lines. To realize safe regenerative medicine, we established a system for tracking the dynamics of transplanted cells using PET-CT imaging. By identifying tumorigenesis after transplantation, early intervention such as surgical excision becomes possible, and the safety of cell transplantation therapy can be guaranteed. Based on the results of these fundamental studies, we are steadily preparing for the world’s first clinical study on SCI using iPSC-NPC transplantation. Clinical iPSCs are supplied from Kyoto University, and we have already established the method of inducing NPCs with full evaluation of their efficacy and safety. This clinical research targets patients in the subacute phase of SCI, and the protocol of this study has already been approved by the Certified Special Committee for Regenerative Medicine. After approval from the Health Science Council, we plan to start this project in 2019.

Effects of UBE3A Overexpression in Sprague Dawley Rats

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Angelman syndrome (AS) is a disease characterized by severe intellectual disability, speech and movement problems, abnormal personality, seizures, and disrupted long-term potentiation. Interruption in expression or function of the paternally imprinted UBE3A gene is thought to be the sole cause of AS. Due to this single gene alteration and lack of changes in brain structure during development, it is believed that a gene therapy approach could offer a therapeutic treatment. We have previously shown that introduction of the full-length UBE3A protein using adeno-associated virus (rAAV) along with stereotactic surgery recovers the deficits present in AS mouse and rat models. Although gene therapy is a promising potential treatment, there is some concern that supraphysiological levels of UBE3A may cause a separate set of cognitive deficits. Evidence for this comes from studies of a syndrome closely related to AS known as chromosome 15q11.2-13.1 duplication syndrome, or Dup15q. This syndrome results from a duplication of the region containing the UBE3A gene resulting in increased levels of the UBE3A protein. Therefore, to investigate the effects of overexpression of UBE3A, hippocampi of 2-month-old Sprague Dawley rats were inoculated with 10¹⁰(vg) rAAV encoding UBE3A. After aging the rats for 3 months, western blotting was performed on hippocampal tissue from a subset of animals, which showed a 33% increase in UBE3A levels in the inoculated group compared with uninoculated controls with wild-type UBE3A levels. Behavioral tests including open field, elevated plus maze, y-maze, novel object recognition, sociability, Morris water maze, rotarod, fear conditioning, and Digi-gait were performed. No change was found in any behavioral test other than sociability and some locomotion measures. Electrophysiological characterization of hippocampal long-term potentiation was also measured and will be presented. Early data demonstrate that a gene therapy approach using rAAV-UBE3A will likely not cause detrimental side effects.

The Role of Interleukin-6 in Murine Estrogen Deficiency-Associated Cerebral Aneurysm Rupture


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Cerebral aneurysms are balloon-like dilations that occur at weakened areas of vasculature and affect up to 5% of the population. Rupture of a cerebral aneurysm results in subarachnoid hemorrhage, which has a mortality rate of approximately 50%. While estrogen deficiency is associated with cerebral aneurysm rupture, the precise mechanism is unknown. Healthy post-menopausal women have increased levels of interleukin-6 (IL-6), a cytokine with pro-inflammatory activity. We hypothesized that IL-6 promotes murine estrogen deficiency-associated cerebral aneurysm rupture. To investigate the clinical relevance of our hypothesis, we analyzed IL-6 expression in human cerebral aneurysm samples compared with superficial temporal artery
controls. Using our previously established model, we induced cerebral aneurysms in estrogen-deficient female C57BL/6 mice. Two different methods of inducing estrogen deficiency were studied, 4-vinlycyclohexene diepoxide (VCD) treatment and bilateral ovariectomy (OVE). Mice were blindly randomized to selective IL-6 inhibition (IL-6 receptor (IL-6 R) neutralizing antibody, n=25) or control (isotype-matched IgG, n=28). Murine cerebral arteries at the circle of Willis were assessed for aneurysm formation, rupture, and macrophage infiltration. Results demonstrated that IL-6 was expressed in human cerebral aneurysm samples but not in superficial temporal artery controls. In both methods of inducing estrogen deficiency, selective IL-6 R inhibition significantly decreased cerebral aneurysm rupture compared with control treatments (VCD: 31.6% vs. 70.0%, p=0.026; OVE: 28.6% vs. 65.2%, p=0.019). Cerebral aneurysm formation was not significantly affected by IL-6 R inhibition (VCD: 90.4% vs. 95%, p=1.000; OVE: 84% vs. 82.1%, p=1.000). Selective IL-6 R inhibition significantly reduced F4/80+ macrophage infiltration at the circle of Willis compared with controls (7.6±0.8 vs. 22.9±2.5 stained cells / 100 μm², p<0.001). Our findings suggest IL-6 promotes murine estrogen deficiency-associated cerebral aneurysm rupture via enhanced macrophage infiltration at the circle of Willis. Similar to our previous studies, the mechanisms of estrogen deficiency affect cerebral aneurysm rupture but do not affect aneurysm formation.

Assessing In Vivo Neuronal Reprogramming by Automated Resonance-Scanned Confocal Stereology

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Direct in vivo reprogramming of non-neuronal cells in the mature central nervous system (CNS) into phenotypically correct neurons can be achieved through forced expression of pioneering transcription factors, such as neurogenin 2 (Ngn2), neurogenic differentiation 1 (NeuroD1), and achaete-scute family bHLH transcription factor 1 (Ascl1), that normally act to direct neuronal fate specification during development. This process produces a variable population of induced neurons that can be identified through their expression of reporter genes tied to the induction process and expression of various neuronal phenotypic markers, requiring detection of multiple fluorescence labels with resolution by confocal microscopy. Following in vivo gene delivery of lineage instruction factors, the number of infected cells and their distribution present some challenges for accurate quantification by design-based stereological sampling. Generally too many cells are infected to directly count with accuracy across histological sections, arguing for subsampling of the population by stereological principles. However, the cell density varies widely from the site of injection to the most distant infected cells. This means that sampling frequency density must be high to reduce estimator variance to an acceptable level. Traditional acquisition of confocal stacks is time consuming and inefficient. The recent availability of resonance scanning confocal microscopes permits the rapid generation of virtual section data sets. Efficient sampling design can now follow complete image acquisition of the histological material. The application of artificial intelligence to detecting cells with different label combinations within the virtual section data set makes it possible to automate cell counting if detection criteria can be achieved. However, cell detection must be combined with stereological sampling principles to account for sectioning and other artifacts and to accommodate fractionated sampling. These approaches are appropriate for other “rare” cell populations, such as grafted cells, and could be extended to dense cell populations if shown to be efficient.

Human Neural Stem Cell Transplantation in Chronic Spinal Cord Injury

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Spinal cord injury (SCI) is a devastating, lifelong neurological condition associated with multiple secondary medical complications, and high economic and social costs. To date, no established treatment to alleviate the loss of function after SCI exists. Accordingly, development of new therapeutic interventions in chronic SCI models is a high priority. Chronic SCI is an appealing time point for potential therapy, not only due to the large number of clinical subjects, but also the reduced probability for spontaneous recovery. In accordance, delayed treatment in clinical trial testing may yield more reliable safety and efficacy data with lower numbers of subjects. Our studies have focused on human fetal neural stem cells (hF-NSC), which represent a multipotent cell population capable of differentiating into neurons, oligodendrocytes, or astrocytes, and which can integrate into the central nervous system (CNS). Critically, hF-NSCs have demonstrated safety and efficacy after delayed transplantation, which is critical to the chronic SCI patient population. In addition, our data suggest that multiple variables such as transplant location, cell dose, or vertebral level need to be taken into consideration while developing clinical cell transplantation protocols. However, a critical variable for clinical success are the intrinsic properties of the target stem cell.
The Role of eIF5A in TDP-43 Pathology in FTD; The Tip of the Iceberg?
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TAR DNA-binding protein 43 (TDP-43) is a nuclear RNA/DNA-binding protein that associates with frontotemporal disorders. The clinical manifestations include motor neuron degeneration such as that in amyotrophic lateral sclerosis (ALS) and cognitive decline such as that in frontotemporal dementia (FTD-TDP-43). FTD remains the second most common form of early-onset dementia after Alzheimer’s disease (AD). The hallmark of TDP-43 proteinopathy is nuclear loss-of-function and accumulation of nuclear and cytoplasmic TDP-43 inclusions, which acquire toxic gain-of-function. The unique post-translational modification of eIF5A, hypusination (eIF5AhypK50), within the hypusination loop denotes its activation and cytoplasmic localization where it further interacts with specific RNA binding proteins. eIF5A is implicated in translational elongation and translation silencing of certain mRNA in stress granules (SG). Together with our findings we posit that active eIF5A is positioned as a stress-response protein. Our data show aberrant increases in enzymes responsible for hypusination in brain tissue from an AD patient, TDP-43 animal models, and arsenite-induced stress cellular models, suggesting that aberrant hypusination underlies the progression of the disease. Further, we show that arsenite-induced stress induces interactions between eIF5AhypK50 and cytoplasmic TDP-43. We also found that eIF5AhypK50 binds TDP-43 and SG protein TIA-1 during pathology and arsenite-induced stress. Importantly, we found that pharmacological inhibition of hypusination and sited-directed mutagenesis induces acetylation of eIF5A at lysine 47 (eIF5AacK47), resulting in significant reduction of phosphorylated and total TDP-43 in the cytoplasm and SG. We further confirm that potentiation of spermidine/spermine N1-acetyltransferase 1 (SSAT1) acetyl-ylates eIF5A and reduces the TDP-43 phenotype in cellular models. Hence, we argue that post-translational modifications, specifically hypusination vs. acetylation, increases or subverts TDP-43 pathology, respectively. We predict that eIF5AhypK50 regulates TDP-43 fate via several potential mechanisms, including protein–protein binding properties, increasing TDP-43 cytoplasmic retention or perturbing the nucleocytoplasmic shuttling of TDP-43 via affecting the nuclear transport machinery. Here, we discuss the strategies and approaches that we have employed to dissect the mechanism of action through which eIF5A affects TDP-43 pathology in FTD disorders and related dementia.

Intermittent Hypercapnia Training to Improve Respiratory Plasticity Following Cervical Spinal Cord Injury
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Cervical spinal cord injury (SCI) frequently leads to severe respiratory dysfunction due to damage of the spinal phrenic motor system which controls the diaphragm—the primary muscle for respiration. While some spontaneous functional plasticity does occur following cervical SCI, the extent is limited and diaphragm paresis persists. The goal of this ongoing research is to test whether a novel activity-based therapy—daily acute intermittent exposures to hypercapnia—can enhance respiratory plasticity and diaphragm recovery after cervical SCI. We hypothesized that rehabilitation with this respiratory-specific activity-based therapy will stimulate anatomical and functional phrenic plasticity and improve diaphragm function following a moderate mid-cervical contusion injury in the adult rat. Anatomical plasticity following injury and treatment was investigated using transynaptic tracing and immunohistochemistry. Pseudorabies virus (PRV) was used to retrogradely and transneuronally trace the spinal phrenic circuitry ipsilateral to injury and assess integration of premotor spinal interneurons with phrenic motoneurons. Immunohistochemistry and western blot analysis were performed to assess changes in serotonin (5-HT) and brain-derived neurotrophic factor (BDNF) expression, and axonal growth, rostral and caudal to injury.
Functional plasticity and respiratory recovery following dAIHc training was assessed with terminal diaphragm electromyography (dEMG). Hypercapnia-trained animals showed a greater density of serotonergic axons within the spinal cord, yet surprisingly had increased BDNF expression within the medulla, when compared with untreated and air control animals. It was also found that 2 weeks of dAIHc training resulted in a greater recruitment of interneurons into ipsilateral phrenic circuitry when compared with untreated and air controls. Diaphragm EMG of the dAIHc-trained animals resulted in modest improvement of the ipsilateral and contralateral diaphragm inspiratory amplitude as well as response to respiratory challenge. These results therefore suggest that dAIHc is able to promote plasticity within the phrenic network following cervical SCI.

Gene Expression, Morphological and Behavioral Changes Associated with a Mouse Model of Mild Repetitive TBI and Treatment with Activators of Nrf2 and PPARγ Transcription Factors

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The incidence of traumatic brain injury (TBI) is estimated at 0.5% per year worldwide, with a much higher frequency among military personnel and athletes. The majority of TBIs are mild, but these can result in deleterious cognitive effects for which there is currently no effective treatment. We have demonstrated improved outcomes in both in vitro and in vivo models of brain injury following treatment with tert-butyldihydroquinone (tBHQ), an activator of the inflammation-responsive transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and downstream neuroprotective factors. Additionally, the peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, pioglitazone, has been shown to have neuroprotective effects in models of neurodegenerative disease and TBI. To better understand the underlying mechanisms of injury, we tested mice receiving closed head injuries once per week for 5 weeks along with potentially synergistic treatment by tBHQ and pioglitazone. At acute and chronic timepoints, we evaluated gene expression, cognitive changes, dendritic changes, and immunohistochemistry for microglial changes. mRNA samples from the ipsilateral hippocampi 1 day post-injury were evaluated.

Our initial examination (4 groups, n=6 per group) indicated that genes displayed a variety of expression patterns. For example, there was downregulation of secreted phosphoprotein 1 (SPP1) and growth hormone (GH) in response to injury, while treatment resulted in a significant upregulation of these two factors. Conversely, we saw an increase in tumor necrosis factor receptor subfamily 25 (TNFRSF25) with injury and a subsequent decrease with treatment. Two months post-injury, Golgi staining has revealed significant changes in dendritic spines within the hippocampi of injured mice, which is not present with treatment. Behaviorally, we have shown that object recognition memory is impaired 2 months following injury and that this is ameliorated by treatment. Through these approaches, we hope to better define inflammatory responsive transcription factor signaling pathways and identify factors that could be targeted to produce neuroprotection and improve outcomes for TBI patients.

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The Effect of hMSCs on Cell Death Pathways in the Spinal Cord of SOD1G93A Rats

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The most recent clinical investigations in patients with amyotrophic lateral sclerosis (ALS) who underwent bone marrow mesenchymal stem cell (hMSC) transplantation
have shown procedural safety and clinical proof of principle with modest neurological benefits. However, the mechanisms responsible for this beneficial effect are not fully understood. In this study, we aimed to investigate the effect of repeated intrathecal applications of hMSCs in a superoxide dismutase 1 (SOD1) transgenic rat model of ALS. As a delivery route we chose lumbar puncture and/or intramuscular injection. Finally, we studied the effect of the applied therapies on apoptosis, necroptosis, and autophagy. All the animals were behaviorally tested (Grip strength test, BBB, rotarod), and the tissue was analyzed immunohistochemically, by qPCR and western blot. All symptomatic SOD1 rats treated with hMSCs (into the spinal canal or in combination with intramuscular injection) had a significantly increased lifespan, improved motor activity and reduced number of TUNEL-positive cells. Moreover, a combined hMSC delivery increased motor neuron survival, maintained neuromuscular junctions, and substantially reduced the levels of proteins involved in necroptosis (Rip1, MLKL, cl-casp8), apoptosis (cl-casp 9), and autophagy (beclin 1). Furthermore, astrogliosis and elevated levels of Connexin 43 were decreased after combined hMSC treatment. The repeated application of intramuscular injections alone improved motor activity; however, this improvement was not supported by changes at the molecular level. We conclude that a combination of repeated intrathecal and intramuscular hMSC applications protects motor neurons and neuromuscular junctions mainly through a reduction of the necroptosis pathway, which is significantly involved in cell death in rodent SOD1 model of ALS.

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Use of Dendrimer Nanoparticles Encapsulated Curcumin as a Potential Therapy for Glioblastoma in Mice

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Glioblastoma (GB), a grade-4 astrocytoma, is an aggressive form of brain tumor with no cure, having high mortality and morbidity rates (12 and 15 months after diagnosis). Most of the anti-cancer drugs do not cross the blood–brain barrier (BBB). The only treatments for GB are chemotherapy/radiotherapy, and the average survival is 14.6 months. Previous studies found increased expression of pro-inflammatory markers in the GB brain, which results in metastasis and inflammation. Therefore, decreasing pro-inflammatory signals and cell proliferation are potential targets for GB. Curcumin (Cur), a natural phytochemical, is known to have anti-inflammatory, anti-oxidant, and anti-carcinogenic properties and can cross the BBB; however, it is not water soluble, which makes its delivery challenging. Some studies use lipid formulation of Cur; however, they were found to be toxic and to increase inflammation in the brain. Therefore, we used a generation 4 (G4) surface-modified dendrimer nanoparticle [10% amine surface (positive charge) and 90% hydroxyl surface (neutral charge), known as D] encapsulated curcumin (D-ECur) that is water soluble and show effective release of Cur, having a safety profile in vitro and in vivo. Moreover, D has a cystamine (Cys) core that can split to give dendrons facilitating the better release of Cur to the cells. We used the D-ECur to test the therapeutic effect of Cur and D in mouse-derived glioblastoma cell lines (GI261) and in GB mice. The GB mice were injected with the GI261 cells to initiate tumor and the D-ECur was injected into the tumor 1 week later. Our data show that D-ECur (1) are water soluble; (2) specifically kill GB cells in vitro, sparing the neurons and glial cells; (3) reduce inflammation in vitro and in vivo in GB brain; and (4) increase the survival of the GB mice by ~25%. The future aspect involves injecting D-ECur systemically into the GB mice.

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Investigating the Role of Serotonergic Hyperinnervation of the Prefrontal Cortex on Parkinson’s Disease Non-Motor Symptoms

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Parkinson’s disease (PD) is a common neurodegenerative disease characterized by the loss of dopaminergic (DA) neurons in the substantia nigra. The gold standard pharmacological treatment, levodopa (L-DOPA), can compensate for the reduced striatal DA. However, over time, the drug loses its efficacy and >90% of treated patients develop a secondary set of aberrant motor symptoms called L-DOPA-induced dyskinesia (LID). We recently demonstrated that LID occurs as a result of “false DA neurotransmission” from dorsal
raphe serotonin (5-HT) neurons, which is facilitated by a significant anatomical reorganization and hyperinnervation of the raphe-striatal circuitry. Interestingly, L-DOPA is also associated with cognitive symptoms such as psychosis or hallucinations, implicating an involvement of additional brain areas. We hypothesize that akin to the role of 5-HT hyperinnervation in LID development described in striatum, that 5-HT innervation in the prefrontal cortex (PFC) is responsible for these non-motor features of PD. Quantification of 5-HT transporter (SERT)-positive projections indeed demonstrated hyperinnervation in PD versus healthy cases. Next, we aimed to assess these results in the context of synaptic activity through 3D reconstruction of SERT projections together with pre- and post-synaptic markers. Using Huygens deconvolution and Imaris Software we are performing a volumetric and synaptic analysis of 5-HT projections. Our data demonstrate that the 5-HT hyperinnervation is associated with significant “functional” changes such as different composition of 5-HT synapses. We are determining whether there is any correlation between any of these histological measures and reported cognitive symptoms as indicated in the Unified Parkinson’s Disease Rating Scale part 1 (UPDRS1). Our data suggest that the same hyperinnervation that gives rise to LID is also present in other areas of the PD brain. It is therefore possible that additional features of PD are caused by DA release from this circuitry, providing a novel target for the improved treatment of PD symptoms.

**Subcellular Compartmentalization of Alpha-Synuclein Alters Histone Post-Translational Modification Patterns and Chromatin State in SH-SY5Y Cells**

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It is known that the protein alpha-synuclein (αSyn) plays a crucial role in Parkinson’s disease (PD), but the mechanisms leading to cell death are still unclear. The main goal of this project is to determine if localization of αSyn to the nucleus of dopaminergic (DA) neurons exerts toxicity by altering histone modification patterns and transcriptional regulation. To identify epigenetic changes associated with nuclear αSyn we successfully developed four stable inducible SH-SY5Y cell lines for controlled expression of wild-type αSyn, αSyn tagged with a nuclear localization signal (αSyn-NLS) or with a nuclear export signal (αSyn-NES), and blue fluorescent protein (BFP) as a control. We found significantly increased global histone acetylation with cytoplasmic αSyn expression, and reduced levels with nuclear αSyn expression. Using ELISA-based assays we have identified several specific histone modifications that are selectively affected, including H3K14Ac which has been reported to be altered in the brain of PD patients. ChiP-Seq experiments are ongoing to identify genes and pathways affected by these epigenetic changes. Furthermore, we developed a series of rAAV vectors for in vivo testing: AAVs expressing αSyn, αSyn-NLS, αSyn-NES, or BFP, as well as GFP tagged with a KASH domain (nuclear envelope localization domain) for identification of nuclei of positively transduced cells. Vectors were delivered directly into the substantia nigra of young (3 months old) rats by stereotaxic injection and histological analysis is ongoing to determine whether compartmentalization of αSyn (nuclear vs cytoplasmic) has any differential effects on DA cell death.

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**Viral Overexpression of Nurr1 Induces Severe LID in Resistant Rats and Promotes Dyskinesia-Like Neuronal Signaling**

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Levodopa (L-DOPA) is the gold standard pharmacotherapy for treating the motor symptoms of Parkinson’s disease (PD). Unfortunately, chronic treatment with L-DOPA leads to the inevitable development of L-DOPA-induced dyskinesia (LID) in the majority of patients. LID has debilitating and disruptive motor symptoms comprising chorea, dystonia, and hyperkinesia. Much research is aimed at better understanding LID development. The transcription factor nuclear receptor related-1 (Nurr1) has been identified by our group, and others, as being significantly upregulated in the striatum of dyskinetic rats. Notably, Nurr1 is not normally expressed in the striatum. In the present study, we sought to characterize whether Nurr1 is a causative factor in LID expression. To examine this we overexpressed Nurr1 (green fluorescent protein (GFP) as a control) in the parkinsonian striatum of LID-resistant Lewis or LID-prone Fischer 344 (F344) rats using rAAV2/5 prior to chronic treatment with L-DOPA. F344 rats—which are predisposed to developing severe LID—did not show exacerbated LID in response to Nurr1
surprisingly, the contact sites between microglia and neuronal somas in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) declined with aging while cell body size increased. Quantitative morphometry analyses revealed microglial complexity and the projection area does not change thereafter. We conducted stereological analyses to measure age-dependent changes in microglial somas in the SNc and VTA of 1-, 6-, 9- and 18-month-old rats. Finally, we also determined that striatal Nurr1 overexpression increases corticostriatal field potentials and increases firing activity in dopamine-depleted striatal direct pathway neurons of L-DOPA-naïve rats, mimicking the activity seen in dyskinetic rats. Together, this research supports Nurr1 as a molecular driver of LID that is capable of inducing physiological changes required for these abnormal behaviors to develop.

Aging Increases Microglia Senescence in the Midbrain Region: A Risk Factor in Parkinson’s Disease


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As highly specialized and dynamic brain-resident macrophages, microglia are responsible for maintaining tissue homeostasis, neuronal support, and protection. There is growing evidence to support microglial heterogeneity within the central nervous system, including midbrain regions containing the highest number of dopaminergic neurons in the brain. Since aging is the major risk factor for neurodegenerative diseases, including Parkinson’s disease (PD), we hypothesized that the ratio of microglia to dopaminergic neurons is altered and change with aging in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). To address this hypothesis, we conducted stereological analyses to measure age-dependent changes in microglial and dopaminergic neurons in the SNc and VTA of 1-, 6-, 9- and 18-month-old C57BL/6J mouse models. Quantification of the anatomical features of microglia, we stained coronal sections of the midbrain with tyrosine hydroxylase (TH) and ionized calcium-binding adaptor molecule 1 (Iba1), and performed stereological image analysis. In both brain regions, we found an increased number of microglia at 18 months, whereas the number of TH+ cells reaches a plateau after 1 month and does not change thereafter. Quantitative morphometry analyses revealed microglial complexity and the projection area declined with aging while cell body size increased. Quite surprisingly, the contact sites between microglia and dopaminergic neurons in both regions increased in aged mice, suggesting an age-dependent increase in microglial support of dopaminergic neurons. In conclusion, increases in microglial cell number, the ratio of microglia to dopaminergic neurons, as well as physical contact sites, suggest these innate biological mechanisms may compensate for the age-dependent decline of microglial complexity (senescence) for continued neuronal support in aging. The morphological microglial heterogeneity between adjacent dopaminergic regions of SNc and VTA found in our studies may describe the susceptibility of SNc dopaminergic neurons in neurodegenerative diseases such as PD.

In Vivo Delivery of Large Plasmids and CRISPR-Cas9 to Edit GFP Gene Using Dendrimer Nanoparticles


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Dendrimers are 3-dimensional branched nanoparticles having applications in the field of medicine. The commonly used G4 PAMAM dendrimers with 100% amine surface (G4-NH2) are highly toxic to cells due to their positive charged surface. Therefore, it is necessary to modify the dendrimer surface having 10% -NH2 and 90% hydroxyl group (G4-90/10), thereby reducing the total number of positive charges. Our data show that these surface-modified dendrimers (1) are taken up by neurons and glial cells in vitro and in vivo; (2) are non-toxic to cells; (3) can cross the blood–brain barrier (BBB) following systemic injection; and (4) carry and deliver large plasmids of various sizes. We have delivered a ~10 kb plasmid with dendrimer nanoparticles in vitro and in vivo, which is currently not possible with the viral vectors. In addition, dendrimers can carry gene-editing plasmids, including CRISPR-Cas9 plasmids. Current strategies to deliver CRISPR-Cas9 rely on predominately viral transduction, electroporation, and hydrodynamic injection. All of these systems have severe
The Role of rs6265 BDNF SNP on Functional Remodeling of the Parkinsonian Striatum Following Dopamine Neuron Grafting in CRISPR Knock-in Rats

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Up to 40% of the general population possesses a single nucleotide polymorphism rs6265 in the Bdnf gene, which results in diminished neuronal release of brain-derived neurotrophic factor (BDNF) and significantly reduces levodopa efficacy in Parkinson’s disease (PD) patients. BDNF plays a critical role in nigral and striatal neuron maturation and function. We hypothesize that dysfunctional BDNF is an unrecognized contributor to the discordant finding of abundant survival of grafted dopamine (DA) neurons and lack of behavioral efficacy reported in a subpopulation of PD subjects, and in the development of graft-induced dyskinesias (GID). To test this hypothesis, we generated a CRISPR knock-in rat model of the human rs6265 BDNF variant to evaluate the impact of this risk allele on the function and synaptic integration of new DA terminals in the parkinsonian striatum. In this study we compared embryonic DA neuron graft survival, integration, and behavioral efficacy between val68val wild-type (WT, “BDNF normal”) and homozygous met68met (“BDNF reduced”) parkinsonian rats. Rats were rendered unilaterally parkinsonian and primed with levodopa (12 mg/kg, M-Fr) to induce stable expression of levodopa-induced dyskinesias (LID), which was the primary behavioral endpoint for graft efficacy. Amphetamine-induced behaviors served as secondary endpoints for graft efficacy and assessment of GID. Analyses thus far indicate that contrary to our hypothesis, the Met68Met subjects show significantly enhanced graft-derived benefit (i.e., amelioration of LID behavior) compared with WT subjects. However, consistent with our hypothesis, only Met68Met subjects exhibited aberrant GID behaviors. Post-mortem analyses demonstrate that survival of grafted DA neurons does not differ between the Val68Val and Met68Met subjects; however, the Met68Met subjects have significantly more robust neurite outgrowth, with enhanced presence of varicosities. Ongoing post-mortem analyses will include quantitative assessment of synapse phenotype and degree of connectivity between grafted dopaminergic neurites and host neurons.

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Role of T Cells in an Alpha-Synuclein Model of Parkinson’s Disease

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Parkinson’s disease (PD) is the second most prevalent movement disorder. It is characterized by up to 70% loss of dopamine (DA)-secreting neurons and accumulation of Lewy bodies, deposits composed of α-synuclein (α-syn), in the remaining DA neurons of the substantia nigra pars compacta (SNpc). Accumulation of α-syn activates microglia, the immune cells of the central nervous system (CNS). Microglial over-activation causes inflammation and subsequently leads to neurodegeneration and tissue destruction. Inflammation caused by the activated microglia and dendritic cells has been associated with the pathogenesis of PD and several other neurodegenerative disorders. Recently, apart from microglia, CD4 and CD8 T cells have been shown to be recruited to the area of damage where they may either mediate neurodegeneration or act in a neuroprotective manner. The communication pattern between T cells, microglia, and dendritic cells in PD patients is unknown. Previous work has shown that injection of human α-syn into the SNpc of the brain failed to induce neurotoxicity in MHCII (the marker for activated microglia) deficient mice, suggesting that T-cell and microglial communication is necessary for the neurotoxic process. Here, we assessed the role of T cells in an α-syn model of PD in T-cell-deficient (male athymic
nude) and T-cell-competent (male heterozygous nude) rats. Injection of AAV9 expressing human \( \alpha \)-syn unilaterally to the SN of heterozygous nude rats at 3 months of age caused deficits in a cylinder test for paw bias in comparison to green fluorescent protein (GFP)-treated controls when the rats were tested 2 months post-injection. On the other hand, nude rats injected with \( \alpha \)-syn showed no deficit in the cylinder test when compared with the controls. The percentage of tyrosine hydroxylase (TH) positive neurons in SNpc was significantly lower in \( \alpha \)-syn injected T-cell-competent rats when compared with their T-cell-deficient rat counterparts. These data suggest that T cells may play a major role in DA neuronal loss and confirm that T-cell communication with microglia is necessary for \( \alpha \)-syn-mediated neurodegeneration in Parkinson’s disease.

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Distinct Neuronal Ensembles within the Memory Engram Regulate Memory Discrimination and Generalization

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Memories of previous experiences affect behavioral responses via balancing discrimination between different and generalization across similar stimuli. An imbalance between discrimination and generalization, for example maladaptive generalization of fear memories, can lead to anxiety disorders such as posttraumatic stress disorder (PTSD) and panic disorder. However, the cellular and circuit mechanisms underlying these two processes are largely unknown. Here we show that individual contextual fear memories are represented in the hippocampal dentate gyrus by multiple functionally distinct neuronal ensembles defined by the \( \text{Fos} \)- or \( \text{Npas4} \)-dependent transcriptional pathways, and that these ensembles bi-directionally regulate the discrimination–generalization balance. The \( \text{Fos} \)-dependent ensemble promotes memory generalization and recruits excitatory inputs from the medial entorhinal cortex, which mediates generalization. The \( \text{Npas4} \)-dependent ensemble promotes memory discrimination and recruits inhibitory drive from local cholecystokinin-expressing interneurons, the activity of which is required for discrimination. Thus we demonstrate for the first time functional heterogeneity of the memory engram and uncover novel cellular and circuit mechanisms regulating the discrimination–generalization balance. These findings may provide new therapeutic strategies to treat anxiety disorders.

Effect of Chronic Neuroinflammation on the Clearance of Macromolecules from the Rat Brain

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Two hypotheses have been proposed to explain the removal of macromolecular waste from the brain extracellular space (ECS): the classic diffusion model and the recently proposed ‘glymphatic’ model. Both models propose that cerebrospinal fluid (CSF) facilitates the clearance of these solutes, albeit by different mechanisms. We hypothesized that chronic neuroinflammation is sufficient to impair macromolecular clearance from the brain and may result in the toxic accumulation of extracellular solutes over time. To test this, we measured the effect of induced lipopolysaccharide (LPS)-induced chronic neuroinflammation on the clearance kinetics of two fluorescently tagged dextran tracers from the rat brain using densitometry and spectrophotometry. We also assessed its effects on the quantity and distribution of aquaporin 4 (AQP4), glial fibrillary acidic protein (GFAP), and amyloid-beta (A\( \beta \)) proteins within the brain, and behavioral measures of cognitive function. Relative to controls, neuroinflammation impaired the clearance of these tracers such that, at corresponding time points, tracer concentrations were significantly increased in the brain parenchyma and significantly decreased in blood serum. Consistent with previous reports, chronic neuroinflammation disrupted AQP4 channel distribution on astrocytic end-feet processes within the prefrontal cortex and hippocampus; however, this did not impair the rate of CSF influx into the parenchyma. Rather, consistent with the diffusion model, we saw a size-dependent influx of tracer into the parenchyma in both LPS and control groups. Chronic neuroinflammation was also associated with elevated A\( \beta \) in the hippocampus, with punctate A\( \beta \) deposits within perivascular space. Behavioral analysis showed enhanced fear memory and a trend toward context generalization in the LPS group, both consistent with chronic inflammation-induced stress. Our results are generally consistent with the diffusion model of waste clearance from the brain, and demonstrate that chronic neuroinflammation is sufficient to impair the elimination of macromolecular solutes from the brain, which over time may result in the accumulation of toxic peptides such as A\( \beta \), contributing to cognitive impairment and neuropathology.
Prolyl Oligopeptidase and its Inhibition on Alpha-Synuclein Toxicity—A Possibility for Disease-Modifying Therapy for Parkinson’s Disease


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In Parkinson’s disease (PD), dopaminergic neurons in the nigrostriatal pathway degenerate, leading to imbalance in neuronal transmission and clinical symptoms. Although the causes leading to PD are not completely known, misfolding and aggregation of alpha-synuclein (aSyn) has been identified as one of the key players for cellular toxicity in PD. Aggregated aSyn is the main component for Lewy bodies, and aSyn oligomers that are formed during the aggregation process can damage cellular organelles while fibrils can propagate aSyn toxicity by cell-to-cell transfer. Several factors can increase aSyn aggregation, and one of them is a serine protease, prolyl oligopeptidase (PREP). We have shown that PREP forms a direct protein–protein interaction with aSyn, leading to increased aSyn dimerization and formation of soluble aSyn oligomers in vitro and in vivo. Strong colocalization between aSyn and PREP is also seen in post-mortem PD brain. KYP-2047, a small-molecule PREP inhibitor, modifies this interaction and reduces aSyn dimerization. Additionally, PREP inhibition increases macroautophagy by activating protein phosphatase 2A (PP2A) and decreases the levels of aSyn oligomers in cells and in vivo. That in turn ameliorates aSyn toxicity in cell culture models, and our study showed that post-symptomatic 4-week treatment by KYP-2047 restored behavioral deficit, decreased aSyn oligomers, and halted damages in dopaminergic neurotransmission in a mouse PD model based on nigral AAV-aSyn injection. To assess the impact of PREP and its inhibition on aSyn propagation, we used aSyn pre-formed fibrils (PFFs) with Cy3-tag in a neuronal cell culture. After exposing cells to PFFs, 2-day incubation with KYP-2047 significantly reduced intracellular PFFs compared with control, and the same was observed in PREP knock-out cells, suggesting that PREP inhibition or deletion improves PFF processing in cells. Therefore, we propose that PREP inhibition could be a multi-targeting drug therapy having effect on aSyn aggregation, clearance, and propagation.

Sequential Combined Treatment of Pifithrin-α and Posiphen Enhances Neurogenesis and Functional Recovery After Stroke

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Although cerebral ischemia can activate endogenous reparative processes, such as proliferation of endogenous neural stem cells (NSCs) in the subventricular zone (SVZ) and subgranular zone (SGZ), the majority of these

Limited NG2 Glial Tropism of Recombinant Adeno-Associated Viral (rAAV)-Mediated Gene Delivery for In Vivo Neuronal Reprogramming

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Direct reprogramming of cell identity from a glial to neuronal phenotype has been demonstrated both in vitro and in vivo. The induction of phenotypic neurons from glial cell populations follows forced expression of pioneering transcription factors, such as neurogenin 2 (Ngn2), neuronal differentiation 1 (NeuroD1), and achaete-scute family bHLH transcription factor 1 (Ascl1), that are normally expressed during development to specify neuronal fate. Viral vectors can be used to deliver reprogramming transcription factors to glial cells. The efficiency of vector tropism has a major impact on the feasibility to reprogram a sufficient number of new neurons to achieve a meaningful functional integration. NG2 glia, also known as oligodendrocyte progenitor cells (OPCs), represent one cellular population that could be an ideal target for neuronal induction for repair. NG2 glia, understood to represent a reserve cell population to replace oligodendrocytes, are actively dividing in the mature central nervous system (CNS), perform no known critical neural activity, and respond to injury by proliferation and subsequent population homeostasis. Previously, we have successfully targeted and reprogrammed NG2 glia to neurons in vitro and in vivo using retroviral vectors to target the actively proliferating NG2 cell population. rAAV vectors are an alternative delivery platform frequently used to achieve efficient and widespread in vivo gene delivery. However, no systematic assessment of AAV serotype efficiency in targeting NG2 glia has been reported. We delivered CMV-eGFP constructs into rat cortex and striatum (n=3 animals per site) using the following AAV serotypes: 1, 2, 4, 5, 6, 6.2, 8, 9, rh10, DJ, PHP.s, PHP.B, PHP.eB. Analysis of infected cell types, 3 weeks post-injection, revealed most serotypes were substantially neurotropic with tropism for OPCs also observed with AAV5 and AAV8. None of these serotypes infected NG2 glia to any extent. These data suggest that targeting reprogramming constructs to NG2 glia will require designing new and specific AAV serotypes or utilizing alternatives to AAV.
new cells die shortly after injury and do not appropriately differentiate into neurons, or migrate and functionally integrate into the brain. The purpose of this study was to examine a novel strategy for treatment of stroke after injury by optimizing the survival of ischemia-induced endogenous NSCs in the SVZ and SGZ. Adult SVZ and SGZ NSCs were grown as neurospheres in culture and treated with a P53 inactivator, pifithrin-α (PFT-α), and an amyloid precursor protein (APP)-lowering drug, posiphen, and effects on neurosphere number, size, and neuronal differentiation were evaluated. This combined sequential treatment approach was then evaluated in mice challenged with middle cerebral artery occlusion (MCAo). Locomotor behavior and cognition were evaluated at 4 weeks, and the number of new surviving neurons was quantified in nestin creERT2-YFP mice. PFT-α and posiphen enhanced the self-renewal, proliferation rate, and neuronal differentiation of adult SVZ and SGZ NSCs in culture. Their sequential combination in mice challenged with MCAo-induced stroke mitigated locomotor and cognitive impairments and increased the survival of SVZ and SGZ NSCs cells. PFT-α and the combined posiphen+PFT-α treatment similarly improved locomotion behavior in stroke-challenged mice. Notably, however, the combined treatment provided significantly more potent cognitive function enhancement in stroke mice, as compared with PFT-α single treatment. Delayed combined sequential treatment with an inhibitor of P53 dependent apoptosis (PFT-α) and APP synthesis (posiphen) proved able to enhance stroke-induced endogenous neurogenesis and improve the functional recovery in stroke animals.

**Intranasal Administration of Exosomes from Human iPSC-Derived NSCs Enhances Neural Stem/Progenitor Cell Proliferation and Neurogenesis in the Normal Adult Hippocampus**

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Exosomes are tiny extracellular vesicles secreted by cells. Exosomes derived from stem cells such as mesenchymal stem cells (MSCs) and neural stem cells (NSCs) have neuroprotective and anti-inflammatory properties. Such exosomes may also have neurogenic properties. The use of stem cell-derived exosomes for enhancing brain function in normal and disease conditions is attractive as they can be harvested in large quantities and characterized. Their small size makes them particularly amenable for administration to the brain via relatively non-invasive routes, and also avoids several potential safety hazards linked with cells such as the risk for tumors. Our previous study has shown that intranasally administered exosomes can enter virtually all regions of the forebrain and get incorporated into neurons and microglia (Castro et al., SFN abstracts, 2017). Here, we examined the neurogenic property of exosomes generated from human induced pluripotent stem cell (hiPSC)-derived NSCs. The conditioned media containing NSC-derived exosomes was first processed as described elsewhere (Kim et al., PNAS, 2016) and further purified through size exclusion chromatography. The hNSC-derived exosomes expressed the exosome marker CD63. We administered these exosomes intranasally to adult (5–6 months old) male rats (~50 billion exosomes/nosstril, ~100 billion/rat) and examined NSC behavior and neurogenesis in the subgranular zone (SGZ) of the hippocampus 14 days after administration. Measurement of Ki-67 expressing cells in the SGZ revealed the proliferation of increased numbers of putative NSCs in animals receiving intranasal (IN) exosomes, in comparison to animals receiving IN vehicle (p<0.01). Moreover, Ki-67+ cell clusters in the SGZ of exosome-treated animals were more substantial, more frequent and comprised a higher number of cells per cluster (p<0.01). Doublecortin (DCX+) immunostaining also revealed increased numbers of DCX+ clusters containing higher amounts of immature neuroblasts (p<0.05). Thus, hiPSC-NSC-derived exosomes have neurogenic properties. These exosomes may be suitable as biologics for enhancing hippocampal neurogenesis in conditions such as aging and disease.

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**Maximum Tolerated Dose of Exosomes derived from Mesenchymal Stem Cells via Intra-arterial Dosing in a Rat Stroke Model**

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Cell therapy is emerging as a promising novel therapy for ischemic stroke. Exosomes are endosomal-origin small-membrane vesicles with a size of 40 to 100 nm in diameter, secreted from many types of cells, for example,
Micro RNA-29b Can Effectively Inhibit Alzheimer’s Disease Symptoms and Maintain Memory

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Alzheimer’s disease (AD) is a degenerative disease of the brain; its main symptoms include amyloid accumulation and hyperphosphorylation of the protein. We screened the initial efficacy of the drug using induced pluripotent stem cells (iPSCs) that produce symptoms of AD. Among them, Butyrideneanthalide (EF-005) can effectively reduce the production of amyloid, total protein and phosphorylated protein in cells at an appropriate concentration. Using miRNA microarray biochip assays, we found that when AD-iPSCs were treated with EF-005, miRNA-29b increased significantly. The literature also pointed out that increased expression of PSEN-1 and 2 genes causes amyloid production in the brains of Alzheimer’s patients. We speculate that miRNA-29b can bind to the gene sequence fragment of PSEN and inhibit protein production. This was confirmed using the C6-C99 cell model which can produce amyloid protein. The PSEN protein was significantly decreased after the addition of EF-005, and with direct inhibition of miRNA-29b, EF-005 will not be able to affect the performance of PSEN protein. The dual luminescence system demonstrated that miRNA-29b binds to specific gene segments of PSEN and interferes with gene expression. The gene transfer mouse-3xTg mice with Alzheimer’s disease were treated with EF-005, and the changes in amyloid accumulation in the mice brains were traced through Florbetaben-18F for a long time. After 1 year of tracking, the oral EF-005 treatment group showed a significant reduction in amyloid protein in the brain compared with the control group. In electrophysiological examination of the long-term enhancement of the brain, the treatment group was also superior to the control group. The learning and memory ability was measured by the water maze, and the treatment group was also significantly better than the untreated group. We propose that EF-005 can reduce PSEN protein by increasing miRNA-29b, thereby reducing the aggregation of amyloid. Inhibiting excessive accumulation of amyloid in the brain by promoting miRNA drugs can be a strategy for preventing and treating AD.

Neural Progenitor Cell Survival and Expression of Parvalbumin and Proenkephalin in a Jaundiced Rat Model of Kernicterus

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Neonatal hyperbilirubinemia targets specific brain regions and can lead to kernicterus. Dystonia in kernicterus is caused by bilirubin toxicity in primarily GABAergic neurons of the globus pallidus (GP). Targeting GP with neuronal stem cells to restore damaged cells is a promising approach to treat dystonia in kernicterus. Our goal was to investigate long-term survival and development of transplanted medial ganglion eminence (MGE)-like (MGE is the major embryologic origin of GP neurons) neuronal progenitor cells (NPCs) in the Gunn rat model of kernicterus. At P10, jaundiced (jj) Gunn rats were injected with 50 mg or 70 mg/kg of sulfonamide, which promotes the transfer of bilirubin into brain tissue to cause kernicterus. jj rats and non-jaundiced (Nj) littermates then received WA09 human embryonic stem cells differentiated into GABAergic MGE-like NPCs into the GP. Animals were kept for 3 or 7 weeks post
Transplantation. Cyclosporine A (10 mg/kg, sc) was given 1 day before and 3 weeks after surgery; thereafter it was mixed in drinking water (50 μl/ml). We then conducted immunohistochemical analyses of brain sections to identify grafted cells and fibers, and the expression of parvalbumin (PV) and proenkephalin (PENK), the two most prevalent neuropeptides in the GP, in the graft. We found that MGE-like NPCs survived and generated various types of fibers in jij brains, even after hyperbilirubinemia exacerbation. More mature grafted cells were observed in brains of the 7-week group than in the 3-week group. PV-ir neurons were more abundant and mature 7 weeks after grafting than 3 weeks. Grafted PENK-ir neurons were observed in vivo for the first time in both the 3- and 7-week groups. Our results reveal that MGE-like NPCs survive in kernicterus brain and generate connecting fibers, with a gradually mature appearance, and express major neuropeptides as in the normal GP. These results support the feasibility of stem cell therapy in kernicterus.

Combining Neural Transplantation with Therapeutic Intermittent Hypoxia to Treat the Injured Spinal Cord

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There is a growing interest in the use of neural progenitor cells (NPCs) to repair the injured spinal cord. Despite extensive preclinical research, it remains unclear as to how donor cells develop, differentiate, and integrate with host injured circuitry, and if integration can be enhanced and/or guided using noninvasive means such as activity-based therapy. With a focus on the phrenic circuit and respiratory dysfunction after cervical spinal cord injury (SCI), the present work tests the hypothesis that pairing cellular transplantation with a clinical rehabilitation strategy (daily acute intermittent hypoxia, dAIH) will enhance neuroplasticity and promote donor–host connectivity. Cultured NPCs isolated from green fluorescent protein (GFP) rats yielded neuronal and glial restricted progenitor cells. While the phenotype of these progenitors is being determined in ongoing work, excitatory, inhibitory, and modulatory neuronal precursors are known to be present. These cells were then transplanted into a clinically relevant cervical (C3/4) contusion injury in adult Sprague Dawley rats, 1 week after injury. Animals received 4 weeks of dAIH (10 × 5 min exposures to 10% oxygen intermittent with normoxia, 5 days a week), beginning 1 week post-transplantation. Donor cells survive, differentiate, and integrate with the host spinal cord as assessed with transynaptic pseudorabies virus tracing (PRV) and immunohistochemistry. Respiratory training resulted in significantly enhanced donor–host connectivity to ipsi- and contralateral-to-injury phrenic circuit, compared with untrained transplant recipients. At least a subset of these newly integrated donor spinal interneurons are cholinergic. Preliminary data suggest the underlying mechanism for directing donor cell outgrowth toward phrenic inter- and motoneurons is in part mediated via brain-derived neurotrophic factor (BDNF) expression within the cervical spinal cord. Transplant recipients, with and without dAIH training, showed greater muscle (diaphragm) recovery than vehicle-controls, as measured by terminal electromyography. Interestingly, transplant and dAIH training recipients demonstrated a greater ability to respond to hypoxic but not hypercapnic respiratory challenge. Ongoing experiments are focused on identifying donor neural phenotypes that become connected to ipsi- and contralateral-to-injury host circuitry. These experiments suggest that rehabilitative strategies such as dAIH may be an effective way for enhancing donor cell outgrowth and connectivity.

Acute Treatment of a Hypothermic Compound Attenuates Psychological Deficits Chronically Developed in Mice with a Focal Ischemic Stroke

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Stroke is one of the leading causes of disability in the world and around 30% of patients develop psychological liabilities, such as post-stroke depression (PSD) and post-stroke anxiety. Basic and translational research on psychological disorders after stroke is limited. A stroke mouse model with a focal ischemic stroke in the sensorimotor cortex was used in our study, which showed sensorimotor functional deficits several days after stroke. The mice underwent spontaneous recovery in the adhesive removal test and corner test in 2–4 weeks. However, in our study, these animals gradually developed depressive/anxious behaviors 4 weeks after stroke and deteriorated until 8 weeks post-surgery in a series of behavioral examinations, such as the forced swim test, tail suspension, sucrose preference, and the open field and water maze tests. Altered neuronal plasticity, suppressed brain-derived neurotrophic factor (BDNF) and oxytocin signaling, and disturbed dopamine regulation were detected in the prefrontal cortex (PFC) of these stroke animals at 6–8 weeks post-surgery. Antidepressants are common clinical treatments for PSD; however, their efficacy is far from satisfactory. Emerging evidence has shown that therapeutic hypothermia is a promising acute protective therapy after stroke; meanwhile, its effect on post-stroke psychological
symptoms has rarely been examined. We have developed a pharmacological hypothermia treatment using neurotensin receptor 1 (NTR1) agonists and demonstrated brain protective effects against stroke and traumatic brain injury (TBI). In the present investigation, we inspected the potential antagonism of acute post-stroke treatment using the hypothermic drug HPI363 on chronic psychological disorders in a mouse model of stroke. In stroke mice that received 6 h hypothermic treatment of HPI363 acutely after stroke, depressive and anxious phenotypes were noticeably attenuated at 6 weeks after stroke, accompanied by restored BDNF and oxytocin signaling. Our data suggest that an acute hypothermia treatment induced with PIH363 has a delayed benefit of attenuating chronically developed post-stroke psychological disorders in mice.

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Extending Cord Blood to Regenerative Therapies for the Brain
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Studies in children with selective inborn errors of metabolism have shown that cord blood cells, administered intravenously after myeloablative therapy, engraft in the brain. DUOC-01, a cord blood derived cellular therapy that promotes myelination, is undergoing testing to augment standard umbilical cord blood treatment in children with leukodystrophies. These observations led us to hypothesize that cord blood cells might also have efficacy treating patients with acquired brain injuries. Clinical studies to date have been performed to demonstrate safety and efficacy of intravenous infusions of autologous cord blood in babies with hypoxic ischemic encephalopathy, young children with cerebral palsy, congenital hydrocephalus and autism, and adults with acute ischemic stroke. Further development of these therapies using allogeneic cord blood products can provide access to these therapies for all.

Learning Objectives
1. The history and evolution of cord blood banking and transplantation
2. Quality measures of cord blood units
3. New clinical applications for cord blood therapies

Human iPSC-Derived Corticospinal Neuronal Grafts To Repair Cervical Spinal Cord Injury
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Traumatic spinal cord injuries (SCIs) at the cervical-level sever corticospinal tract axons disrupt the motor circuit responsible for voluntary movement and pose both an immediate and long-term burden on the patient and health care system. Whereas the predominant amount of grafts in preclinical trials are comprised of undifferentiated and uncharacterized neural progenitor cells, our laboratory has developed a well-characterized human induced pluripotent stem cell-derived corticospinal motor neuron (iPSC-CSMN) transplant relay system to treat cervical SCI. Herein, human iPSCs underwent a directed differentiation towards a CSMN fate and were then implanted in immunodeficient nude rats following a C5 unilateral hemisection injury. Cells were phenotypically characterized and monitored within the host corticospinal tract following implantation for growth, migration, synapse formation, and effect on the injury. Additionally, anatomical changes were correlated with extent of functional recovery. At 12-32 weeks post transplantation, iPSC-CSMN grafts survived, integrated, did not proliferate or migrate, extend axons long distances, reduce inflammation and lesion size, allowed for serotonergic and corticospinal tract axon innervation, and led to significant improvements in forelimb function. Further investigation is underway to understand supraspinal innervation of the CSMN grafts and examining how these fully differentiated iPSC-CSMN grafts respond to chronic SCIs. Elucidating how to better integrate transplanted cells into the host neural circuitry to repair and restore function is a future path of investigation.

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