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Stem Cell

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# Stem Cell Technology Research Literatures

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**Abstract**: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.
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**Key words**: stem cell; technology; life; research; literature

## Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Acharya, M. M., et al. (2013). "Comparing the functional consequences of human stem cell transplantation in the irradiated rat brain." Cell Transplant **22**(1): 55-64.

Radiotherapy is a frontline treatment for the clinical management of CNS tumors. Although effective in eradicating tumor cells, radiotherapy also depletes neural stem and progenitor cells in the hippocampus that are important for neurogenesis and cognitive function. Consequently, the use of radiation to control primary and metastatic brain tumors often leads to debilitating and progressive cognitive decrements in surviving patients, representing a serious medical condition that, to date, has no satisfactory, long-term solutions. As a result, we have explored the use of stem cells as therapeutic agents to improve cognition after radiotherapy. Our past work has demonstrated the capability of cranially transplanted human embryonic (hESCs) and neural (hNSCs) stem cells to functionally restore cognition in rats 1 and 4 months after head-only irradiation. We have now expanded our cognitive analyses with hESCs and quantified both survival and differentiated fates of engrafted cells at 1 and 4 months after irradiation. Our findings indicate the capability of hESC transplantation to ameliorate radiation-induced cognitive dysfunction 1 month following cranial irradiation, using a hippocampal-dependent novel place recognition task. Irradiated animals not engrafted with stem cells experienced prolonged and significant cognitive dysfunction. Stereological estimates indicated that 35% and 17% of the transplanted hESCs survived at 1 and 4 months postgrafting, respectively. One month after irradiation and grafting, phenotypic analyses revealed that 26% and 31% of the hESCs differentiated into neurons and astrocytes, while at the 4-month time, neuronal and astrocytic differentiation was 7% and 46%, respectively. Comparison between present and past data with hESCs and hNSCs demonstrates equivalent cognitive restoration and a preference of hNSCs to commit to neuronal versus astrocytic lineages over extended engraftment times. Our data demonstrate the functional utility of human stem cell replacement strategies for ameliorating the adverse effects of cranial irradiation on cognition.

Acharya, M. M., et al. (2014). "Long-term cognitive effects of human stem cell transplantation in the irradiated brain." Int J Radiat Biol **90**(9): 816-820.

PURPOSE: Radiotherapy remains a primary treatment modality for the majority of central nervous system tumors, but frequently leads to debilitating cognitive dysfunction. Given the absence of satisfactory solutions to this serious problem, we have used human stem cell therapies to ameliorate radiation-induced cognitive impairment. Here, past studies have been extended to determine whether engrafted cells provide even longer-term benefits to cognition. MATERIALS AND METHODS: Athymic nude rats were cranially irradiated (10 Gy) and subjected to intrahippocampal transplantation surgery 2 days later. Human embryonic stem cells (hESC) or human neural stem cells (hNSC) were transplanted, and animals were subjected to cognitive testing on a novel place recognition task 8 months later. RESULTS: Grafting of hNSC was found to provide long lasting cognitive benefits over an 8-month post-irradiation interval. At this protracted time, hNSC grafting improved behavioral performance on a novel place recognition task compared to irradiated animals not receiving stem cells. Engrafted hESC previously shown to be beneficial following a similar task, 1 and 4 months after irradiation, were not found to provide cognitive benefits at 8 months. CONCLUSIONS: Our findings suggest that hNSC transplantation promotes the long-term recovery of the irradiated brain, where intrahippocampal stem cell grafting helps to preserve cognitive function.

Aday, S., et al. (2016). "Stem Cell-Based Human Blood-Brain Barrier Models for Drug Discovery and Delivery." Trends Biotechnol **34**(5): 382-393.

The development of novel neuropharmaceuticals requires the evaluation of blood-brain barrier (BBB) permeability and toxicity. Recent studies have highlighted differences in the BBB among different species, with the most important differences involving the expression of P-glycoprotein (P-gp), multidrug resistance-associated proteins, transporters, and claudins. In addition, functional studies have shown that brain pharmacokinetics of P-glycoprotein substrates are different in humans and rodents. Therefore, human BBB models may be an important platform for initial drug screening before in vivo studies. This strategy might help to reduce costs in drug development and failures in clinical studies. We review the differences in the BBB among species, recent advances in the generation of human BBB models, and their applications in drug discovery and delivery.

Al Nimer, F., et al. (2004). "MHC expression after human neural stem cell transplantation to brain contused rats." Neuroreport **15**(12): 1871-1875.

Human neural stem cells survive and improve motor function after transplantation to the contused brain. However, the transplants might be rejected and that depends on the graft immunogenicity, the host immunological status and the immunosuppression strategy. We transplanted human neural stem cells to rats with brain contusion and analyzed the donor and host MHC antigen expression and the effect of a short-term immunosuppression with cyclosporine. In vitro human neural stem cells expressed only MHC-II antigens. This expression was down-regulated 6 weeks after transplantation. The host response was characterized by an increased MHC-II expression which was down-regulated by a longer term of immunosuppression. These findings are novel and necessary in order to understand the immunogenicity of human neural stem cell grafts.

Al-Ahmad, A. J. (2023). "Human-Induced Pluripotent Stem Cell-Based Model of the Blood-Brain at 10 Years: A Retrospective on Past and Current Disease Models." Handb Exp Pharmacol.

The initial discovery and derivation of induced pluripotent stem cells (iPSCs) by Yamanaka and colleagues in 2006 revolutionized the field of personalized medicine, as it opened the possibility to model diseases using patient-derived stem cells. A decade of adoption of iPSCs within the community of the blood-brain barrier (BBB) significantly opened the door for modeling diseases at the BBB, a task until then considered challenging, if not impossible.In this book chapter, we provided an extensive review of the literature on the use of iPSC-based models of the human BBB to model neurological diseases including infectious diseases (COVID-19, Streptococcus, Neisseria) neurodevelopmental diseases (adrenoleukodystrophy, Allan-Herndon-Dudley Syndrome, Batten's disease, GLUT1 deficiency syndrome), and neurodegenerative diseases (Alzheimer's disease, the current findings and observations, but also the challenges and limitations inherent to the use of iPSC-based models in reproducing the human BBB during health and diseases in a Petri dish.

Arthur, P., et al. (2022). "Bioengineering Human Pluripotent Stem Cell-Derived Retinal Organoids and Optic Vesicle-Containing Brain Organoids for Ocular Diseases." Cells **11**(21).

Retinal organoids are three-dimensional (3D) structures derived from human pluripotent stem cells (hPSCs) that mimic the retina's spatial and temporal differentiation, making them useful as in vitro retinal development models. Retinal organoids can be assembled with brain organoids, the 3D self-assembled aggregates derived from hPSCs containing different cell types and cytoarchitectures that resemble the human embryonic brain. Recent studies have shown the

development of optic cups in brain organoids. The cellular components of a developing optic vesicle-containing organoids include primitive corneal epithelial and lens-like cells, retinal pigment epithelia, retinal progenitor cells, axon-like projections, and electrically active neuronal networks. The importance of retinal organoids in ocular diseases such as age-related macular degeneration, Stargardt disease, retinitis pigmentosa, and diabetic retinopathy are described in this review. This review highlights current developments in retinal organoid techniques, and their applications in ocular conditions such as disease modeling, gene therapy, drug screening and development. In addition, recent advancements in utilizing extracellular vesicles secreted by retinal organoids for ocular disease treatments are summarized.

Bakhtiary, M., et al. (2011). "Combination of stem cell mobilized by granulocyte-colony stimulating factor and human umbilical cord matrix stem cell: therapy of traumatic brain injury in rats." Iran J Basic Med Sci **14**(4): 327-339.

OBJECTIVES: Clinical studies of treating traumatic brain injury (TBI) with autologous adult stem cells led us to examine the impression of a combination therapy. This was performed by intravenous injection of human umbilical cord matrix stem cell (hUCMSC-Wharton(,)s jelly stem cell) with bone marrow cell mobilized by granulocytecolony stimulating factor (G-CSF) in rats injured with cortical compact device. MATERIALS AND METHODS: Adult male Wistar rats (n= 50) were injured with controlled cortical impact device and divided into five groups. All injections were performed 1 day after injury into the tail veins of rats. Neurological functional evaluation of animals was performed before and after injury using modified neurological severity scores (mNSS). Animals were sacrificed 42 days after TBI and brain sections were stained by Brdu immunohistochemistry. RESULTS: Statistically significant improvement in functional outcome was observed in treatment groups when compared with control (P< 0.01). mNSS showed no significant differences among the hUCMSC and G-CSF treated groups at any time point (end of trial). Rats with hUCMSC + G-CSF treatment had a significant improvement on mNSS at 5 and 6 week compared to other treatment group (P< 0.01). CONCLUSION: Histological analysis in G-CSF+ hUCMSC treated traumatic rats exhibited significant increase in numbers of Brdu immunoreactive cells in their traumatic core compared with other labeled group.

Bauersachs, H. G., et al. (2022). "N-methyl-d-aspartate Receptor-mediated Preconditioning Mitigates Excitotoxicity in Human Induced Pluripotent Stem Cell-derived Brain Organoids." Neuroscience **484**: 83-97.

Studies in rodent models of acute and chronic neurodegenerative disorders have uncovered that glutamate-induced excitotoxic cell death is mediated primarily by extrasynaptic N-methyl-d-aspartate receptors (NMDARs). Rodent neurons can also build up in an activity-dependent manner a protective shield against excitotoxicity. This form of acquired neuroprotection is induced by preconditioning with low doses of NMDA or by activation of synaptic NMDARs triggered by bursts of action potentials. Whether NMDARs in human neurons have similar dichotomous actions in cell death and survival is unknown. To investigate this, we established an induced pluripotent stem cell (iPSC)-derived forebrain organoid model for excitotoxic cell death and explored conditions of NMDAR activation that promote neuronal survival when applied prior to a toxic insult. We found that glutamate-induced excitotoxicity in human iPSC-derived neurons is mediated by NMDARs. Treatment of organoids with high concentrations of glutamate or NMDA caused the typical excitotoxicity pathology, comprising structural disintegration, neurite blebbing, shut-off of the transcription factor CRE binding protein (CREB), and cell death. In contrast, bath-applied low doses of NMDA elicited synaptic activity, a robust and sustained increase in CREB phosphorylation as well as function, and upregulation of immediate-early genes, including neuroprotective genes. Moreover, we found that conditions of enhanced synaptic activity increased survival of human iPSC-derived neurons if applied as pre-treatment before toxic NMDA application. These results revealed that both toxic and protective actions of NMDARs are preserved in human neurons. The experimental platform described in this study may prove useful for the validation of neuroprotective gene products and drugs in human neurons.

Beretta, S., et al. (2017). "Effects of Human ES-Derived Neural Stem Cell Transplantation and Kindling in a Rat Model of Traumatic Brain Injury." Cell Transplant **26**(7): 1247-1261.

Traumatic brain injury (TBI) is one of the leading causes of death and disability in the population worldwide, with a broad spectrum of symptoms and disabilities. Posttraumatic hyperexcitability is one of the most common neurological disorders that affect people after a head injury. A reliable animal model of posttraumatic hyperexcitability induced by TBI which allows one to test effective treatment strategies is yet to be developed. To address these issues, in the present study, we tested human embryonic stem cell-derived neural stem cell (NSC) transplantation in an animal model of posttraumatic hyperexcitability in which the brain injury was produced in one hemisphere of

immunodeficient athymic nude rats by controlled cortical impact, and spontaneous seizures were produced by repeated electrical stimulation (kindling) in the contralateral hemisphere. At 14 wk posttransplantation, we report human NSC (hNSC) survival and differentiation into all 3 neural lineages in both sham and injured animals. We observed twice as many surviving hNSCs in the injured versus sham brain, and worse survival on the kindled side in both groups, indicating that kindling/seizures are detrimental to survival or proliferation of hNSCs. We also replicated our previous finding that hNSCs can ameliorate deficits on the novel place recognition task,(33) but such improvements are abolished following kindling. We found no significant differences pre- or post-kindling on the elevated plus maze. No significant correlations were observed between hNSC survival and cognitive performance on either task. Together these findings suggest that Shef6-derived hNSCs may be beneficial as a therapy for TBI, but not in animals or patients with posttraumatic hyperexcitability.

Bhan, A., et al. (2021). "Human induced pluripotent stem cell-derived platelets loaded with lapatinib effectively target HER2+ breast cancer metastasis to the brain." Sci Rep **11**(1): 16866.

Prognosis of patients with HER2+ breast-to-brain-metastasis (BBM) is dismal even after current standard-of-care treatments, including surgical resection, whole-brain radiation, and systemic chemotherapy. Radiation and systemic chemotherapies can also induce cytotoxicity, leading to significant side effects. Studies indicate that donor-derived platelets can serve as immune-compatible drug carriers that interact with and deliver drugs to cancer cells with fewer side effects, making them a promising therapeutic option with enhanced antitumor activity. Moreover, human induced pluripotent stem cells (hiPSCs) provide a potentially renewable source of clinical-grade transfusable platelets that can be drug-loaded to complement the supply of donor-derived platelets. Here, we describe methods for ex vivo generation of megakaryocytes (MKs) and functional platelets from hiPSCs (hiPSC-platelets) in a scalable fashion. We then loaded hiPSC-platelets with lapatinib and infused them into BBM tumor-bearing NOD/SCID mouse models. Such treatment significantly increased intracellular lapatinib accumulation in BBMs in vivo, potentially via tumor cell-induced activation/aggregation. Lapatinib-loaded hiPSC-platelets exhibited normal morphology and function and released lapatinib pH-dependently. Importantly, lapatinib delivery to BBM cells via hiPSC-platelets inhibited tumor growth and prolonged survival of tumor-bearing mice. Overall, use of lapatinib-loaded hiPSC-platelets effectively reduced adverse effects of free lapatinib and enhanced its therapeutic efficacy, suggesting that they represent a novel means to deliver chemotherapeutic drugs as treatment for BBM.

Bhargav, H., et al. (2015). "Effect of Mobile Phone-Induced Electromagnetic Field on Brain Hemodynamics and Human Stem Cell Functioning: Possible Mechanistic Link to Cancer Risk and Early Diagnostic Value of Electronphotonic Imaging." J Stem Cells **10**(4): 287-294.

The mobile phones (MP) are low power radio devices which work on electromagnetic fields (EMFs), in the frequency range of 900-1800 MHz. Exposure to MPEMFs may affect brain physiology and lead to various health hazards including brain tumors. Earlier studies with positron emission tomography (PET) have found alterations in cerebral blood flow (CBF) after acute exposure to MPEMFs. It is widely accepted that DNA double-strand breaks (DSBs) and their misrepair in stem cells are critical events in the multistage origination of various leukemia and tumors, including brain tumors such as gliomas. Both significant misbalance in DSB repair and severe stress response have been triggered by MPEMFs and EMFs from cell towers. It has been shown that stem cells are most sensitive to microwave exposure and react to more frequencies than do differentiated cells. This may be important for cancer risk assessment and indicates that stem cells are the most relevant cellular model for validating safe mobile communication signals. Recently developed technology for recording the human bio-electromagnetic (BEM) field using Electron photonic Imaging (EPI) or Gas Discharge Visualisation (GDV) technique provides useful information about the human BEM. Studies have recorded acute effects of Mobile Phone Electromagnetic Fields (MPEMFs) using EPI and found quantifiable effects on human BEM field. Present manuscript reviews evidences of altered brain physiology and stem cell functioning due to mobile phone/cell tower radiations, its association with increased cancer risk and explores early diagnostic value of EPI imaging in detecting EMF induced changes on human BEM.

Blair, J. D. and H. S. Bateup (2020). "New frontiers in modeling tuberous sclerosis with human stem cell-derived neurons and brain organoids." Dev Dyn **249**(1): 46-55.

Recent advances in human stem cell and genome engineering have enabled the generation of genetically defined human cellular models for brain disorders. These models can be established from a patient's own cells and can be genetically engineered to generate isogenic, controlled systems for mechanistic studies. Given the challenges of obtaining and working

with primary human brain tissue, these models fill a critical gap in our understanding of normal and abnormal human brain development and provide an important complement to animal models. Recently, there has been major progress in modeling the neuropathophysiology of the canonical "mTORopathy" tuberous sclerosis complex (TSC) with such approaches. Studies using two- and three-dimensional cultures of human neurons and glia have provided new insights into how mutations in the TSC1 and TSC2 genes impact human neural development and function. Here we discuss recent progress in human stem cell-based modeling of TSC and highlight challenges and opportunities for further efforts in this area.

Bouyer, C., et al. (2016). "A Bio-Acoustic Levitational (BAL) Assembly Method for Engineering of Multilayered, 3D Brain-Like Constructs, Using Human Embryonic Stem Cell Derived Neuro-Progenitors." Adv Mater **28**(1): 161-167.

A bio-acoustic levitational assembly method for engineering of multilayered, 3D brainlike constructs is presented. Acoustic radiation forces are used to levitate neuroprogenitors derived from human embryonic stem cells in 3D multilayered fibrin tissue constructs. The neuro-progenitor cells are subsequently differentiated in neural cells, resulting in a 3D neuronal construct with inter and intralayer neurite elongations.

Bye, C. R., et al. (2019). "Transcriptional Profiling of Xenogeneic Transplants: Examining Human Pluripotent Stem Cell-Derived Grafts in the Rodent Brain." Stem Cell Reports **13**(5): 877-890.

Human pluripotent stem cells are a valuable resource for transplantation, yet our ability to profile xenografts is largely limited to low-throughput immunohistochemical analysis by difficulties in readily isolating grafts for transcriptomic and/or proteomic profiling. Here, we present a simple methodology utilizing differences in the RNA sequence between species to discriminate xenograft from host gene expression (using qPCR or RNA sequencing [RNA-seq]). To demonstrate the approach, we assessed grafts of undifferentiated human stem cells and neural progenitors in the rodent brain. Xenograft-specific qPCR provided sensitive detection of proliferative cells, and identified germ layer markers and appropriate neural maturation genes across the graft types. Xenograft-specific RNA-seq enabled profiling of the complete transcriptome and an unbiased characterization of graft composition. Such xenograft-specific profiling will be crucial for pre-clinical characterization of grafts and batch-testing of therapeutic cell preparations to ensure safety and functional predictability prior to translation.

Canfield, S. G., et al. (2019). "Correction to: An isogenic neurovascular unit model comprised of human induced pluripotent stem cell-derived brain microvascular endothelial cells, pericytes, astrocytes, and neurons." Fluids Barriers CNS **16**(1): 31.

Following publication of the original article [1], the author has reported that in Figure 1 (b and c) the y-axis TEER ((c) x cm(2)) should be replaced with TEER (Omega x cm(2)).

Canfield, S. G., et al. (2019). "An isogenic neurovascular unit model comprised of human induced pluripotent stem cell-derived brain microvascular endothelial cells, pericytes, astrocytes, and neurons." Fluids Barriers CNS **16**(1): 25.

BACKGROUND: Brain microvascular endothelial cells (BMECs) astrocytes, neurons, and pericytes form the neurovascular unit (NVU). Interactions with NVU cells endow BMECs with extremely tight barriers via the expression of tight junction proteins, a host of active efflux and nutrient transporters, and reduced transcellular transport. To recreate the BMEC-enhancing functions of NVU cells, we combined BMECs, astrocytes, neurons, and brain pericyte-like cells. METHODS: BMECs, neurons, astrocytes, and brain like pericytes were differentiated from human induced pluripotent stem cells (iPSCs) and placed in a Transwell-type NVU model. BMECs were placed in co-culture with neurons, astrocytes, and/or pericytes alone or in varying combinations and critical barrier properties were monitored. RESULTS: Co-culture with pericytes followed by a mixture of neurons and astrocytes (1:3) induced the greatest barrier tightening in BMECs, supported by a significant increase in junctional localization of occludin. BMECs also expressed active P-glycoprotein (PGP) efflux transporters under baseline BMEC monoculture conditions and continued to express baseline active PGP efflux transporters regardless of co-culture conditions. Finally, brain-like pericyte co-culture significantly reduced the rate of non-specific transcytosis across BMECs. CONCLUSIONS: Importantly, each cell type in the NVU model was differentiated from the same donor iPSC source, yielding an isogenic model that could prove enabling for enhanced personalized modeling of the NVU in human health and disease.

Chang, D. J., et al. (2013). "Contralaterally transplanted human embryonic stem cell-derived neural precursor cells (ENStem-A) migrate and improve brain functions in stroke-damaged rats." Exp Mol Med **45**(11): e53.

The transplantation of neural precursor cells (NPCs) is known to be a promising approach to ameliorating behavioral deficits after stroke in a rodent

model of middle cerebral artery occlusion (MCAo). Previous studies have shown that transplanted NPCs migrate toward the infarct region, survive and differentiate into mature neurons to some extent. However, the spatiotemporal dynamics of NPC migration following transplantation into stroke animals have yet to be elucidated. In this study, we investigated the fates of human embryonic stem cell (hESC)-derived NPCs (ENStem-A) for 8 weeks following transplantation into the side contralateral to the infarct region using 7.0T animal magnetic resonance imaging (MRI). T2- and T2*-weighted MRI analyses indicated that the migrating cells were clearly detectable at the infarct boundary zone by 1 week, and the intensity of the MRI signals robustly increased within 4 weeks after transplantation. Afterwards, the signals were slightly increased or unchanged. At 8 weeks, we performed Prussian blue staining and immunohistochemical staining using human-specific markers, and found that high percentages of transplanted cells migrated to the infarct boundary. Most of these cells were CXCR4-positive. We also observed that the migrating cells expressed markers for various stages of neural differentiation, including Nestin, Tuj1, NeuN, TH, DARPP-32 and SV38, indicating that the transplanted cells may partially contribute to the reconstruction of the damaged neural tissues after stroke. Interestingly, we found that the extent of gliosis (glial fibrillary acidic protein-positive cells) and apoptosis (TUNEL-positive cells) were significantly decreased in the cell-transplanted group, suggesting that hESC-NPCs have a positive role in reducing glia scar formation and cell death after stroke. No tumors formed in our study. We also performed various behavioral tests, including rotarod, stepping and modified neurological severity score tests, and found that the transplanted animals exhibited significant improvements in sensorimotor functions during the 8 weeks after transplantation. Taken together, these results strongly suggest that hESC-NPCs have the capacity to migrate to the infarct region, form neural tissues efficiently and contribute to behavioral recovery in a rodent model of ischemic stroke.

Chang, J., et al. (2015). "A meta-analysis of efficacy in pre-clinical human stem cell therapies for traumatic brain injury." Exp Neurol **273**: 225-233.

OBJECTIVES: Evaluate the preclinical evidence for human cell therapies for the treatment of traumatic brain injury (TBI), determine behavioral effect sizes for modified and non-modified cells, and identify variables that correlate with greater effect sizes. METHODS: A literature search identified 58 animal studies of TBI using human stem cells. Each study received a Quality Index (QI) score based on existing guidelines. Effect sizes for cell therapies were determined for the most common behavioral endpoints: Morris Water Maze (MWM) latency/correct quadrant, and modified Neurological Severity Score (mNSS). RESULTS: 50 studies reported significant behavioral and/or histological improvement. The mean effect size for MWM latency was -1.08 for non-modified cells and -3.35 for modified cells. The mean effect size for MWM percent time in the correct quadrant was 1.66 for non-modified cells and 4.36 for modified cells. The mean effect size on the mNSS was -1.56 for non-modified cells and -4.46 for modified cells. No significant associations were found between methodological variables and effect sizes other than route of administration, where intra-lesional delivery resulted in larger effect sizes than i.v. or ventricular delivery. Studies with higher QI had smaller effect sizes; studies with larger effect sizes had greater standard errors. QI was not associated with journal impact factor. CONCLUSIONS: Although human cell therapy studies report improved behavioral outcomes in the majority of preclinical literature, the methods are too heterogeneous to facilitate direct comparisons and bias was detected. Replication and standardization are needed to identify procedural variables to yield the best results. We encourage the use of quality criteria and rigor for future studies of human cell therapy in animal models of TBI.

Charlebois, C., et al. (2022). "Development of a Blood-Brain Barrier Permeability Assay Using Human Induced Pluripotent Stem Cell Derived Brain Endothelial Cells." Methods Mol Biol **2454**: 397-410.

The development of translational and predictive models in vitro for assessing blood-brain barrier (BBB) delivery has become an important requirement in preclinical testing of CNS-targeting therapeutics. Here we describe a directed monolayer differentiation strategy to generate a population of brain endothelial-like cells (BECs) from human induced pluripotent stem cell (iPSC) with robust BBB properties. To generate BBB permeability assays, the BECs are seeded as a monolayer on a semipermeable Transwell insert placed inside a companion plate to generate a two-compartment Transwell model. The BECs provide a BBB-like separation between the luminal (blood) and abluminal (brain) compartments to assess BBB permeability of CNS-targeting therapeutics.

Chen, K. H., et al. (2019). "Human induced pluripotent stem cell-derived mesenchymal stem cell therapy effectively reduced brain infarct volume and preserved neurological function in rat after acute intracranial hemorrhage." Am J Transl Res **11**(9): 6232-6248.

We tested the hypothesis that human induced pluripotent stem cell-derived mesenchymal stem cell (iPSC-MSC) therapy could effectively reduce brain-

infarct volume (BIV) and improve neurological function in rat after acute intracranial hemorrhage (ICH) induced by a weight-drop device. Adult-male SD rats (n=40) were equally divided into group 1 (sham-operated control), group 2 (ICH), group 3 (ICH + hyaluronic acid (HA)/intracranial injection at 3 h after ICH), group 4 [ICH + HA + iPSC-MSC (1.2 x 10(6) cells/intracranial injection at 3 h after ICH)] and euthanized by day 28 after ICH procedure. In vitro study showed that hemorrhagic-brain tissue augmented protein expressions of inflammation (HMGB1/MyD88/TLR-4/TLR-2/NF-kappaB/TNF-alpha/iNOS/IL-1beta) in cultured neurons that were significantly inhibited by iPSC-MSC treatment (all P<0.001). By days 7 and 14 after ICH procedure, circulating inflammatory levels of TNF-alpha/IL-6/MPO expressed were lowest in group 1, highest in group 2 and significantly lower in group 4 than in group 3 (all P<0.0001). By day 14 after ICH procedure, neurological function and BIV expressed an opposite pattern, whereas protein expressions of inflammation (HMGB1/MyD88/TLR-4/TLR-2/NF-kappaB/I-kB/TNF-alpha/iNOS/IL-1beta/MMP-9), oxidative stress (NOX-1/NOX-2/oxidized protein) and apoptosis (mitochondrial-Bax/cleaved-caspase-2/PARP) in brain exhibited an identical pattern to circulating inflammation among the four groups (all P<0.001). Microscopy demonstrated that the number of vascular remodeling and GFAP+/53BP1+/gamma-H2AX+ cells displayed an identical pattern of inflammation, whereas the NeuN+ cells displayed an opposite pattern of inflammation among the four groups (all P<0.001). In conclusion, iPSC-MSC therapy markedly reduced BIV and preserved neurological function mainly by inhibiting inflammatory/oxidative-stress generation.

Chen, K. H., et al. (2020). "Human Umbilical Cord-Derived Mesenchymal Stem Cell Therapy Effectively Protected the Brain Architecture and Neurological Function in Rat After Acute Traumatic Brain Injury." Cell Transplant **29**: 963689720929313.

Intracranial hemorrhage from stroke and head trauma elicits a cascade of inflammatory and immune reactions detrimental to neurological integrity and function at cellular and molecular levels. This study tested the hypothesis that human umbilical cord-derived mesenchymal stem cell (HUCDMSC) therapy effectively protected the brain integrity and neurological function in rat after acute traumatic brain injury (TBI). Adult male Sprague-Dawley rats (n = 30) were equally divided into group 1 (sham-operated control), group 2 (TBI), and group 3 [TBI + HUCDMSC (1.2 x 10(6) cells/intravenous injection at 3 h after TBI)] and euthanized by day 28 after TBI procedure. The results of corner test and inclined plane test showed the neurological function was significantly progressively improved from days 3, 7, 14, and 28 in groups 1 and 3 than in group 2, and group 1 than in group 3 (all P < 0.001). By day 28, brain magnetic resonance imaging brain ischemic volume was significantly increased in group 2 than in group 3 (P < 0.001). The protein expressions of apoptosis [mitochondrial-bax positive cells (Bax)/cleaved-caspase3/cleaved-poly(adenosine diphosphate (ADP)-ribose) polymerase], fibrosis (Smad3 positive cells (Smad3)/transforming growth factor-beta), oxidative stress (NADPH Oxidase 1 (NOX-1)/NADPH Oxidase 2 (NOX-2)/oxidized-protein/cytochrome b-245 alpha chain (p22phox)), and brain-edema/deoxyribonucleic acid (DNA)-damaged biomarkers (Aquaporin-4/gamma H2A histone family member X ( (gamma-H2AX)) displayed an identical pattern to neurological function among the three groups (all P < 0.0001), whereas the protein expressions of angiogenesis biomarkers (vascular endothelial growth factor/stromal cell-derived factor-1alpha/C-X-C chemokine receptor type 4 (CXCR4)) significantly increased from groups 1 to 3 (all P < 0.0001). The cellular expressions of inflammatory biomarkers (cluster of differentiation 14 (+) cells (CD14+)/glial fibrillary acidic protein positive cells (GFAP+)/ a member of a new family of EGF-TM7 molecules positive cells (F4/80+)) and DNA-damaged parameter (gamma-H2AX) exhibited an identical pattern, whereas cellular expressions of neural integrity (hexaribonucleotide Binding Protein-3 positive cells (NeuN+)/nestin+/doublecortin+) exhibited an opposite pattern of neurological function among the three groups (all P < 0.0001). Xenogeneic HUCDMSC therapy was safe and it significantly preserved neurological function and brain architecture in rat after TBI.

Cho, A. N., et al. (2019). "Aligned Brain Extracellular Matrix Promotes Differentiation and Myelination of Human-Induced Pluripotent Stem Cell-Derived Oligodendrocytes." ACS Appl Mater Interfaces **11**(17): 15344-15353.

Myelination by oligodendrocytes (OLs) is a key developmental milestone in terms of the functions of the central nervous system (CNS). Demyelination caused by defects in OLs is a hallmark of several CNS disorders. Although a potential therapeutic strategy involves treatment with the myelin-forming cells, there is no readily available source of these cells. OLs can be differentiated from pluripotent stem cells; however, there is a lack of efficient culture systems that generate functional OLs. Here, we demonstrate biomimetic approaches to promote OL differentiation from human-induced pluripotent stem cells (iPSCs) and to enhance the maturation and myelination capabilities of iPSC-derived OL (iPSC-OL). Functionalization of culture substrates using the brain extracellular matrix (BEM)

derived from decellularized human brain tissue enhanced the differentiation of iPSCs into myelin-expressing OLs. Co-culture of iPSC-OL with induced neuronal (iN) cells on BEM substrates, which closely mimics the in vivo brain microenvironment for myelinated neurons, not only enhanced myelination of iPSC-OL but also improved electrophysiological function of iN cells. BEM-functionalized aligned electrospun nanofibrous scaffolds further promoted the maturation of iPSC-OLs, enhanced the production of myelin sheath-like structures by the iPSC-OL, and enhanced the neurogenesis of iN cells. Thus, the biomimetic strategy presented here can generate functional OLs from stem cells and facilitate myelination by providing brain-specific biochemical, biophysical, and structural signals. Our system comprising stem cells and brain tissue from human sources could help in the establishment of human demyelination disease models and the development of regenerative cell therapy for myelin disorders.

Cho, Y. H., et al. (2006). "The behavioral effect of human mesenchymal stem cell transplantation in cold brain injured rats." Acta Neurochir Suppl **99**: 125-132.

We investigated the effect of stereotaxically transplanted human mesenchymal stem cells (hMSCs) on behavioral change after traumatic cold brain injury in adult rats. Cortical lesions (n= 20) were induced by touching a metal stamp, cooled with liquid nitrogen, to the dura over the forelimb motor cortex of adult rats. The procedure produced a localized lesion, and the animals showed significant motor deficits. hMSCs were freshly isolated from human iliac bone and cultured in tissue culture flasks with 10 ml Dulbecco's modified Eagle's medium. The animals received hMSC grafts (3 x 10(5) hMSCs) 6 days after cold lesion (n = 10). All rats were sacrificed 3 or 7 weeks after cold injury, and immunohistochemical staining was performed on brain sections to identify donor hMSCs. Neurological evaluations were performed with the forepaw adjusting step test and modified neurological scoring. Treatment with 3 x 10(5) hMSCs improved the rat's neurological functions. We also found that the transplanted cells successfully migrated into the injured brain, preferentially localized around the injury site, and expressed the neuronal and astrocyte marker. These data suggest that hMSCs may be a potential therapeutic tool for brain injuries.

Choe, M. S., et al. (2020). "A simple metastatic brain cancer model using human embryonic stem cell-derived cerebral organoids." FASEB J **34**(12): 16464-16475.

Every year, hundreds of thousands of people die because of metastatic brain cancer. Most metastatic cancer research uses 2D cell culture or animal models, but they have a few limitations, such as difficulty reproducing human tissue structures. This study developed a simple 3D in vitro model to better replicate brain metastasis using human cancer cells and human embryonic stem cell-derived cerebral organoids (metastatic brain cancer cerebral organoid [MBCCO]). The MBCCO model successfully reproduced metastatic cancer processes, including cell adhesion, proliferation, and migration, in addition to cell-cell interactions. Using the MBCCO model, we demonstrated that lung-specific X protein (LUNX) plays an important role in cell proliferation and migration or invasion. We also observed astrocyte accumulation around and their interaction with cancer cells through connexin 43 in the MBCCO model. We analyzed whether the MBCCO model can be used to screen drugs by measuring the effects of gefitinib, a well-known anticancer agent. We also examined the toxicity of gefitinib using normal cerebral organoids (COs). Therefore, the MBCCO model is a powerful tool for modeling human metastatic brain cancer in vitro and can also be used to screen drugs.

Clark, P. A., et al. (2016). "Analysis of Cancer-Targeting Alkylphosphocholine Analogue Permeability Characteristics Using a Human Induced Pluripotent Stem Cell Blood-Brain Barrier Model." Mol Pharm **13**(9): 3341-3349.

Cancer-targeting alkylphosphocholine (APC) analogues are being clinically developed for diagnostic imaging, intraoperative visualization, and therapeutic applications. These APC analogues derived from chemically synthesized phospholipid ethers were identified and optimized for cancer-targeting specificity using extensive structure-activity studies. While they strongly label human brain cancers associated with disrupted blood-brain barriers (BBB), APC permeability across intact BBB remains unknown. Three of our APC analogues, CLR1404 (PET radiotracer), CLR1501 (green fluorescence), and CLR1502 (near-infrared fluorescence), were tested for permeability across a BBB model composed of human induced pluripotent stem cell-derived brain microvascular endothelial cells (iPSC-derived BMECs). This in vitro BBB system has reproducibly consistent high barrier integrity marked by high transendothelial electrical resistance (TEER > 1500 Omega-cm(2)) and functional expression of drug efflux transporters. The radioiodinated and fluorescent APC analogues demonstrated fairly low permeability across the iPSC-BMEC (35 +/- 5.7 (CLR1404), 54 +/- 3.2 (CLR1501), and 26 +/- 4.9 (CLR1502) x 10(-5) cm/min) compared with BBB-impermeable sucrose (13 +/- 2.5) and BBB-permeable diazepam (170 +/- 29). Only the fluorescent APC analogues (CLR1501, CLR1502) underwent BCRP and MRP polarized drug efflux transport in the

brain-to-blood direction of the BBB model, and this efflux can be specifically blocked with pharmacological inhibition. None of the tested APC analogues appeared to undergo substantial P-gp transport. Limited permeability of the APC analogues across an intact BBB into normal brain likely contributes to the high tumor to background ratios observed in initial human trials. Moreover, addition of fluorescent moieties to APCs resulted in greater BMEC efflux via MRP and BCRP, and may affect fluorescence-guided applications. Overall, the characterization of APC analogue permeability across human BBB is significant for advancing future brain tumor-targeted applications of these agents.

Clervius, H., et al. (2019). "Human neural stem cell transplants to address multiple pathologies associated with traumatic brain injury." Neural Regen Res **14**(10): 1699-1700.

Coletti, A. M., et al. (2018). "Characterization of the ventricular-subventricular stem cell niche during human brain development." Development **145**(20).

Human brain development proceeds via a sequentially transforming stem cell population in the ventricular-subventricular zone (V-SVZ). An essential, but understudied, contributor to V-SVZ stem cell niche health is the multi-ciliated ependymal epithelium, which replaces stem cells at the ventricular surface during development. However, reorganization of the V-SVZ stem cell niche and its relationship to ependymogenesis has not been characterized in the human brain. Based on comprehensive comparative spatiotemporal analyses of cytoarchitectural changes along the mouse and human ventricle surface, we uncovered a distinctive stem cell retention pattern in humans as ependymal cells populate the surface of the ventricle in an occipital-to-frontal wave. During perinatal development, ventricle-contacting stem cells are reduced. By 7 months few stem cells are detected, paralleling the decline in neurogenesis. In adolescence and adulthood, stem cells and neurogenesis are not observed along the lateral wall. Volume, surface area and curvature of the lateral ventricles all significantly change during fetal development but stabilize after 1 year, corresponding with the wave of ependymogenesis and stem cell reduction. These findings reveal normal human V-SVZ development, highlighting the consequences of disease pathologies such as congenital hydrocephalus.

Cui, L., et al. (2022). "Human umbilical cord mesenchymal stem cell-derived exosomes promote neurological function recovery in rat after traumatic brain injury by inhibiting the activation of microglia and astrocyte." Regen Ther **21**: 282-287.

Traumatic brain injury (TBI) is a serious neurological disorder with increasing worldwide incidence. Emerging evidence has shown a significant therapeutic role of mesenchymal stem cells (MSCs) derived exosomes on traumatic brain injury with broad application prospects as a cell-free therapy. However, a comprehensive understanding of its underlying mechanism remained elusive. In this study, umbilical cord mesenchymal stem cells (UCMSCs)-derived exosomes (UC-MSCs-Exo) were isolated by ultracentrifugation and injected intraventricularly in a rat model of TBI. Our results showed that UC-MSCs-Exo promoted functional recovery and reduced neuronal apoptosis in TBI rats. Moreover, UC-MSCs-Exo inhibited the activation of microglia and astrocytes during brain injury, thereby promoting functional recovery. However, the effect of UC-MSCs-Exo on the content of plasma inflammatory factors in rats was not significant. Collectively our study suggested that UC-MSCs-Exo promotes the recovery of neurological function in TBI rats by inhibiting the activation of microglia and astrocytes, providing a theoretical basis for new therapeutic strategies for central nervous system diseases.

Daadi, M. M., et al. (2010). "Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury." Stroke **41**(3): 516-523.

BACKGROUND AND PURPOSE: Hypoxic-ischemic (HI) brain injury in newborn infants represents a major cause of cerebral palsy, development delay, and epilepsy. Stem cell-based therapy has the potential to rescue and replace the ischemic tissue caused by HI and to restore function. However, the mechanisms by which stem cell transplants induce functional recovery are yet to be elucidated. In the present study, we sought to investigate the efficacy of human neural stem cells derived from human embryonic stem cells in a rat model of neonatal HI and the mechanisms enhancing brain repair. METHODS: The human neural stem cells were genetically engineered for in vivo molecular imaging and for postmortem histological tracking. Twenty-four hours after the induction of HI, animals were grafted with human neural stem cells into the forebrain. Motor behavioral tests were performed the fourth week after transplantation. We used immunocytochemistry and neuroanatomical tracing to analyze neural differentiation, axonal sprouting, and microglia response. Treatment-induced changes in gene expression were investigated by microarray and quantitative polymerase chain reaction. RESULTS: Bioluminescence imaging permitted real time longitudinal tracking of grafted human neural stem cells. HI transplanted animals significantly improved in

their use of the contralateral impeded forelimb and in the Rotorod test. The grafts showed good survival, dispersion, and differentiation. We observed an increase of uniformly distributed microglia cells in the grafted side. Anterograde neuroanatomical tracing demonstrated significant contralesional sprouting. Microarray analysis revealed upregulation of genes involved in neurogenesis, gliogenesis, and neurotrophic support. CONCLUSIONS: These results suggest that human neural stem cell transplants enhance endogenous brain repair through multiple modalities in response to HI.

Daadi, M. M., et al. (2009). "Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain." Mol Ther **17**(7): 1282-1291.

Real-time imaging of transplanted stem cells is essential for understanding their interactions in vivo with host environments, for tracking cell fate and function and for successful delivery and safety monitoring in the clinical setting. In this study, we used bioluminescence (BLI) and magnetic resonance imaging (MRI) to visualize the fate of grafted human embryonic stem cell (hESC)-derived human neural stem cells (hNSCs) in stroke-damaged rat brain. The hNSCs were genetically engineered with a lentiviral vector carrying a double fusion (DF) reporter gene that stably expressed enhanced green fluorescence protein (eGFP) and firefly luciferase (fLuc) reporter genes. The hNSCs were self-renewable, multipotent, and expressed markers for neural stem cells. Cell survival was tracked noninvasively by MRI and BLI for 2 months after transplantation and confirmed histologically. Electrophysiological recording from grafted GFP(+) cells and immuno-electronmicroscopy demonstrated connectivity. Grafted hNSCs differentiated into neurons, into oligodendrocytes in stroke regions undergoing remyelination and into astrocytes extending processes toward stroke-damaged vasculatures. Our data suggest that the combination of BLI and MRI modalities provides reliable real-time monitoring of cell fate.

Dabrowska, S., et al. (2019). "Human bone marrow mesenchymal stem cell-derived extracellular vesicles attenuate neuroinflammation evoked by focal brain injury in rats." J Neuroinflammation **16**(1): 216.

BACKGROUND: Ischemic stroke is the major cause of long-term severe disability and death in aged population. Cell death in the infarcted region of the brain induces immune reaction leading to further progression of tissue damage. Immunomodulatory function of mesenchymal stem cells (MSCs) has been shown in multiple preclinical studies; however, it has not been successfully translated to a routine clinical practice due to logistical, economical, regulatory, and intellectual property obstacles. It has been recently demonstrated that therapeutic effect of intravenously administered MSCs can be recapitulated by extracellular vesicles (EVs) derived from them. However, in contrast to MSCs, EVs were not capable to decrease stroke-induced neuroinflammation. Therefore, the aim of the study was to investigate if intra-arterial delivery of MSC-derived EVs will have stronger impact on focal brain injury-induced neuroinflammation, which mimics ischemic stroke, and how it compares to MSCs. METHODS: The studies were performed in adult male Wistar rats with focal brain injury induced by injection of 1 mul of 50 nmol ouabain into the right hemisphere. Two days after brain insult, 5 x 10(5) human bone marrow MSCs (hBM-MSCs) labeled with Molday ION or 1.3 x 10(9) EVs stained with PKH26 were intra-arterially injected into the right hemisphere under real-time MRI guidance. At days 1, 3, and 7 post-transplantation, the rats were decapitated, the brains were removed, and the presence of donor cells or EVs was analyzed. The cellular immune response in host brain was evaluated immunohistochemically, and humoral factors were measured by multiplex immunoassay. RESULTS: hBM-MSCs and EVs transplanted intra-arterially were observed in the rat ipsilateral hemisphere, near the ischemic region. Immunohistochemical analysis of brain tissue showed that injection of hBM-MSCs or EVs leads to the decrease of cell activation by ischemic injury, i.e., astrocytes, microglia, and infiltrating leucocytes, including T cytotoxic cells. Furthermore, we observed significant decrease of pro-inflammatory cytokines and chemokines after hBM-MSC or EV infusion comparing with non-treated rats with focal brain injury. CONCLUSIONS: Intra-arterially injected EVs attenuated neuroinflammation evoked by focal brain injury, which mimics ischemic stroke, and this effect was comparable to intra-arterial hBM-MSC transplantation. Thus, intra-arterial injection of EVs might be an attractive therapeutic approach, which obviates MSC-related obstacles.

Darkazalli, A., et al. (2016). "Use of human mesenchymal stem cell treatment to prevent anhedonia in a rat model of traumatic brain injury." Restor Neurol Neurosci **34**(3): 433-441.

PURPOSE: Major depression and related mood disorders are the most common long-term outcomes associated with traumatic brain injury (TBI). Given the potentially debilitating consequences of depression, and the fact that TBI patients are frequently refractory to antidepressant drugs, new therapies are clearly needed. We hypothesized that human bone marrow-derived mesenchymal stem cells (hMSC), delivered intravenously, can effectively treat TBI-

induced depression and other behavioral deficits associated with TBI. METHODS: Rats (n = 8 per group) were subjected to experimental TBI or control sham operation. Six hours post TBI, rats were treated with 1x106 hMSC or vehicle control. Immediately after TBI and prior to hMSC or control treatment, rats were subjected to either targeted precision x-ray irradiation to eliminate subventricular zone (SVZ) proliferation or sham irradiation. One week after TBI, SVZ irradiation, and hMSC treatment, rats were evaluated for the depression-like behavior, anhedonia, using the two-bottle saccharin preference paradigm; and for working memory using the novel object recognition test. RESULTS: TBI resulted in a 54% (p</=0.05) decrease in saccharin preference scores while treatment of TBI with hMSC fully prevented this anhedonic behavior. TBI was also found to produce a 73% (p</=0.05) decrease in novel object interaction time, indicating impaired working memory, and was similarly improved by treatment with hMSC. The ability of hMSC to prevent TBI-associated depression and working memory impairment was eliminated when SVZ proliferation was inhibited by irradiation. CONCLUSIONS: This work has identified a possible role for hMSC in the treatment of TBI-induced depression and other behaviors and suggests a mechanistic role for proliferative cells of the SVZ proliferation in hMSC efficacy.

Darkazalli, A., et al. (2017). "Human Mesenchymal Stem Cell Treatment Normalizes Cortical Gene Expression after Traumatic Brain Injury." J Neurotrauma **34**(1): 204-212.

Traumatic brain injury (TBI) results in a progressive disease state with many adverse and long-term neurological consequences. Mesenchymal stem cells (MSCs) have emerged as a promising cytotherapy and have been previously shown to reduce secondary apoptosis and cognitive deficits associated with TBI. Consistent with the established literature, we observed that systemically administered human MSCs (hMSCs) accumulate with high specificity at the TBI lesion boundary zone known as the penumbra. Substantial work has been done to illuminate the mechanisms by which MSCs, and the bioactive molecules they secrete, exert their therapeutic effect. However, no such work has been published to examine the effect of MSC treatment on gene expression in the brain post-TBI. In the present study, we use high-throughput RNA sequencing (RNAseq) of cortical tissue from the TBI penumbra to assess the molecular effects of both TBI and subsequent treatment with intravenously delivered hMSCs. RNAseq revealed that expression of almost 7000 cortical genes in the penumbra were differentially regulated by TBI. Pathway analysis using the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database revealed that TBI regulated a large number of genes belonging to pathways involved in metabolism, receptor-mediated cell signaling, neuronal plasticity, immune cell recruitment and infiltration, and neurodegenerative disease. Remarkably, hMSC treatment was found to normalize 49% of all genes disrupted by TBI, with notably robust normalization of specific pathways within the categories mentioned above, including neuroactive receptor-ligand interactions (57%), glycolysis and gluconeogenesis (81%), and Parkinson's disease (100%). These data provide evidence in support of the multi-mechanistic nature of stem cell therapy and suggest that hMSC treatment is capable of simultaneously normalizing a wide variety of important molecular pathways that are disrupted by brain injury.

Darsalia, V., et al. (2011). "Cell number and timing of transplantation determine survival of human neural stem cell grafts in stroke-damaged rat brain." J Cereb Blood Flow Metab **31**(1): 235-242.

Neural stem cells (NSCs) derived from human fetal striatum and transplanted as neurospheres survive in stroke-damaged striatum, migrate from the implantation site, and differentiate into mature neurons. Here, we investigated how various steps of neurogenesis are affected by intrastriatal transplantation of human NSCs at different time points after stroke and with different numbers of cells in each implant. Rats were subjected to middle cerebral artery occlusion and then received intrastriatal transplants of NSCs. Transplantation shortly after stroke (48 hours) resulted in better cell survival than did transplantation 6 weeks after stroke, but the delayed transplantation did not influence the magnitude of migration, neuronal differentiation, and cell proliferation in the grafts. Transplanting greater numbers of grafted NSCs did not result in a greater number of surviving cells or increased neuronal differentiation. A substantial number of activated microglia was observed at 48 hours after the insult in the injured striatum, but reached maximum levels 1 to 6 weeks after stroke. Our findings show that the best survival of grafted human NSCs in stroke-damaged brain requires optimum numbers of cells to be transplanted in the early poststroke phase, before the inflammatory response is established. These findings, therefore, have direct clinical implications.

Del Fiacco, M., et al. (2002). "Topographical localization of glial cell line-derived neurotrophic factor in the human brain stem: an immunohistochemical study of prenatal, neonatal and adult brains." J Chem Neuroanat **23**(1): 29-48.

As a step towards the identification of the neuronal populations responsive to glial cell line-

derived neurotrophic factor (GDNF) in the human nervous system and their changes with age, this study reports on the immunohistochemical localization of the protein GDNF in the autoptic normal human brain stem of pre- and full-term newborns and adult subjects. Two different anti-GDNF polyclonal antibodies were used. Western blot analysis on homogenates of human and rat brain and recombinant human GDNF resulted in differential detection of monomeric and dimeric forms of the proteins. The ABC immunohistochemical technique on cryostat tissue sections showed an uneven distribution of GDNF-like immunoreactive nerve fibers and terminals and neuronal cell bodies. Immunoreactive elements were mainly localized to the spinal trigeminal, cuneate, solitary, vestibular, and cochlear sensory nuclei, dorsal motor nucleus of the vagus nerve, ventral grey column, hypoglossal nucleus, dorsal and ventrolateral medullary reticular formation, pontine subventricular grey and locus coeruleus, lateral regions of the rostral pontine tegmentum, tectal plate, trochlear nucleus, dorsal and median raphe nuclei, caudal and rostral linear nuclei, cuneiform nucleus, and substantia nigra. Comparison between pre- and full-term newborns and adult subjects revealed changes with age in density of positive innervation and frequency of immunoreactive perikarya. The results obtained provide detailed information on the occurrence of GDNF-like immunoreactive neurons in the human brain stem and suggest that the protein is present in a variety of neuronal systems, which subserve different functional activities, at developmental ages and in adult brains.

Di Marco, A., et al. (2020). "Establishment of an in Vitro Human Blood-Brain Barrier Model Derived from Induced Pluripotent Stem Cells and Comparison to a Porcine Cell-Based System." Cells **9**(4).

The blood-brain barrier (BBB) is responsible for the homeostasis between the cerebral vasculature and the brain and it has a key role in regulating the influx and efflux of substances, in healthy and diseased states. Stem cell technology offers the opportunity to use human brain-specific cells to establish in vitro BBB models. Here, we describe the establishment of a human BBB model in a two-dimensional monolayer culture, derived from human induced pluripotent stem cells (hiPSCs). This model was characterized by a transendothelial electrical resistance (TEER) higher than 2000 Omega·cm(2) and associated with negligible paracellular transport. The hiPSC-derived BBB model maintained the functionality of major endothelial transporter proteins and receptors. Some proprietary molecules from our central nervous system (CNS) programs were evaluated revealing comparable permeability in the human model and in the model from primary porcine brain endothelial cells (PBECs).

Durens, M., et al. (2020). "High-throughput screening of human induced pluripotent stem cell-derived brain organoids." J Neurosci Methods **335**: 108627.

BACKGROUND: The need for scalable high-throughput screening (HTS) approaches for 3D human stem cell platforms remains a central challenge for disease modeling and drug discovery. We have developed a workflow to screen cortical organoids across platforms. NEW METHOD: We used serum-free embryoid bodies (SFEBs) derived from human induced pluripotent stem cells (hiPSCs) and employed high-content imaging (HCI) to assess neurite outgrowth and cellular composition within SFEBs. We multiplexed this screening assay with both multi-electrode arrays (MEAs) and single-cell calcium imaging. RESULTS: HCI was used to assess the number of excitatory neurons (VGlut(+)) in experimental replicates of hiPSC-derived SFEBs, demonstrating experiment-to-experiment consistency. Neurite detection using HCI was applied to assess neurite morphology. MEA analysis showed that firing and burst rates in SFEBs decreased with blockade of NMDARs and AMPARs and increased with GABAR blockade. We also demonstrate effective combination of both MEA and HCI to analyze VGlut(+) populations surrounding electrodes within MEAs. HCI-based (Ca(2+)) transient analysis revealed firing in individual cells surrounding active MEA electrodes. COMPARISON WITH EXISTING METHODS: Current methods to generate neural organoids show high degrees of variability, and often require sectioning or special handling for analysis. The protocol outlined in this manuscript generates SFEBs with high degree of consistency making them amenable to complex assays combining HTS and electrophysiology allowing for an in-depth, unbiased analysis. CONCLUSIONS: SFEBs can be used in combination with HTS to compensate for experimental variability common in 3D cultures, while significantly decreasing processing speed, making this an efficient starting point for phenotypic drug screening.

Espuny-Camacho, I., et al. (2017). "Hallmarks of Alzheimer's Disease in Stem-Cell-Derived Human Neurons Transplanted into Mouse Brain." Neuron **93**(5): 1066-1081 e1068.

Human pluripotent stem cells (PSCs) provide a unique entry to study species-specific aspects of human disorders such as Alzheimer's disease (AD). However, in vitro culture of neurons deprives them of their natural environment. Here we transplanted human PSC-derived cortical neuronal precursors into the brain of a murine AD model. Human neurons differentiate and integrate into the brain, express 3R/4R Tau splice forms, show abnormal phosphorylation and

conformational Tau changes, and undergo neurodegeneration. Remarkably, cell death was dissociated from tangle formation in this natural 3D model of AD. Using genome-wide expression analysis, we observed upregulation of genes involved in myelination and downregulation of genes related to memory and cognition, synaptic transmission, and neuron projection. This novel chimeric model for AD displays human-specific pathological features and allows the analysis of different genetic backgrounds and mutations during the course of the disease.

Fattorelli, N., et al. (2021). "Stem-cell-derived human microglia transplanted into mouse brain to study human disease." Nat Protoc **16**(2): 1013-1033.

Microglia are critically involved in complex neurological disorders with a strong genetic component, such as Alzheimer's disease, Parkinson's disease and frontotemporal dementia. Although mouse microglia can recapitulate aspects of human microglia physiology, they do not fully capture the human genetic aspects of disease and do not reproduce all human cell states. Primary cultures of human microglia or microglia derived from human induced pluripotent stem cells (PSCs) are difficult to maintain in brain-relevant cell states in vitro. Here we describe MIGRATE (microglia in vitro generation refined for advanced transplantation experiments, which provides a combined in vitro differentiation and in vivo xenotransplantation protocol to study human microglia in the context of the mouse brain. This article details an accurate, step-by-step workflow that includes in vitro microglia differentiation from human PSCs, transplantation into the mouse brain and quantitative analysis of engraftment. Compared to current differentiation and xenotransplantation protocols, we present an optimized, faster and more efficient approach that yields up to 80% chimerism. To quantitatively assess engraftment efficiency by flow cytometry, access to specialized flow cytometry is required. Alternatively, the percentage of chimerism can be estimated by standard immunohistochemical analysis. The MIGRATE protocol takes ~40 d to complete, from culturing PSCs to engraftment efficiency assessment.

Feng, Y., et al. (2014). "The involvement of CXCL11 in bone marrow-derived mesenchymal stem cell migration through human brain microvascular endothelial cells." Neurochem Res **39**(4): 700-706.

Bone marrow-derived mesenchymal stem cells (MSCs) transplant into the brain, where they play a potential therapeutic role in neurological diseases. However, the blood-brain barrier (BBB) is a native obstacle for MSCs entry into the brain. Little is known about the mechanism behind MSCs migration across the BBB. In the present study, we modeled the interactions between human MSCs (hMSCs) and human brain microvascular endothelial cells (HBMECs) to mimic the BBB microenvironment. Real-time PCR analysis indicated that the chemokine CXCL11 is produced by hMSCs and the chemokine receptor CXCR3 is expressed on HBMECs. Further results indicate that CXCL11 secreted by hMSCs may interact with CXCR3 on HBMECs to induce the disassembly of tight junctions through the activation of ERK1/2 signaling in the endothelium, which promotes MSCs transendothelial migration. These findings are relevant for understanding the biological responses of MSCs in BBB environments and helpful for the application of MSCs in neurological diseases.

Froslev, P., et al. (2022). "Highly cationic cell-penetrating peptides affect the barrier integrity and facilitates mannitol permeation in a human stem cell-based blood-brain barrier model." Eur J Pharm Sci **168**: 106054.

The blood-brain barrier (BBB) allows passive permeation of only a limited number of, primarily lipophilic, low-molecular weight drugs that obey the so-called "rule of CNS likeness". Therefore, novel strategies to facilitate drug delivery across the BBB are needed. Cell-penetrating peptides (CPPs) enable delivery of various therapeutic cargoes into cells and may potentially serve as shuttles for delivery of brain-specific drugs across the BBB. The CPPs Tat(47-57) and penetratin are prototypical cationic CPPs, whereas apidaecin and oncocin belong to the group of proline-rich cationic antimicrobial peptides displaying CPP-like properties. The aim of the present study was to investigate the potential of Tat(47-57), penetratin, apidaecin, and oncocin for interaction with and permeation of the BBB in vitro. We also studied whether the CPPs facilitated permeation of the paracellular flux marker mannitol as well as the transcellular flux marker propranolol. The peptides were labelled with the fluorophore 6-TAMRA (T) for visualization and quantification purposes. CPP membrane-adherence, membrane-embedding, and cellular uptake as well as barrier-permeation were evaluated in murine brain capillary endothelial cells (bEND3) and human induced pluripotent stem cell-derived (Bioni-010c) brain capillary endothelial-like monolayers. The cationic and the proline-rich cationic CPPs were taken up into the Bioni-010c monolayers. T-Tat(47-57), T-apidaecin, and T-oncocin also permeated Bioni-010c monolayers, whereas T-penetratin did not. However, both T-Tat(47-57) and T-penetratin affected the barrier integrity to a degree that facilitated permeation of (14)C-mannitol. These results may therefore pave the way for future CPP-mediated brain delivery of small drugs that do not obey the "rule of CNS likeness".

Furmanski, O., et al. (2019). "Controlled Cortical Impact Model of Mouse Brain Injury with Therapeutic Transplantation of Human Induced Pluripotent Stem Cell-Derived Neural Cells." J Vis Exp(149).

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality worldwide. Disease pathology due to TBI progresses from the primary mechanical insult to secondary injury processes, including apoptosis and inflammation. Animal modeling has been valuable in the search to unravel injury mechanisms and evaluate potential neuroprotective therapies. This protocol describes the controlled cortical impact (CCI) model of focal, open-head TBI. Specifically, parameters for producing a mild unilateral cortical injury are described. Behavioral consequences of CCI are analyzed using the adhesive tape removal test of bilateral sensorimotor integration. Regarding experimental therapy for TBI pathology, this protocol also illustrates a process for transplanting cultured cells into the brain. Neural cell cultures derived from human induced pluripotent stem cells (hiPSCs) were chosen for their potential to show superior functional restoration in human TBI patients. Chronic survival of hiPSCs in the host mouse brain tissue is detected using a modified DAB immunohistochemical process.

Gallagher, E., et al. (2016). "In vitro characterization of pralidoxime transport and acetylcholinesterase reactivation across MDCK cells and stem cell-derived human brain microvascular endothelial cells (BC1-hBMECs)." Fluids Barriers CNS **13**(1): 10.

BACKGROUND: Current therapies for organophosphate poisoning involve administration of oximes, such as pralidoxime (2-PAM), that reactivate the enzyme acetylcholinesterase. Studies in animal models have shown a low concentration in the brain following systemic injection. METHODS: To assess 2-PAM transport, we studied transwell permeability in three Madin-Darby canine kidney (MDCKII) cell lines and stem cell-derived human brain microvascular endothelial cells (BC1-hBMECs). To determine whether 2-PAM is a substrate for common brain efflux pumps, experiments were performed in the MDCKII-MDR1 cell line, transfected to overexpress the P-gp efflux pump, and the MDCKII-FLuc-ABCG2 cell line, transfected to overexpress the BCRP efflux pump. To determine how transcellular transport influences enzyme reactivation, we developed a modified transwell assay where the inhibited acetylcholinesterase enzyme, substrate, and reporter are introduced into the basolateral chamber. Enzymatic activity was inhibited using paraoxon and parathion. RESULTS: The permeability of 2-PAM is about 2 x 10(-6) cm s(-1) in MDCK cells and about 1 x 10(-6) cm s(-1) in BC1-hBMECs. Permeability is not influenced by pre-treatment with atropine. In addition, 2-PAM is not a substrate for the P-gp or BCRP efflux pumps. CONCLUSIONS: The low permeability explains poor brain penetration of 2-PAM and therefore the slow enzyme reactivation. This elucidates one of the reasons for the necessity of sustained intravascular (IV) infusion in response to organophosphate poisoning.

Gao, J., et al. (2016). "Human Neural Stem Cell Transplantation-Mediated Alteration of Microglial/Macrophage Phenotypes after Traumatic Brain Injury." Cell Transplant **25**(10): 1863-1877.

Neural stem cells (NSCs) promote recovery from brain trauma, but neuronal replacement is unlikely the sole underlying mechanism. We hypothesize that grafted NSCs enhance neural repair at least partially through modulating the host immune response after traumatic brain injury (TBI). C57BL/6 mice were intracerebrally injected with primed human NSCs (hNSCs) or vehicle 24 h after a severe controlled cortical impact injury. Six days after transplantation, brain tissues were collected for Western blot and immunohistochemical analyses. Observations included indicators of microglia/macrophage activation, M1 and M2 phenotypes, axonal injury detected by amyloid precursor protein (APP), lesion size, and the fate of grafted hNSCs. Animals receiving hNSC transplantation did not show significant decreases of brain lesion volumes compared to transplantation procedures with vehicle alone, but did show significantly reduced injury-dependent accumulation of APP. Furthermore, intracerebral transplantation of hNSCs reduced microglial activation as shown by a diminished intensity of Iba1 immunostaining and a transition of microglia/macrophages toward the M2 anti-inflammatory phenotype. The latter was represented by an increase in the brain M2/M1 ratio and increases of M2 microglial proteins. These phenotypic switches were accompanied by the increased expression of anti-inflammatory interleukin-4 receptor alpha and decreased proinflammatory interferon-gamma receptor beta. Finally, grafted hNSCs mainly differentiated into neurons and were phagocytized by either M1 or M2 microglia/macrophages. Thus, intracerebral transplantation of primed hNSCs efficiently leads host microglia/macrophages toward an anti-inflammatory phenotype that presumably contributes to stem cell-mediated neuroprotective effects after severe TBI in mice.

Gao, X., et al. (2021). "Protective effect of human umbilical cord mesenchymal stem cell-derived exosomes on rat retinal neurons in hyperglycemia

through the brain-derived neurotrophic factor/TrkB pathway." Int J Ophthalmol **14**(11): 1683-1689.

AIM: To explore whether human umbilical cord mesenchymal stem cell (hUCMSC)-derived exosomes (hUCMSC-Exos) protect rat retinal neurons in high-glucose (HG) conditions by activating the brain-derived neurotrophic factor (BDNF)-TrkB pathway. METHODS: hUCMSC-Exos were collected with differential ultracentrifugation methods and observed by transmission electron microscopy. Enzyme-linked immunosorbent assays (ELISAs) was used to quantify BDNF in hUCMSC-Exos, and Western blot was used to identify surface markers of hUCMSC-Exos. Rat retinal neurons were divided into 4 groups. Furthermore, cell viability, cell apoptosis, and TrkB protein expression were measured in retinal neurons. RESULTS: hUCMSCs and isolated hUCMSC-Exos were successfully cultured. All hUCMSC-Exos showed a diameter of 30 to 150 nm and had a phospholipid bimolecular membrane structure, as observed by transmission electron microscopy. ELISA showed the BDNF concentration of hUCMSCs-Exos was 2483.16+/-281.75. hUCMSCs-Exos effectively reduced the apoptosis of retinal neuron rate and improved neuron survival rate, meanwhile, the results of immunofluorescence verified the fluorescence intensity of TrKB in neurons increased. And all above effects were reduced by treated hUCMSCs-Exos with BDNF inhibitors. hUCMSC-Exos effectively reduced the apoptosis rate of retinal neurons by activating the BDNF-TrkB pathway in a HG environment. CONCLUSION: In the HG environment, hUCMSC-Exos could carry BDNF into rat retinal neurons, inhibiting neuronal apoptosis by activating the BDNF-TrkB pathway.

Gastfriend, B. D., et al. (2021). "Differentiation of Brain Pericyte-Like Cells from Human Pluripotent Stem Cell-Derived Neural Crest." Curr Protoc **1**(1): e21.

Brain pericytes regulate diverse aspects of neurovascular development and function, including blood-brain barrier (BBB) induction and maintenance. Primary brain pericytes have been widely employed in coculture-based in vitro models of the BBB, and a method to generate brain pericytes from human pluripotent stem cells (hPSCs) could provide a renewable, genetically tractable source of cells for BBB modeling and studying pericyte roles in development and disease. Here, we describe a protocol to differentiate hPSCs to NG2(+) PDGFRbeta(+) alphaSMA(low) brain pericyte-like cells in 22-25 days through a p75-NGFR(+) HNK-1(+) neural crest intermediate, which mimics the developmental origin of forebrain pericytes. The resulting brain pericyte-like cells have molecular and functional attributes of brain pericytes. We also provide protocols for maintenance, cryopreservation, and recovery of the neural crest intermediate, and for molecular and functional characterization of the resulting cells. (c) 2021 Wiley Periodicals LLC. Basic Protocol 1: Differentiation of hPSCs to neural crest Basic Protocol 2: Differentiation of neural crest to brain pericyte-like cells Support Protocol 1: Flow cytometry analysis of neural crest cells Support Protocol 2: Maintenance, cryopreservation, and recovery of neural crest cells Support Protocol 3: Molecular characterization of brain pericyte-like cells Support Protocol 4: Cord formation assay with endothelial cells and brain pericyte-like cells.

Girard, S. D., et al. (2023). "High and low permeability of human pluripotent stem cell-derived blood-brain barrier models depend on epithelial or endothelial features." FASEB J **37**(2): e22770.

The search for reliable human blood-brain barrier (BBB) models represents a challenge for the development/testing of strategies aiming to enhance brain delivery of drugs. Human-induced pluripotent stem cells (hiPSCs) have raised hopes in the development of predictive BBB models. Differentiating strategies are thus required to generate endothelial cells (ECs), a major component of the BBB. Several hiPSC-based protocols have reported the generation of in vitro models with significant differences in barrier properties. We studied in depth the properties of iPSCs byproducts from two protocols that have been established to yield these in vitro barrier models. Our analysis/study reveals that iPSCs derivatives endowed with EC features yield high permeability models while the cells that exhibit outstanding barrier properties show principally epithelial cell-like (EpC) features. We found that models containing EpC-like cells express tight junction proteins, transporters/efflux pumps and display a high functional tightness with very low permeability, which are features commonly shared between BBB and epithelial barriers. Our study demonstrates that hiPSC-based BBB models need extensive characterization beforehand and that a reliable human BBB model containing EC-like cells and displaying low permeability is still needed.

Goldeman, C., et al. (2021). "Human induced pluripotent stem cells (BIONi010-C) generate tight cell monolayers with blood-brain barrier traits and functional expression of large neutral amino acid transporter 1 (SLC7A5)." Eur J Pharm Sci **156**: 105577.

The barrier properties of the brain capillary endothelium, the blood-brain barrier (BBB) restricts uptake of most small and all large molecule drug compounds to the CNS. There is a need for predictive human in vitro models of the BBB to enable studies of

brain drug delivery. Here, we investigated whether human induced pluripotent stem cell (hiPSC) line (BIONi010-C) could be differentiated to brain capillary endothelial- like cells (BCEC) and evaluated their potential use in drug delivery studies. BIONi010-C hIPSCs were differentiated according to established protocols. BCEC monolayers displayed transendothelial electrical resistance (TEER) values of 5,829+/-354 Omega·cm(2), a P(app),(mannitol) of 1.09+/-0.15 · 10(-6) cm·s(-1) and a P(app,diazepam) of 85.7 +/- 5.9 · 10(-6) cm ·s(-1). The P(diazepam)/P(mannitol) ratio of ~80, indicated a large dynamic passive permeability range. Monolayers maintained their integrity after medium exchange. Claudin-5, Occludin, Zonulae Occludens 1 and VE-Cadherin were expressed at the cell-cell contact zones. Efflux transporters were present at the mRNA level, but functional efflux of substrates was not detected. Transferrin-receptor (TFR), Low density lipoprotein receptor-related protein 1 (LRP1) and Basigin receptors were expressed at the mRNA-level. The presence and localization of TFR and LRP1 were verified at the protein level. A wide range of BBB-expressed solute carriers (SLC's) were detected at the mRNA level. The presence and localization of SLC transporters GLUT1 and LAT1 was verified at the protein level. Functional studies revealed transport of the LAT1 substrate [(3)H]-L-Leucine and the LRP1 substrate angiopep-2. In conclusion, we have demonstrated that BIONi010-C-derived BCEC monolayers exhibited, BBB properties including barrier tightness and integrity, a high dynamic range, expression of some of the BBB receptor and transporter expression, as well as functional transport of LAT1 and LRP1 substrates. This suggests that BIONi010-C-derived BCEC monolayers may be useful for studying the roles of LAT-1 and LRP1 in brain drug delivery.

Gomi, M., et al. (2012). "Functional recovery of the murine brain ischemia model using human induced pluripotent stem cell-derived telencephalic progenitors." Brain Res **1459**: 52-60.

Induced pluripotent stem (iPS) cells possess the properties of self-renewal and pluripotency, similar to embryonic stem cells. They are a good candidate as a source of suitable cells for cell replacement therapy. In this study, we transplanted human iPS cell-derived neural progenitors into an ischemic mouse brain. Human iPS cells were differentiated into neuronal progenitors by serum-free culture of embryoid body-like aggregates (SFEBs). Focal cerebral ischemia was induced by occluding the middle cerebral artery using the intraluminal filament technique. Donor cells were transplanted into the ischemic lateral striatum 1 week after ischemia induction. Cells survived at the transplantation site, with migration of a proportion of cells along the external capsule and corpus callosum. Cells that were positive for the basal telencephalon marker, Nkx2.1, migrated into the basal part of the telencephalon. The pallial telencephalon marker, Emx1, was detected in cells that had migrated into the pallial part of the telencephalon. SFEBs differentiated into various types of neurons, and a retrograde tracer labeling study showed that differentiated cells integrated into host neural circuitry. Behavioral recovery was significantly enhanced in the transplanted group. Our results suggest that human iPS cell-derived neuronal progenitors survive and migrate in the ischemic brain, and contribute toward functional recovery via neural circuit reconstitution.

Gong, B., et al. (2019). "Intravenous Transplants of Human Adipose-Derived Stem Cell Protect the Rat Brain From Ischemia-Induced Damage." J Stroke Cerebrovasc Dis **28**(3): 595-603.

BACKGROUND: Survival following cardiac arrest (CA) and subsequent cardiopulmonary resuscitation (CPR), to a great extent, depends on brain damage. Adipose-derived stem cells (ADSCs), as a source of paracrine growth factors and the capacity of neural differentiation may reduce this brain damage. OBJECTIVE: The purpose of this study is to evaluate the protection of ADSCs to brain damage following CPR. METHODS: Rats were divided into 3 groups, sham, CA, and ADSCs group. Rats in sham group went through sham surgery. Rats in CA group went through CA, CPR, and injection PBS (phosphate buffer saline). Rats in ADSCs group went through CA, CPR, and intravenous injection of ADSCs. Rats in sham group were sacrificed immediately after operation. At 24, 72, and 168 hours after return of spontaneous circulation operation, rats in CA and ADSCs group were randomly selected and sacrificed. Brain damage was evaluated by using Neurological Deficit Scale (NDS) score, hippocampal pathology, serum level of S100beta, and apoptosis ratio of hippocampal neurons. Protein of brain derived neurotrophic factor (BDNF) and IL-6 (interleukin-6) in the hippocampus were detected. RESULTS: Compared with sham group, CA and ADSCs group showed a decrease in NDS score, an increased apoptosis ratio of hippocampal nerve cells, increased serum level of S100-beta, and a significant increase in neuroprotective IL-6 and BDNF. In comparison to CA group, ADSCs group had a mild degree of brain damage and higher expression of IL-6 and BDNF. CONCLUSIONS: In the acute stage of cerebral injury following CA, ADSCs might improve the prognosis of brain damage by stimulating the expression of neuroprotective IL-6 and BDNF.

Guillaume, D. J., et al. (2006). "Human embryonic stem cell-derived neural precursors develop into

neurons and integrate into the host brain." J Neurosci Res **84**(6): 1165-1176.

Whether and how in-vitro-produced human neural precursors mature and integrate into the brain are crucial to the utility of human embryonic stem (hES) cells in treating neurological disorders. After transplantation into the ventricles of neonatal immune-deficient mice, hES-cell-derived neural precursors stopped expressing the cell division marker Ki67, except in neurogenic areas, and differentiated into neurons and then glia in a temporal course intrinsic to that of human cells regardless of location. The human cells located in the gray matter became neurons in the olfactory bulb and striatum, whereas those in the white matter produced exclusively glia. Importantly, the grafted human cells formed synapses. Thus, the in-vitro-produced human neural precursors follow their intrinsic temporal program to produce neurons and glia and, in response to environmental signals, generate cells appropriate to their target regions and integrate into the brain.

Gupta, N., et al. (2012). "Neural stem cell engraftment and myelination in the human brain." Sci Transl Med **4**(155): 155ra137.

Pelizaeus-Merzbacher disease (PMD) is a rare leukodystrophy caused by mutation of the proteolipid protein 1 gene. Defective oligodendrocytes in PMD fail to myelinate axons, causing global neurological dysfunction. Human central nervous system stem cells (HuCNS-SCs) can develop into oligodendrocytes and confer structurally normal myelin when transplanted into a hypomyelinating mouse model. A 1-year, open-label phase-1 study was undertaken to evaluate safety and to detect evidence of myelin formation after HuCNS-SC transplantation. Allogeneic HuCNS-SCs were surgically implanted into the frontal lobe white matter in four male subjects with an early-onset severe form of PMD. Immunosuppression was administered for 9 months. Serial neurological evaluations, developmental assessments, and cranial magnetic resonance imaging (MRI) and MR spectroscopy, including high-angular resolution diffusion tensor imaging (DTI), were performed at baseline and after transplantation. The neurosurgical procedure, immunosuppression regimen, and HuCNS-SC transplantation were well tolerated. Modest gains in neurological function were observed in three of the four subjects. No clinical or radiological adverse effects were directly attributed to the donor cells. Reduced T1 and T2 relaxation times were observed in the regions of transplantation 9 months after the procedure in the three subjects. Normalized DTI showed increasing fractional anisotropy and reduced radial diffusivity, consistent with myelination, in the region of transplantation compared to control white matter regions remote to the transplant sites. These phase 1 findings indicate a favorable safety profile for HuCNS-SCs in subjects with PMD. The MRI results suggest durable cell engraftment and donor-derived myelin in the transplanted host white matter.

Han, D., et al. (2017). "Human Cytomegalovirus IE2 Protein Disturbs Brain Development by the Dysregulation of Neural Stem Cell Maintenance and the Polarization of Migrating Neurons." J Virol **91**(17).

Despite the high incidence of severe defects in the central nervous system caused by human cytomegalovirus (HCMV) congenital infection, the mechanism of HCMV neuropathogenesis and the roles of individual viral genes have not yet been fully determined. In this study, we show that the immediate-early 2 (IE2) protein may play a key role in HCMV-caused neurodevelopmental disorders. IE2-transduced neural progenitor cells gave rise to neurospheres with a lower frequency and produced smaller neurospheres than control cells in vitro, indicating reduction of self-renewal and expansion of neural progenitors by IE2. At 2 days after in utero electroporation into the ventricle of the developing brain, a dramatically lower percentage of IE2-expressing cells was detected in the ventricular zone (VZ) and cortical plate (CP) compared to control cells, suggesting that IE2 concurrently dysregulates neural stem cell maintenance in the VZ and neuronal migration to the CP. In addition, most IE2(+) cells in the lower intermediate zone either showed multipolar morphology with short neurites or possessed nonradially oriented processes, whereas control cells had long, radially oriented monopolar or bipolar neurites. IE2(+) callosal axons also failed to cross the midline to form the corpus callosum. Furthermore, we provide molecular evidence that the cell cycle arrest and DNA binding activities of IE2 appear to be responsible for the increased neural stem cell exit from the VZ and cortical migrational defects, respectively. Collectively, our results demonstrate that IE2 disrupts the orderly process of brain development in a stepwise manner to further our understanding of neurodevelopmental HCMV pathogenesis.IMPORTANCE HCMV brain pathogenesis has been studied in limited experimental settings, such as in vitro HCMV infection of neural progenitor cells or in vivo murine CMV infection of the mouse brain. Here, we show that IE2 is a pivotal factor that contributes to HCMV-induced abnormalities in the context of the embryonic brain using an in utero gene transfer tool. Surprisingly, IE2, but not HCMV IE1 or murine CMV ie3, interferes pleiotropically with key neurodevelopmental processes, including neural stem cell regulation, proper positioning of migrating neurons, and the callosal axon projections important for communication between the hemispheres. Our data

suggest that the wide spectrum of clinical outcomes, ranging from mental retardation to microcephaly, caused by congenital HCMV infection can be sufficiently explained in terms of IE2 action alone.

Harberts, J., et al. (2020). "Toward Brain-on-a-Chip: Human Induced Pluripotent Stem Cell-Derived Guided Neuronal Networks in Tailor-Made 3D Nanoprinted Microscaffolds." ACS Nano **14**(10): 13091-13102.

Brain-on-a-chip (BoC) concepts should consider three-dimensional (3D) scaffolds to mimic the 3D nature of the human brain not accessible by conventional planar cell culturing. Furthermore, the essential key to adequately address drug development for human pathophysiological diseases of the nervous system, such as Parkinson's or Alzheimer's, is to employ human induced pluripotent stem cell (iPSC)-derived neurons instead of neurons from animal models. To address both issues, we present electrophysiologically mature human iPSC-derived neurons cultured in BoC applicable microscaffolds prepared by direct laser writing. 3D nanoprinted tailor-made elevated cavities interconnected by freestanding microchannels were used to create defined neuronal networks-as a proof of concept-with two-dimensional topology. The neuronal outgrowth in these nonplanar structures was investigated, among others, in terms of neurite length, size of continuous networks, and branching behavior using z-stacks prepared by confocal microscopy and cross-sectional scanning electron microscopy images prepared by focused ion beam milling. Functionality of the human iPSC-derived neurons was demonstrated with patch clamp measurements in both current- and voltage-clamp mode. Action potentials and spontaneous excitatory postsynaptic currents-fundamental prerequisites for proper network signaling-prove full integrity of these artificial neuronal networks. Considering the network formation occurring within only a few days and the versatile nature of direct laser writing to create even more complex scaffolds for 3D network topologies, we believe that our study offers additional approaches in human disease research to mimic the complex interconnectivity of the human brain in BoC studies.

Hartlaub, A. M., et al. (2019). "Modeling Human Brain Circuitry Using Pluripotent Stem Cell Platforms." Front Pediatr **7**: 57.

Neural circuits are the underlying functional units of the human brain that govern complex behavior and higher-order cognitive processes. Disruptions in neural circuit development have been implicated in the pathogenesis of multiple neurodevelopmental disorders such as autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and schizophrenia. Until recently, major efforts utilizing neurological disease modeling platforms based on human induced pluripotent stem cells (hiPSCs), investigated disease phenotypes primarily at the single cell level. However, recent advances in brain organoid systems, microfluidic devices, and advanced optical and electrical interfaces, now allow more complex hiPSC-based systems to model neuronal connectivity and investigate the specific brain circuitry implicated in neurodevelopmental disorders. Here we review emerging research advances in studying brain circuitry using in vitro and in vivo disease modeling platforms including microfluidic devices, enhanced functional recording interfaces, and brain organoid systems. Research efforts in these areas have already yielded critical insights into pathophysiological mechanisms and will continue to stimulate innovation in this promising area of translational research.

Hermanto, Y., et al. (2018). "Transplantation of feeder-free human induced pluripotent stem cell-derived cortical neuron progenitors in adult male Wistar rats with focal brain ischemia." J Neurosci Res **96**(5): 863-874.

The use of human induced pluripotent stem cells (hiPSCs) eliminates the ethical issues associated with fetal or embryonic materials, thus allowing progress in cell therapy research for ischemic stroke. Strict regulation of cell therapy development requires the xeno-free condition to eliminate clinical complications. Maintenance of hiPSCs with feeder-free condition presents a higher degree of spontaneous differentiation in comparison with conventional cultures. Therefore, feeder-free derivation might be not ideal for developing transplantable hiPSC derivatives. We developed the feeder-free condition for differentiation of cortical neurons from hiPSCs. Then, we evaluated the cells' characteristics upon transplantation into the sham and focal brain ischemia on adult male Wistar rats. Grafts in lesioned brains demonstrated polarized reactivity toward the ischemic border, indicated by directional preferences in axonal outgrowth and cellular migration, with no influence on graft survival. Following the transplantation, forelimb asymmetry was better restored compared with controls. Herein, we provide evidence to support the use of the xeno-free condition for the development of cell therapy for ischemic stroke.

Higgins, D. M., et al. (2013). "Brain tumor stem cell multipotency correlates with nanog expression and extent of passaging in human glioblastoma xenografts." Oncotarget **4**(5): 792-801.

Glioblastoma multiforme (GBM) is the most common primary brain tumor, with a median survival of only 15 months. A subpopulation of cells, the brain tumor stem cells (BTSCs), may be responsible for the

malignancy of this disease. Xenografts have proven to be a robust model of human BTSCs, but the effects of long-term passaging have yet to be determined. Here we present a study detailing changes in BTSC multipotency, invasive migration, and proliferation after serial passaging of human GBM xenografts. Immunocytochemistry and tumorsphere formation assays demonstrated the presence of BTSCs in both early generation (EG-BTSCs; less than 15 passages) and late generation (LG-BTSCs; more than 24 passages) xenografts. The EG-BTSCs upregulated expression of lineage markers for neurons and oligodendrocytes upon differentiation, indicating multipotency. In contrast, the LG-BTSCs were restricted to an astrocytic differentiation. Quantitative migration and proliferation assays showed that EG-BTSCs are more migratory and proliferative than LG-BTSCs. However, both populations respond similarly to the chemokine SDF-1 by increasing invasive migration. These differences between the EG- and LG-BTSCs were correlated with a significant decrease in nanog expression as determined by qRT-PCR. Mice implanted intracranially with EG-BTSCs showed shorter survival when compared to LG-BTSCs. Moreover, differentiation prior to implantation of EG-BTSCs, but not LG-BTSCs, led to increased survival. Thus, nanog may identify multipotent BTSCs. Furthermore, limited passaging of xenografts preserves these multipotent BTSCs, which may be an essential underlying feature of GBM lethality.

Honmou, O., et al. (2001). "[Neural stem cells derived from adult human brain: implications for a cell therapy for CNS diseases]." No Shinkei Geka **29**(4): 293-304.

Hovakimyan, M., et al. (2012). "Survival of transplanted human neural stem cell line (ReNcell VM) into the rat brain with and without immunosuppression." Ann Anat **194**(5): 429-435.

Functional replacement of specific neuronal populations through transplantation of neural tissue represents an attractive therapeutic strategy for treating neurodegenerative disorders like Parkinson's disease (PD). Even though the brain is a partially immune privileged site, immunosuppression is still needed for the prevention of host immune response, and thus, xenograft rejection. Here, we investigated the fate of human ventral mesencephalon derived immortalized cell line ReNcell VM upon unilateral transplantation into the intact rat striatum with or without immunosuppression with cyclosporine A (CsA). The status of xenografted human ReNcell VM cells was analysed by immunohistochemistry/immunofluorescence 4 and 6weeks after transplantation. Four weeks after transplantation, ReNcell VM cells could be detected in both groups, although the number of survived cells was significantly higher in brains of immunosuppressed rats. In contrast, only 2 out of 6 brains grafted without immunosuppression revealed human ReNcell VM cells 6weeks post grafting, whereas a considerable number of human cells could still be found in all the brains of immunosuppressed rats. Immunohistochemical analysis of grafted cells showed almost no evidence of neuronal differentiation, but rather astroglial development. In summary, we have shown that the immunosuppression is needed for the survival of human VM derived progenitor cells in the rat striatum. CsA affected cell survival, but not differentiation capacity: in both groups, grafted either with or without immunosuppression, the ReNcell VM cells lacked neuronal phenotype and developed preferentially into astroglia.

Hu, Z., et al. (2020). "Human neural stem cell transplant location-dependent neuroprotection and motor deficit amelioration in rats with penetrating traumatic brain injury." J Trauma Acute Care Surg **88**(4): 477-485.

BACKGROUND: Penetrating traumatic brain injury induces chronic inflammation that drives persistent tissue loss long after injury. Absence of endogenous reparative neurogenesis and effective neuroprotective therapies render injury-induced disability an unmet need. Cell replacement via neural stem cell transplantation could potentially rebuild the tissue and alleviate penetrating traumatic brain injury disability. The optimal transplant location remains to be determined. METHODS: To test if subacute human neural stem cell (hNSC) transplant location influences engraftment, lesion expansion, and motor deficits, rats (n = 10/group) were randomized to the following four groups (uninjured and three injured): group 1 (Gr1), uninjured with cell transplants (sham+hNSCs), 1-week postunilateral penetrating traumatic brain injury, after establishing motor deficit; group 2 (Gr2), treated with vehicle (media, no cells); group 3 (Gr3), hNSCs transplanted into lesion core (intra); and group 4 (Gr4), hNSCs transplanted into tissue surrounding the lesion (peri). All animals were immunosuppressed for 12 weeks and euthanized following motor assessment. RESULTS: In Gr2, penetrating traumatic brain injury effect manifests as porencephalic cyst, 22.53 +/- 2.87 (% of intact hemisphere), with p value of <0.0001 compared with uninjured Gr1. Group 3 lesion volume at 17.44 +/- 2.11 did not differ significantly from Gr2 (p = 0.36), while Gr4 value, 9.17 +/- 1.53, differed significantly (p = 0.0001). Engraftment and neuronal differentiation were significantly lower in the uninjured Gr1 (p < 0.05), compared with injured groups. However, there were no differences between Gr3 and Gr4. Significant increase in cortical tissue sparing (p =

0.03), including motor cortex (p = 0.005) was observed in Gr4 but not Gr3. Presence of transplant within lesion or in penumbra attenuated motor deficit development (p < 0.05) compared with Gr2. CONCLUSION: In aggregate, injury milieu supports transplanted cell proliferation and differentiation independent of location. Unexpectedly, cortical sparing is transplant location dependent. Thus, apart from cell replacement and transplant mediated deficit amelioration, transplant location-dependent neuroprotection may be key to delaying onset or preventing development of injury-induced disability. LEVEL OF EVIDENCE: Preclinical study evaluation of therapeutic intervention, level VI.

Huang, J., et al. (2022). "Human pluripotent stem cell-derived ectomesenchymal stromal cells promote more robust functional recovery than umbilical cord-derived mesenchymal stromal cells after hypoxic-ischaemic brain damage." Theranostics **12**(1): 143-166.

Aims: Hypoxic-ischaemic encephalopathy (HIE) is one of the most serious complications in neonates and infants. Mesenchymal stromal cell (MSC)-based therapy is emerging as a promising treatment avenue for HIE. However, despite its enormous potential, the clinical application of MSCs is limited by cell heterogeneity, low isolation efficiency and unpredictable effectiveness. In this study, we examined the therapeutic effects and underlying mechanisms of human pluripotent stem cell-derived ectomesenchymal stromal cells (hPSC-EMSCs) in a rat model of HIE. Methods: hPSC-EMSCs were induced from either human embryonic stem cells or induced pluripotent stem cells. Stem cells or the conditioned medium (CM) derived from stem cells were delivered intracranially or intranasally to neonatal rats with HIE. Human umbilical cord-derived MSCs (hUC-MSCs) were used as the therapeutic comparison control and phosphate-buffered saline (PBS) was used as a negative control. Lesion size, apoptosis, neurogenesis, astrogliosis and microgliosis were evaluated. The rotarod test and Morris water maze were used to determine brain functional recovery. The PC-12 cell line, rat primary cortical neurons and neural progenitor cells were used to evaluate neurite outgrowth and the neuroprotective and neurogenesis effects of hPSC-EMSCs/hUC-MSCs. RNA-seq and enzyme-linked immunosorbent assays were used to determine the secretory factors that were differentially expressed between hPSC-EMSCs and hUC-MSCs. The activation and suppression of extracellular signal-regulated kinase (ERK) and cAMP response element-binding protein (CREB) were characterised using western blotting and immunofluorescent staining. Results: hPSC-EMSCs showed a higher neuroprotective potential than hUC-MSCs, as demonstrated by a more significant reduction in lesion size and apoptosis in the rat brain following hypoxia-ischaemia (HI). Compared with PBS treatment, hPSC-EMSCs promoted endogenous neurogenesis and alleviated astrogliosis and microgliosis. hPSC-EMSCs were more effective than hUC-MSCs. hPSC-EMSCs achieved a greater recovery of brain function than hUC-MSCs and PBS in rats with HIE. CM derived from hPSC-EMSCs had neuroprotective and neurorestorative effects in vitro through anti-apoptotic and neurite outgrowth- and neurogenesis-promoting effects. Direct comparisons between hPSC-EMSCs and hUC-MSCs revealed the significant enrichment of a group of secretory factors in hPSC-EMSCs, including nerve growth factor (NGF), platelet-derived growth factor-AA and transforming growth factor-beta(2), which are involved in neurogenesis, synaptic transmission and neurotransmitter transport, respectively. Mechanistically, the CM derived from hPSC-EMSCs was found to potentiate NGF-induced neurite outgrowth and the neuronal differentiation of NPCs via the ERK/CREB pathway. Suppression of ERK or CREB abolished CM-potentiated neuritogenesis and neuronal differentiation. Finally, intranasal delivery of the CM derived from hPSC-EMSCs significantly reduced brain lesion size, promoted endogenous neurogenesis, mitigated inflammatory responses and improved functional recovery in rats with HIE. Conclusion: hPSC-EMSCs promote functional recovery after HI through multifaceted neuromodulatory activities via paracrine/trophic mechanisms. We propose the use of hPSC-EMSCs for the treatment of HIE, as they offer an excellent unlimited cellular source of MSCs.

Jacob, F., et al. (2020). "Human Pluripotent Stem Cell-Derived Neural Cells and Brain Organoids Reveal SARS-CoV-2 Neurotropism." bioRxiv.

Neurological complications are common in patients with COVID-19. While SARS-CoV-2, the causal pathogen of COVID-19, has been detected in some patient brains, its ability to infect brain cells and impact their function are not well understood, and experimental models using human brain cells are urgently needed. Here we investigated the susceptibility of human induced pluripotent stem cell (hiPSC)-derived monolayer brain cells and region-specific brain organoids to SARS-CoV-2 infection. We found modest numbers of infected neurons and astrocytes, but greater infection of choroid plexus epithelial cells. We optimized a protocol to generate choroid plexus organoids from hiPSCs, which revealed productive SARS-CoV-2 infection that leads to increased cell death and transcriptional dysregulation indicative of an inflammatory response and cellular function deficits. Together, our results provide evidence for SARS-CoV-2 neurotropism and support

use of hiPSC-derived brain organoids as a platform to investigate the cellular susceptibility, disease mechanisms, and treatment strategies for SARS-CoV-2 infection.

Jacob, F., et al. (2020). "Human Pluripotent Stem Cell-Derived Neural Cells and Brain Organoids Reveal SARS-CoV-2 Neurotropism Predominates in Choroid Plexus Epithelium." Cell Stem Cell **27**(6): 937-950 e939.

Neurological complications are common in patients with COVID-19. Although severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causal pathogen of COVID-19, has been detected in some patient brains, its ability to infect brain cells and impact their function is not well understood. Here, we investigated the susceptibility of human induced pluripotent stem cell (hiPSC)-derived monolayer brain cells and region-specific brain organoids to SARS-CoV-2 infection. We found that neurons and astrocytes were sparsely infected, but choroid plexus epithelial cells underwent robust infection. We optimized a protocol to generate choroid plexus organoids from hiPSCs and showed that productive SARS-CoV-2 infection of these organoids is associated with increased cell death and transcriptional dysregulation indicative of an inflammatory response and cellular function deficits. Together, our findings provide evidence for selective SARS-CoV-2 neurotropism and support the use of hiPSC-derived brain organoids as a platform to investigate SARS-CoV-2 infection susceptibility of brain cells, mechanisms of virus-induced brain dysfunction, and treatment strategies.

Jin, M., et al. (2022). "Rag2(-/-) accelerates lipofuscin accumulation in the brain: Implications for human stem cell brain transplantation studies." Stem Cell Reports **17**(11): 2381-2391.

Immunodeficient mice are widely used in human stem cell transplantation research. Recombination activating gene 1 (Rag1) deletion results in immunodeficiency and leads to accelerated aging in zebrafish with increased cytosolic accumulation of lipofuscin (LF). Unlike zebrafish, mammals have two homologs, Rag1 and Rag2, that regulate adaptive immunity. Currently, little is known if and how Rag1(-/-) and Rag2(-/-) may impact aging and LF accumulation in immunodeficient mouse brains and how this may confound results in human neural cell transplantation studies. Here, we demonstrate that in Rag2(-/-) mouse brains, LF appears early, spreads broadly, emits strong autofluorescence, and accumulates with age. LF is found in various types of glial cells, including xenografted human microglia. Surprisingly, in Rag1(-/-) mouse brains, LF autofluorescence is seen at much older ages compared with Rag2(-/-) brains. This study provides direct evidence that Rag2(-/-) expedites LF occurrence and sets a context for studies using aged immunodeficient mice.

Jin, M., et al. (2023). "Rag2(-/-) accelerates lipofuscin accumulation in the brain: Implications for human stem cell brain transplantation studies." Stem Cell Reports **18**(1): 413-415.

Jirak, D., et al. (2019). "Metabolic Changes in Focal Brain Ischemia in Rats Treated With Human Induced Pluripotent Stem Cell-Derived Neural Precursors Confirm the Beneficial Effect of Transplanted Cells." Front Neurol **10**: 1074.

There is currently no treatment for restoring lost neurological function after stroke. A growing number of studies have highlighted the potential of stem cells. However, the mechanisms underlying their beneficial effect have yet to be explored in sufficient detail. In this study, we transplanted human induced pluripotent stem cell-derived neural precursors (iPSC-NPs) in rat temporary middle cerebral artery occlusion (MCAO) model. Using magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) we monitored the effect of cells and assessed lesion volume and metabolite changes in the brain. We monitored concentration changes of myo-inositol (Ins), Taurine (Tau), Glycerophosphocholine+Phosphocholine (GPC+PCh), N-acetyl-aspartate+N-acetyl-aspartyl-glutamate (NAA+NAAG), Creatine+Phosphocreatine (Cr+PCr), and Glutamate+Glutamine (Glu+Gln) in the brains of control and iPSC-NP-transplanted rats. Based on initial lesion size, animals were divided into small lesion and big lesion groups. In the small lesion control group (SCL), lesion size after 4 months was three times smaller than initial measurements. In the small lesion iPSC-NP-treated group, lesion volume decreased after 1 month and then increased after 4 months. Although animals with small lesions significantly improved their motor skills after iPSC-NP transplantation, animals with big lesions showed no improvement. However, our MRI data demonstrate that in the big lesion iPSC-NP-treated (BTL) group, lesion size increased only up until 1 month after MCAO induction and then decreased. In contrast, in the big lesion control group, lesion size increased throughout the whole experiment. Significantly higher concentrations of Ins, Tau, GPC+PCh, NAA+NAAG, Cr+PCr, and Glu+Gln were found in in contralateral hemisphere in BTL animals 4 months after cell injection. Lesion volume decreased at this time point. Spectroscopic results of metabolite concentrations in lesion correlated with volumetric measurements of lesion, with the highest negative correlation observed for NAA+NAAG. Altogether, our

results suggest that iPSC-NP transplantation decreases lesion volume and regulates metabolite concentrations within the normal range expected in healthy tissue. Further research into the ability of iPSC-NPs to differentiate into tissue-specific neurons and its effect on the long-term restoration of lesioned tissue is necessary.

Kaindl, J. and B. Winner (2019). "Disease Modeling of Neuropsychiatric Brain Disorders Using Human Stem Cell-Based Neural Models." Curr Top Behav Neurosci **42**: 159-183.

Human pluripotent stem (PS) cells are a relevant platform to model human-specific neurological disorders. In this chapter, we focus on human stem cell models for neuropsychiatric disorders including induced pluripotent stem (iPS) cell-derived neural precursor cells (NPCs), neurons and cerebral organoids. We discuss crucial steps for planning human disease modeling experiments. We introduce the different strategies of human disease modeling including transdifferentiation, human embryonic stem (ES) cell-based models, iPS cell-based models and genome editing options. Analysis of disease-relevant phenotypes is discussed. In more detail, we provide exemplary insight into modeling of the neurodevelopmental defects in autism spectrum disorder (ASD) and the process of neurodegeneration in Alzheimer's disease (AD). Besides monogenic diseases, iPS cell-derived models also generated data from idiopathic and sporadic cases.

Kappy, N. S., et al. (2018). "Human adipose-derived stem cell treatment modulates cellular protection in both in vitro and in vivo traumatic brain injury models." J Trauma Acute Care Surg **84**(5): 745-751.

BACKGROUND: Traumatic brain injury (TBI) is a common cause of morbidity and mortality in the civilian population. The purpose of this study was to examine the effect(s) of adipose-derived stem cell (ASC) treatment on cellular and functional recovery in TBI via both in vitro and in vivo methods. METHODS: Cultured neuroblastoma cells, SH-SY5Y, were scratched to mimic TBI in an in vitro model. The effect of ASC-conditioned medium (CM) on cell death, mitochondrial function, and expression of inflammatory cytokines (tumor necrosis factor alpha [TNF-alpha], interleukin 1beta [IL-1beta], and IL-6), as well as apoptosis marker FAS, was measured. In our in vivo model, Sprague-Dawley rats underwent TBI via a frontal, closed-head injury model. Animals randomly received either intravenous human-derived ASCs or intravenous saline within 3 hours of injury and were compared with a sham group. Functional recovery was evaluated via accelerating Rotarod method. On post-TBI Day 3, brain tissue was harvested and assessed for cellular damage via enzyme-linked immunosorbent assay for TNF-alpha, as well as immunohistochemical staining for beta-amyloid precursor protein (beta-APP). RESULTS: Our in vitro data show that ASC treatment imparted reduced cell death (ratio to control: 1.21 +/- 0.066 vs. 1.01 +/- 0.056, p = 0.017), increased cell viability (ratio to control: 0.86 +/- 0.009 vs. 1.09 +/- 0.01, p = 0.0001), increased mitochondrial function (percentage of control: 78 +/- 6% vs. 68 +/- 3%), and significantly decreased levels of inflammatory cytokine IL-1beta. In our in vivo study, compared with TBI alone, ASC-treated animals showed no difference in functional recovery, lower levels of expressed TNF-alpha (ratio to total protein, 0.47 +/- 0.01 vs. 0.67 +/- 0.04; p < 0.01), and lower levels of beta-amyloid precursor protein (fluorescence ratio, 0.43 +/- 0.05 vs. 0.69 +/- 0.03; p < 0.01). CONCLUSIONS: Adipose-derived stem cell treatment results in improved cell survival, decreased inflammatory marker release, and decreased evidence of neural injury. No difference in functional recovery was seen. These data suggest the potential for ASC treatment to aid in cellular protection and recovery in neural cells following TBI.

Kelava, I. and M. A. Lancaster (2016). "Stem Cell Models of Human Brain Development." Cell Stem Cell **18**(6): 736-748.

Recent breakthroughs in pluripotent stem cell technologies have enabled a new class of in vitro systems for functional modeling of human brain development. These advances, in combination with improvements in neural differentiation methods, allow the generation of in vitro systems that reproduce many in vivo features of the brain with remarkable similarity. Here, we describe advances in the development of these methods, focusing on neural rosette and organoid approaches, and compare their relative capabilities and limitations. We also discuss current technical hurdles for recreating the cell-type complexity and spatial architecture of the brain in culture and offer potential solutions.

Kikuchi, T., et al. (2011). "Survival of human induced pluripotent stem cell-derived midbrain dopaminergic neurons in the brain of a primate model of Parkinson's disease." J Parkinsons Dis **1**(4): 395-412.

Before induced pluripotent stem cells (iPSCs) can be used to treat neurologic diseases, human iPSC-derived neural cells must be analyzed in the primate brain. In fact, although mouse and human iPSCs have been used to generate dopaminergic (DA) neurons that are beneficial in rat models of Parkinson's disease (PD), human iPSC-derived neural progenitor cells (NPCs) have not been examined in primate brains. Here, we generated NPCs at different stages of predifferentiation using a feeder-free culture method, and grafted them

into the brains of a monkey PD model and NOD-SCID mice. Magnetic resonance imaging (MRI), positron emission tomography (PET), immunocytochemistry, and behavioral analyses revealed that NPCs pretreated with Sonic hedgehog and fibroblast growth factor-8 followed by glial cell-derived neurotrophic factor, brain-derived neurotrophic factor, ascorbic acid, and dibutyryl cyclic AMP resulted in smaller grafts than those without these treatments, and survived as DA neurons in a monkey brain as long as six months. Thus, for the first time, we describe a feeder-free neural differentiation method from human iPSCs and an evaluation system that can be used to assess monkey PD models.

Kim, E. S., et al. (2012). "Human umbilical cord blood-derived mesenchymal stem cell transplantation attenuates severe brain injury by permanent middle cerebral artery occlusion in newborn rats." Pediatr Res **72**(3): 277-284.

BACKGROUND: Severe brain injury induced by neonatal stroke causes significant mortality and disability, and effective therapies are currently lacking. We hypothesized that human umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs) can attenuate severe brain injury induced by permanent middle cerebral artery occlusion (MCAO) in rat pups. METHODS: After confirming severe brain injury involving more than 50% of the ipsilateral hemisphere volume at 1 h after MCAO using diffusion-weighted magnetic resonance imaging (MRI) in postnatal day (P)10 rats, human UCB-derived MSCs were transplanted intraventricularly. The brain MRI was evaluated periodically up to 28 d after MCAO (P38). Sensorimotor function and histology in the peri-infarct tissues were evaluated at the end of the experiment. RESULTS: Severe brain injury induced by permanent MCAO resulted in decreased survival and body weight gain, increased brain infarct volume as measured by MRI, impaired functional tests such as the rotarod and cylinder test, and histologic abnormalities such as increased terminal deoxynucleotidyl transferase nick-end labeling, reactive microglial marker, and glial fibrillary acidic protein-positive cells in the penumbra. All of these abnormalities were significantly improved by MSC transplantation 6 h after MCAO. CONCLUSION: These results suggest that human UCB-derived MSCs are a promising therapeutic candidate for the treatment of severe perinatal brain injury including neonatal stroke.

Kim, J. T., et al. (2022). "Human embryonic stem cell-derived cerebral organoids for treatment of mild traumatic brain injury in a mouse model." Biochem Biophys Res Commun **635**: 169-178.

OBJECTIVE: There are no effective treatments for relieving neuronal dysfunction after mild traumatic brain injury (TBI). Here, we evaluated therapeutic efficacy of human embryonic stem cell-derived cerebral organoids (hCOs) in a mild TBI model, in terms of repair of damaged cortical regions, neurogenesis, and improved cognitive function. METHODS: Male C57BL/6 J mice were randomly divided into sham-operated, mild TBI, and mild TBI with hCO groups. hCOs cultured at 8 weeks were used for transplantation. Mice were sacrificed at 7 and 14 days after transplantation followed by immunofluorescence staining, cytokine profile microarray, and novel object recognition test. RESULTS: 8W-hCOs transplantation significantly reduced neuronal cell death, recovered microvessel density, and promoted neurogenesis in the ipsilateral subventricular zone and dentate gyrus of hippocampus after mild TBI. In addition, increased angiogenesis into the engrafted hCOs was observed. Microarray results of hCOs revealed neuronal differentiation potential and higher expression of early brain development proteins associated with neurogenesis, angiogenesis and extracellular matrix remodeling. Ultimately, 8W-hCO transplantation resulted in reconstruction of damaged cortex and improvement in cognitive function after mild TBI. CONCLUSION: hCO transplantation may be feasible for treating mild TBI-related neuronal dysfunction via reconstruction of damaged cortex and neurogenesis in the hippocampus.

Kim, S. Y., et al. (2022). "NeuroTrace 500/525 identifies human induced pluripotent stem cell-derived brain pericyte-like cells." Mol Brain **15**(1): 11.

In the CNS, pericytes are important for maintaining the blood-brain barrier (BBB) and for controlling blood flow. Recently, several methods were suggested for the differentiation of human pluripotent stem cells (hPSCs) into brain mural cells, specifically pericytes or vascular smooth muscle cells (vSMCs). Unfortunately, identifying the pericytes from among such hPSC-derived mural cells has been challenging. This is due both to the lack of pericyte-specific markers and to the loss of defining anatomical information inherent to culture conditions. We therefore asked whether NeuroTrace 500/525, a newly developed dye that shows cell-specific uptake into pericytes in the mouse brain, can help identify human induced pluripotent stem cell (hiPSC)-derived brain pericyte-like cells. First, we found that NeuroTrace 500/525 specifically stains primary cultured human brain pericytes, confirming its specificity in vitro. Second, we found that NeuroTrace 500/525 specifically labels hiPSC-derived pericyte-like cells, but not endothelial cells or vSMCs derived from the same hiPSCs. Last, we found that neuroectoderm-derived vSMCs, which

have pericyte-like features, also take up NeuroTrace 500/525. These data indicate NeuroTrace 500/525 is useful for identifying pericyte-like cells among hiPSC-derived brain mural cells.

Koenig, L., et al. (2022). "A Human Stem Cell-Derived Brain-Liver Chip for Assessing Blood-Brain-Barrier Permeation of Pharmaceutical Drugs." Cells **11**(20).

Significant advancements in the field of preclinical in vitro blood-brain barrier (BBB) models have been achieved in recent years, by developing monolayer-based culture systems towards complex multi-cellular assays. The coupling of those models with other relevant organoid systems to integrate the investigation of blood-brain barrier permeation in the larger picture of drug distribution and metabolization is still missing. Here, we report for the first time the combination of a human induced pluripotent stem cell (hiPSC)-derived blood-brain barrier model with a cortical brain and a liver spheroid model from the same donor in a closed microfluidic system (MPS). The two model compounds atenolol and propranolol were used to measure permeation at the blood-brain barrier and to assess metabolization. Both substances showed an in vivo-like permeation behavior and were metabolized in vitro. Therefore, the novel multi-organ system enabled not only the measurement of parent compound concentrations but also of metabolite distribution at the blood-brain barrier.

Kurosawa, T., et al. (2022). "Construction and Functional Evaluation of a Three-Dimensional Blood-Brain Barrier Model Equipped With Human Induced Pluripotent Stem Cell-Derived Brain Microvascular Endothelial Cells." Pharm Res **39**(7): 1535-1547.

PURPOSE: The purpose of this study was to construct and validate an in vitro three-dimensional blood-brain barrier (3DBBB) model system equipped with brain microvascular endothelial cells derived from human induced pluripotent stem cells (hiPS-BMECs). METHODS: The 3D-BBB system was constructed by seeding hiPS-BMECs onto the capillary lane of a MIMETAS OrganoPlate((R)) 3-lane coated with fibronectin/collagen IV. hiPS-BMECs were incubated under continuous switchback flow with an OrganoFlow((R)) for 2 days. The 3D capillary structure and expression of tight-junction proteins and transporters were confirmed by immunocytochemistry. The mRNA expression of transporters in the 3D environment was determined using qRT-PCR, and the permeability of endogenous substances and drugs was evaluated under various conditions. RESULTS AND DISCUSSION: The expression of tight-junction proteins, including claudin-5 and ZO-1, was confirmed by immunohistochemistry. The permeability rate constant of lucifer yellow through hiPS-BMECs was undetectably low, indicating that paracellular transport is highly restricted by tight junctions in the 3D-BBB system. The mRNA expression levels of transporters and receptors in the 3D-BBB system differed from those in the 2D-culture system by 0.2- to 5.8-fold. The 3D-cultured hiPS-BMECs showed asymmetric transport of substrates of BCRP, CAT1 and LAT1 between the luminal (blood) and abluminal (brain) sides. Proton-coupled symport function of MCT1 was also confirmed. CONCLUSION: The 3D-BBB system constructed in this study mimics several important characteristics of the human BBB, and is expected to be a useful high-throughput evaluation tool in the development of CNS drugs.

Kurosawa, T., et al. (2022). "Correction to: Construction and Functional Evaluation of a Three-Dimensional Blood-Brain Barrier Model Equipped With Human Induced Pluripotent Stem Cell-Derived Brain Microvascular Endothelial Cells." Pharm Res **39**(7): 1661.

Larimore, J., et al. (2013). "MeCP2 regulates the synaptic expression of a Dysbindin-BLOC-1 network component in mouse brain and human induced pluripotent stem cell-derived neurons." PLoS One **8**(6): e65069.

Clinical, epidemiological, and genetic evidence suggest overlapping pathogenic mechanisms between autism spectrum disorder (ASD) and schizophrenia. We tested this hypothesis by asking if mutations in the ASD gene MECP2 which cause Rett syndrome affect the expression of genes encoding the schizophrenia risk factor dysbindin, a subunit of the biogenesis of lysosome-related organelles complex-1 (BLOC-1), and associated interacting proteins. We measured mRNA and protein levels of key components of a dysbindin interaction network by, quantitative real time PCR and quantitative immunohistochemistry in hippocampal samples of wild-type and Mecp2 mutant mice. In addition, we confirmed results by performing immunohistochemistry of normal human hippocampus and quantitative qRT-PCR of human inducible pluripotent stem cells (iPSCs)-derived human neurons from Rett syndrome patients. We defined the distribution of the BLOC-1 subunit pallidin in human and mouse hippocampus and contrasted this distribution with that of symptomatic Mecp2 mutant mice. Neurons from mutant mice and Rett syndrome patients displayed selectively reduced levels of pallidin transcript. Pallidin immunoreactivity decreased in the hippocampus of symptomatic Mecp2 mutant mice, a feature most prominent at asymmetric synapses as determined by immunoelectron microcopy. Pallidin immunoreactivity decreased concomitantly with reduced BDNF content in the hippocampus of Mecp2

mice. Similarly, BDNF content was reduced in the hippocampus of BLOC-1 deficient mice suggesting that genetic defects in BLOC-1 are upstream of the BDNF phenotype in Mecp2 deficient mice. Our results demonstrate that the ASD-related gene Mecp2 regulates the expression of components belonging to the dysbindin interactome and these molecular differences may contribute to synaptic phenotypes that characterize Mecp2 deficiencies and ASD.

Le Bail, R., et al. (2021). "Learning about cell lineage, cellular diversity and evolution of the human brain through stem cell models." Curr Opin Neurobiol **66**: 166-177.

Here, we summarize the current knowledge on cell diversity in the cortex and other brain regions from in vivo mouse models and in vitro models based on pluripotent stem cells. We discuss the mechanisms underlying cell proliferation and temporal progression that leads to the sequential generation of neurons dedicated to different layers of the cortex. We highlight models of corticogenesis from stem cells that recapitulate specific transcriptional and connectivity patterns from different cortical areas. We overview state-of-the art of human brain organoids modeling different brain regions, and we discuss insights into human cortical evolution from stem cells. Finally, we interrogate human brain organoid models for their competence to recapitulate the essence of human brain development.

Lee, J. Y., et al. (2019). "Human parthenogenetic neural stem cell grafts promote multiple regenerative processes in a traumatic brain injury model." Theranostics **9**(4): 1029-1046.

International Stem Cell Corporation human parthenogenetic neural stem cells (ISC-hpNSC) have potential therapeutic value for patients suffering from traumatic brain injury (TBI). Here, we demonstrate the behavioral and histological effects of transplanting ISC-hpNSC intracerebrally in an animal model of TBI. Methods: Sprague-Dawley rats underwent a moderate controlled cortical impact TBI surgery. Transplantation occurred at 72 h post-TBI with functional readouts of behavioral and histological deficits conducted during the subsequent 3-month period after TBI. We characterized locomotor, neurological, and cognitive performance at baseline (before TBI), then on days 0, 1, 7, 14, 30, 60, and 90 (locomotor and neurological), and on days 28-30, 58-60, and 88-90 (cognitive) after TBI. Following completion of behavioral testing at 3 months post-TBI, animals were euthanized by transcardial perfusion and brains harvested to histologically characterize the extent of brain damage. Neuronal survival was revealed by Nissl staining, and stem cell engraftment and host tissue repair mechanisms such as the anti-inflammatory response in peri-TBI lesion areas were examined by immunohistochemical analyses. Results: We observed that TBI groups given high and moderate doses of ISC-hpNSC had an improved swing bias on an elevated body swing test for motor function, increased scores on forelimb akinesia and paw grasp neurological tests, and committed significantly fewer errors on a radial arm water maze test for cognition. Furthermore, histological analyses indicated that high and moderate doses of stem cells increased the expression of phenotypic markers related to the neural lineage and myelination and decreased reactive gliosis and inflammation in the brain, increased neuronal survival in the peri-impact area of the cortex, and decreased inflammation in the spleen at 90 days post-TBI. Conclusion: These results provide evidence that high and moderate doses of ISC-hpNSC ameliorate TBI-associated histological alterations and motor, neurological, and cognitive deficits.

Li, J. Y., et al. (2008). "Critical issues of clinical human embryonic stem cell therapy for brain repair." Trends Neurosci **31**(3): 146-153.

Embryonic stem cells (ESCs) provide hope as a potential regenerative therapy for neurological conditions such as Parkinson's disease and spinal cord injury. Currently, ESC-based nervous system repair faces several problems. One major hurdle is related to problems in generating large and defined populations of the desired types of neurons from human ESCs (hESCs). Moreover, survival of grafted hESC-derived cells has varied and functional recovery in recipient animals has often been disappointing. Importantly, in clinical trials, adverse effects after surgery, including tumors or vigorous immune reactions, must be avoided. Here we highlight attempts to overcome these hurdles with hESCs intended for central nervous system repair. We focus on hESC-derived dopamine-producing neurons that can be grafted in Parkinson's disease and identify critical experiments that need to be conducted before clinical trials can occur.

Li, Y., et al. (2019). "Traditional Chinese medicine, Kami-Shoyo-San protects ketamine-induced neurotoxicity in human embryonic stem cell-differentiated neurons through activation of brain-derived neurotrophic factor." Neuroreport **30**(16): 1102-1109.

BACKGROUND: Anesthesia-induced neurotoxicity may cause permanent dysfunctions in human brains. In this work, we used a cell-based in-vitro model to demonstrate that traditional Chinese medicine, Kami-Shoyo-San may protect ketamine-induced neuronal apoptosis in human embryonic stem cell-differentiated neurons. METHODS: Human embryonic stem cell-differentiated neurons were

cultured in vitro and treated with high-concentration ketamine to induce neuronal apoptosis. Pre-incubation of Kami-Shoyo-San was conducted to evaluate its neuroprotection on ketamine-injured neurons. Quantitative real-time PCR and western blot assays were used to assess brain-derived neurotrophic factor and its receptor, tropomyosin receptor kinase B, in response to Kami-Shoyo-San and ketamine treatment. Brain-derived neurotrophic factor/tropomyosin receptor kinase B signaling pathway was then deactivated, by siRNA application, to further explore its functional role in Kami-Shoyo-San-mediated protection on ketamine-induced apoptosis among human embryonic stem cell-differentiated neurons. RESULTS: High concentration of ketamine-induced significant apoptosis, whereas pre-incubation of Kami-Shoyo-San markedly rescued ketamine-induced apoptosis, in human embryonic stem cell-differentiated neurons. Kami-Shoyo-San activated brain-derived neurotrophic factor/tropomyosin receptor kinase B signaling pathway by upregulating brain-derived neurotrophic factor and inducing tropomyosin receptor kinase B phosphorylation. Conversely, siRNA-mediated brain-derived neurotrophic factor/tropomyosin receptor kinase B signaling pathway deactivation reversed the neuroprotection of Kami-Shoyo-San in ketamine-injured human embryonic stem cell-differentiated neurons. CONCLUSION: Kami-Shoyo-San could protect ketamine-induced neurotoxicity, and the underlying mechanism may involve brain-derived neurotrophic factor/tropomyosin receptor kinase B signaling pathway.

Li, Y., et al. (2019). "Development of Human in vitro Brain-blood Barrier Model from Induced Pluripotent Stem Cell-derived Endothelial Cells to Predict the in vivo Permeability of Drugs." Neurosci Bull **35**(6): 996-1010.

An in vitro blood-brain barrier (BBB) model is critical for enabling rapid screening of the BBB permeability of the drugs targeting on the central nervous system. Though many models have been developed, their reproducibility and renewability remain a challenge. Furthermore, drug transport data from many of the models do not correlate well with the data for in vivo BBB drug transport. Induced-pluripotent stem cell (iPSC) technology provides reproducible cell resources for in vitro BBB modeling. Here, we generated a human in vitro BBB model by differentiating the human iPSC (hiPSC) line GM25256 into brain endothelial-type cells. The model displayed BBB characteristics including tight junction proteins (ZO-1, claudin-5, and occludin) and endothelial markers (von Willebrand factor and Ulex), as well as high trans-endothelial electrical resistance (TEER) (1560 Omega.cm(2) +/- 230 Omega.cm(2)) and gamma-GTPase activity. Co-culture with primary rat astrocytes significantly increased the TEER of the model (2970 Omega.cm(2) to 4185 Omega.cm(2)). RNAseq analysis confirmed the expression of key BBB-related genes in the hiPSC-derived endothelial cells in comparison with primary human brain microvascular endothelial cells, including P-glycoprotein (Pgp) and breast cancer resistant protein (BCRP). Drug transport assays for nine CNS compounds showed that the permeability of non-Pgp/BCRP and Pgp/BCRP substrates across the model was strongly correlated with rodent in situ brain perfusion data for these compounds (R(2) = 0.982 and R(2) = 0.9973, respectively), demonstrating the functionality of the drug transporters in the model. Thus, this model may be used to rapidly screen CNS compounds, to predict the in vivo BBB permeability of these compounds and to study the biology of the BBB.

Limone, F., et al. (2022). "Pluripotent stem cell strategies for rebuilding the human brain." Front Aging Neurosci **14**: 1017299.

Neurodegenerative disorders have been extremely challenging to treat with traditional drug-based approaches and curative therapies are lacking. Given continued progress in stem cell technologies, cell replacement strategies have emerged as concrete and potentially viable therapeutic options. In this review, we cover advances in methods used to differentiate human pluripotent stem cells into several highly specialized types of neurons, including cholinergic, dopaminergic, and motor neurons, and the potential clinical applications of stem cell-derived neurons for common neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, ataxia, and amyotrophic lateral sclerosis. Additionally, we summarize cellular differentiation techniques for generating glial cell populations, including oligodendrocytes and microglia, and their conceivable translational roles in supporting neural function. Clinical trials of specific cell replacement therapies in the nervous system are already underway, and several attractive avenues in regenerative medicine warrant further investigation.

Lin, H. Y., et al. (2020). "Reprogramming of a human induced pluripotent stem cell (iPSC) line from a patient with neurodegeneration with brain iron accumulation (NBIA) harboring a novel frameshift mutation in C19orf12 gene." Stem Cell Res **49**: 102032.

Mutations in an open-reading frame on chromosome 19 (C19orf12) were identified as one of the causative genes for neurodegeneration with brain iron accumulation (NBIA). Because of the mitochondrial localization of the derived protein, this

variant is referred to as mitochondrial membrane protein-associated neurodegeneration with brain iron accumulation (MPAN). Here, we used the Sendai virus delivery system to generate induced pluripotent stem cells (iPSCs) from peripheral blood mononuclear cells of a female patient with MPAN having a novel heterozygous frameshift mutation caused by an insertion, c.273_274insA (p.P92Tfs*9), in C19orf12. This cellular model could provide a platform for pathophysiological studies of MPAN.

Lin, M. N., et al. (2013). "Involvement of PI3K and ROCK signaling pathways in migration of bone marrow-derived mesenchymal stem cells through human brain microvascular endothelial cell monolayers." Brain Res **1513**: 1-8.

Bone marrow-derived mesenchymal stem cells (MSC) represent an important and easily available source of stem cells for potential therapeutic use in neurological diseases. The entry of circulating cells into the central nervous system by intravenous administration requires, firstly, the passage of the cells across the blood-brain barrier (BBB). However, little is known of the details of MSC transmigration across the BBB. In the present study, we employed an in vitro BBB model constructed using a human brain microvascular endothelial cell monolayer to study the mechanism underlying MSC transendothelial migration. Transmigration assays, transendothelial electrical resistance (TEER) and horseradish peroxidase (HRP) flux assays showed that MSC could transmigrate through human brain microvascular endothelial cell monolayers by a paracellular pathway. Cell fractionation and immunofluorescence assays confirmed the disruption of tight junctions. Inhibition assays showed that a Rho-kinase (ROCK) inhibitor (Y27632) effectively promoted MSC transendothelial migration; conversely, a PI3K inhibitor (LY294002) blocked MSC transendothelial migration. Interestingly, adenovirus-mediated interference with ROCK in MSC significantly increased MSC transendothelial migration, and overexpression of a PI3K dominant negative mutant in MSC cells could block transendothelial migration. Our findings provide clear evidence that the PI3K and ROCK pathways are involved in MSC migration through human brain microvascular endothelial cell monolayers. The information yielded by this study may be helpful in constructing gene-modified mesenchymal stem cells that are able to penetrate the BBB effectively for cell therapy.

Lindvall, O. and Z. Kokaia (2005). "Stem cell therapy for human brain disorders." Kidney Int **68**(5): 1937-1939.

Transplantation of stem cells or their derivatives, and mobilization of endogenous stem cells in the adult brain, have been proposed as future therapies for various brain disorders such as Parkinson's disease and stroke. In support, recent progress shows that neurons suitable for transplantation can be generated from stem cells in culture, and that the adult brain produces new neurons from its own stem cells in response to injury. However, from a clinical perspective, the development of stem cell-based therapies for brain diseases is still at an early stage. Many basic issues remain to be solved and we need to move forward with caution and avoid scientifically ill-founded trials in patients. We do not know the best stem cell source, and research on embryonic stem cells and stem cells from embryonic or adult brain or from other tissues should therefore be performed in parallel. We need to understand how to control stem cell proliferation and differentiation into specific cell types, induce their integration into neural networks, and optimize the functional recovery in animal models closely resembling the human disease. All these scientific efforts are clearly justified because, for the first time, there is now real hope that we in the future can offer patients with currently intractable diseases effective cell-based treatments to restore brain function.

Linville, R. M., et al. (2020). "Three-dimensional induced pluripotent stem-cell models of human brain angiogenesis." Microvasc Res **132**: 104042.

During brain development, chemical cues released by developing neurons, cellular signaling with pericytes, and mechanical cues within the brain extracellular matrix (ECM) promote angiogenesis of brain microvascular endothelial cells (BMECs). Angiogenesis is also associated with diseases of the brain due to pathological chemical, cellular, and mechanical signaling. Existing in vitro and in vivo models of brain angiogenesis have key limitations. Here, we develop a high-throughput in vitro blood-brain barrier (BBB) bead assay of brain angiogenesis utilizing 150 mum diameter beads coated with induced pluripotent stem-cell (iPSC)-derived human BMECs (dhBMECs). After embedding the beads within a 3D matrix, we introduce various chemical cues and extracellular matrix components to explore their effects on angiogenic behavior. Based on the results from the bead assay, we generate a multi-scale model of the human cerebrovasculature within perfusable three-dimensional tissue-engineered blood-brain barrier microvessels. A sprouting phenotype is optimized in confluent monolayers of dhBMECs using chemical treatment with vascular endothelial growth factor (VEGF) and wnt ligands, and the inclusion of pro-angiogenic ECM components. As a proof-of-principle that the bead angiogenesis assay can be applied to study pathological angiogenesis, we show that

oxidative stress can exert concentration-dependent effects on angiogenesis. Finally, we demonstrate the formation of a hierarchical microvascular model of the human blood-brain barrier displaying key structural hallmarks. We develop two in vitro models of brain angiogenesis: the BBB bead assay and the tissue-engineered BBB microvessel model. These platforms provide a tool kit for studies of physiological and pathological brain angiogenesis, with key advantages over existing two-dimensional models.

Lippmann, E. S., et al. (2014). "A retinoic acid-enhanced, multicellular human blood-brain barrier model derived from stem cell sources." Sci Rep **4**: 4160.

Blood-brain barrier (BBB) models are often used to investigate BBB function and screen brain-penetrating therapeutics, but it has been difficult to construct a human model that possesses an optimal BBB phenotype and is readily scalable. To address this challenge, we developed a human in vitro BBB model comprising brain microvascular endothelial cells (BMECs), pericytes, astrocytes and neurons derived from renewable cell sources. First, retinoic acid (RA) was used to substantially enhance BBB phenotypes in human pluripotent stem cell (hPSC)-derived BMECs, particularly through adherens junction, tight junction, and multidrug resistance protein regulation. RA-treated hPSC-derived BMECs were subsequently co-cultured with primary human brain pericytes and human astrocytes and neurons derived from human neural progenitor cells (NPCs) to yield a fully human BBB model that possessed significant tightness as measured by transendothelial electrical resistance (~5,000 Omegaxcm(2)). Overall, this scalable human BBB model may enable a wide range of neuroscience studies.

Lippmann, E. S., et al. (2020). "Commentary on human pluripotent stem cell-based blood-brain barrier models." Fluids Barriers CNS **17**(1): 64.

In 2012, we provided the first published evidence that human pluripotent stem cells could be differentiated to cells exhibiting markers and phenotypes characteristic of the blood-brain barrier (BBB). In the ensuing years, the initial protocols have been refined, and the research community has identified both positive and negative attributes of this stem cell-based BBB model system. Here, we give our perspective on the current status of these models and their use in the BBB community, as well as highlight key attributes that would benefit from improvement moving forward.

Liszewska, E. and J. Jaworski (2018). "Neural Stem Cell Dysfunction in Human Brain Disorders." Results Probl Cell Differ **66**: 283-305.

Neural stem cells (NSCs) give rise to the entire nervous system. Animal models suggest that defects in NSC proliferation and differentiation contribute to several brain disorders (e.g., microcephaly, macrocephaly, autism, schizophrenia, and Huntington's disease). However, animal models of such diseases do not fully recapitulate all disease-related phenotypes because of substantial differences in brain development between rodents and humans. Therefore, additional human-based evidence is required to understand the mechanisms that are involved in the development of neurological diseases that result from human NSC (hNSC) dysfunction. Human-induced pluripotent stem cells provide a new model to investigate the contribution of hNSCs to various neurological pathologies. In this chapter, we review the role of hNSCs in both neurodevelopment- and neurodegeneration-related human brain pathologies, with an emphasis on recent evidence that has been obtained using embryonic stem cell- or induced pluripotent stem cell-derived hNSCs and progenitors.

Lu, T. M., et al. (2021). "Human Induced Pluripotent Stem Cell-Derived Brain Endothelial Cells: Current Controversies." Front Physiol **12**: 642812.

Brain microvascular endothelial cells (BMECs) possess unique properties that are crucial for many functions of the blood-brain-barrier (BBB) including maintenance of brain homeostasis and regulation of interactions between the brain and immune system. The generation of a pure population of putative brain microvascular endothelial cells from human pluripotent stem cell sources (iBMECs) has been described to meet the need for reliable and reproducible brain endothelial cells in vitro. Human pluripotent stem cells (hPSCs), embryonic or induced, can be differentiated into large quantities of specialized cells in order to study development and model disease. These hPSC-derived iBMECs display endothelial-like properties, such as tube formation and low-density lipoprotein uptake, high transendothelial electrical resistance (TEER), and barrier-like efflux transporter activities. Over time, the de novo generation of an organotypic endothelial cell from hPSCs has aroused controversies. This perspective article highlights the developments made in the field of hPSC derived brain endothelial cells as well as where experimental data are lacking, and what concerns have emerged since their initial description.

Luo, J. and P. Li (2021). "Human pluripotent stem cell-derived brain organoids as in vitro models for studying neural disorders and cancer." Cell Biosci **11**(1): 99.

The sheer complexities of brain and resource limitation of human brain tissue greatly hamper our understanding of the brain disorders and cancers. Recently developed three-dimensional (3D) brain

organoids (BOs) are self-organized and spontaneously differentiated from human pluripotent stem cells (hPSCs) in vitro, which exhibit similar features with cell type diversity, structural organization, and functional connectivity as the developing human brain. Based on these characteristics, hPSC-derived BOs (hPDBOs) provide new opportunities to recapitulate the complicated processes during brain development, neurodegenerative disorders, and brain cancers in vitro. In this review, we will provide an overview of existing BO models and summarize the applications of this technology in modeling the neural disorders and cancers. Furthermore, we will discuss the challenges associated with their use as in vitro models for disease modeling and the potential future direction.

Lv, X. S., et al. (2013). "[Effects of sodium selenite on the cell growth inhibition and intracellular reactive oxygen species of human brain glioma stem cells]." Zhongguo Ying Yong Sheng Li Xue Za Zhi **29**(1): 80-81, 85.

Mancuso, R., et al. (2019). "Stem-cell-derived human microglia transplanted in mouse brain to study human disease." Nat Neurosci **22**(12): 2111-2116.

Although genetics highlights the role of microglia in Alzheimer's disease, one-third of putative Alzheimer's disease risk genes lack adequate mouse orthologs. Here we successfully engraft human microglia derived from embryonic stem cells in the mouse brain. The cells recapitulate transcriptionally human primary microglia ex vivo and show expression of human-specific Alzheimer's disease risk genes. Oligomeric amyloid-beta induces a divergent response in human versus mouse microglia. This model can be used to study the role of microglia in neurological diseases.

Marques, E. T. A., et al. (2023). "Herpesvirus Infections in the Human Brain: A Neural Cell Model of the Complement System Derived from Induced Pluripotent Stem Cells." Curr Top Behav Neurosci **61**: 243-264.

BACKGROUND: Herpesviruses alter cognitive functions in humans following acute infections; progressive cognitive decline and dementia have also been suggested. It is important to understand the pathogenic mechanisms of such infections. The complement system - comprising functionally related proteins integral for systemic innate and adaptive immunity - is an important component of host responses. The complement system has specialized functions in the brain. Still, the dynamics of the brain complement system are still poorly understood. Many complement proteins have limited access to the brain from plasma, necessitating synthesis and specific regulation of expression in the brain; thus, complement protein synthesis, activation, regulation, and signaling should be investigated in human brain-relevant cellular models. Cells derived from human-induced pluripotent stem cells (hiPSCs) could enable tractable models. METHODS: Human-induced pluripotent stem cells were differentiated into neuronal (hi-N) and microglial (hi-M) cells that were cultured with primary culture human astrocyte-like cells (ha-D). Gene expression analyses and complement protein levels were analyzed in mono- and co-cultures. RESULTS: Transcript levels of complement proteins differ by cell type and co-culture conditions, with evidence for cellular crosstalk in co-cultures. Hi-N and hi-M cells have distinct patterns of expression of complement receptors, soluble factors, and regulatory proteins. hi-N cells produce complement factor 4 (C4) and factor B (FB), whereas hi-M cells produce complement factor 2 (C2) and complement factor 3 (C3). Thus, neither hi-N nor hi-M cells can form either of the C3-convertases - C4bC2a and C3bBb. However, when hi-N and hi-M cells are combined in co-cultures, both types of functional C3 convertase are produced, indicated by elevated levels of the cleaved C3 protein, C3a. CONCLUSIONS: hiPSC-derived co-culture models can be used to study viral infection in the brain, particularly complement receptor and function in relation to cellular "crosstalk." The models could be refined to further investigate pathogenic mechanisms.

Mayilsamy, K., et al. (2020). "Treatment with shCCL20-CCR6 nanodendriplexes and human mesenchymal stem cell therapy improves pathology in mice with repeated traumatic brain injury." Nanomedicine **29**: 102247.

Traumatic brain injury (TBI) is a devastating neurological disorder, although the underlying pathophysiology is poorly understood. TBI causes blood-brain barrier (BBB) disruption, immune cell trafficking, neuroinflammation and neurodegeneration. CCL20 is an important chemokine mediating neuroinflammation. Human mesenchymal stem cell (hMSC) therapy is a promising regenerative approach but the inflammatory microenvironment in the brain tends to decrease the efficacy of the hMSC transplantation. Reducing the inflammation prior to hMSC therapy improves the outcome. We developed a combined nano-cell therapy by using dendrimers complexed with plasmids (dendriplexes) targeting CCL20 and its sole receptor CCR6 to reduce inflammation followed by hMSC transplantation. Treatment of TBI mice with shRNA conjugated dendriplexes followed by hMSC administration downregulated the inflammatory markers and significantly increased brain-derived neurotrophic factor (BDNF) expression in the cerebral cortex

indicating future possible neurogenesis and improved behavioral deficits. Taken together, this nano-cell therapy ameliorates neuroinflammation and promotes brain tissue repair after TBI.

Mendez, E. F., et al. (2023). "A human stem cell-derived neuronal model of morphine exposure reflects brain dysregulation in opioid use disorder: Transcriptomic and epigenetic characterization of postmortem-derived iPSC neurons." Front Psychiatry **14**: 1070556.

INTRODUCTION: Human-derived induced pluripotent stem cell (iPSC) models of brain promise to advance our understanding of neurotoxic consequences of drug use. However, how well these models recapitulate the actual genomic landscape and cell function, as well as the drug-induced alterations, remains to be established. New in vitro models of drug exposure are needed to advance our understanding of how to protect or reverse molecular changes related to substance use disorders. METHODS: We engineered a novel induced pluripotent stem cell-derived model of neural progenitor cells and neurons from cultured postmortem human skin fibroblasts, and directly compared these to isogenic brain tissue from the donor source. We assessed the maturity of the cell models across differentiation from stem cells to neurons using RNA cell type and maturity deconvolution analyses as well as DNA methylation epigenetic clocks trained on adult and fetal human tissue. As proof-of-concept of this model's utility for substance use disorder studies, we compared morphine- and cocaine-treated neurons to gene expression signatures in postmortem Opioid Use Disorder (OUD) and Cocaine Use Disorder (CUD) brains, respectively. RESULTS: Within each human subject (N = 2, 2 clones each), brain frontal cortex epigenetic age parallels that of skin fibroblasts and closely approximates the donor's chronological age; stem cell induction from fibroblast cells effectively sets the epigenetic clock to an embryonic age; and differentiation of stem cells to neural progenitor cells and then to neurons progressively matures the cells via DNA methylation and RNA gene expression readouts. In neurons derived from an individual who died of opioid overdose, morphine treatment induced alterations in gene expression similar to those previously observed in OUD ex-vivo brain tissue, including differential expression of the immediate early gene EGR1, which is known to be dysregulated by opioid use. DISCUSSION: In summary, we introduce an iPSC model generated from human postmortem fibroblasts that can be directly compared to corresponding isogenic brain tissue and can be used to model perturbagen exposure such as that seen in opioid use disorder. Future studies with this and other postmortem-derived brain cellular models, including cerebral organoids, can be an invaluable tool for understanding mechanisms of drug-induced brain alterations.

Morrison, L. C., et al. (2019). "Embryonic Stem Cell Models of Human Brain Tumors." Methods Mol Biol **1869**: 127-142.

Utilization of human embryonic stem cells (hESCs) as a model system to study highly malignant pediatric cancers has led to significant insight into the molecular mechanisms governing tumor progression and has revealed novel therapeutic targets for these devastating diseases. Here, we describe a method for generating heterogeneous populations of neural precursors from both normal and neoplastic hESCs and the subsequent injection of neoplastic human embryonic neural cells (hENs) into intracerebellar or intracranial xenograft models. Histopathologically, neural tumors derived from neoplastic hENs exhibit features similar to more aggressive medulloblastoma, the most common malignant primary pediatric brain tumor. In this chapter, we will outline the detailed methods for culturing normal and neoplastic neural precursor cells in both adherent and tumorsphere format and the full characterization of the brain tumors generated from these cells in non-obese diabetic severe combined immunodeficiency (NOD SCID) mice.

Moshayedi, P., et al. (2016). "Systematic optimization of an engineered hydrogel allows for selective control of human neural stem cell survival and differentiation after transplantation in the stroke brain." Biomaterials **105**: 145-155.

Stem cell therapies have shown promise in promoting recovery in stroke but have been limited by poor cell survival and differentiation. We have developed a hyaluronic acid (HA)-based self-polymerizing hydrogel that serves as a platform for adhesion of structural motifs and a depot release for growth factors to promote transplant stem cell survival and differentiation. We took an iterative approach in optimizing the complex combination of mechanical, biochemical and biological properties of an HA cell scaffold. First, we optimized stiffness for a minimal reaction of adjacent brain to the transplant. Next hydrogel crosslinkers sensitive to matrix metalloproteinases (MMP) were incorporated as they promoted vascularization. Finally, candidate adhesion motifs and growth factors were systemically changed in vitro using a design of experiment approach to optimize stem cell survival or proliferation. The optimized HA hydrogel, tested in vivo, promoted survival of encapsulated human neural progenitor cells (iPS-NPCs) after transplantation into the stroke core and differentially tuned transplanted cell fate through the promotion of glial, neuronal or

immature/progenitor states. This HA hydrogel can be tracked in vivo with MRI. A hydrogel can serve as a therapeutic adjunct in a stem cell therapy through selective control of stem cell survival and differentiation in vivo.

Mueller, D., et al. (2005). "Transplanted human embryonic germ cell-derived neural stem cells replace neurons and oligodendrocytes in the forebrain of neonatal mice with excitotoxic brain damage." J Neurosci Res **82**(5): 592-608.

Stem cell therapy is a hope for the treatment of some childhood neurological disorders. We examined whether human neural stem cells (hNSCs) replace lost cells in a newborn mouse model of brain damage. Excitotoxic lesions were made in neonatal mouse forebrain with the N-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid (QA). QA induced apoptosis in neocortex, hippocampus, striatum, white matter, and subventricular zone. This degeneration was associated with production of cleaved caspase-3. Cells immunopositive for inducible nitric oxide synthase were present in damaged white matter and subventricular zone. Three days after injury, mice received brain parenchymal or intraventricular injections of hNSCs derived from embryonic germ (EG) cells. Human cells were prelabeled in vitro with DiD for in vivo tracking. The locations of hNSCs within the mouse brain were determined through DiD fluorescence and immunodetection of human-specific nestin and nuclear antigen 7 days after transplantation. hNSCs survived transplantation into the lesioned mouse brain, as evidenced by human cell markers and DiD fluorescence. The cells migrated away from the injection site and were found at sites of injury within the striatum, hippocampus, thalamus, and white matter tracts and at remote locations in the brain. Subsets of grafted cells expressed neuronal and glial cell markers. hNSCs restored partially the complement of striatal neurons in brain-damaged mice. We conclude that human EG cell-derived NSCs can engraft successfully into injured newborn brain, where they can survive and disseminate into the lesioned areas, differentiate into neuronal and glial cells, and replace lost neurons. (c) 2005 Wiley-Liss, Inc.

Mukherjee, S., et al. (2016). "Drosophila Brat and Human Ortholog TRIM3 Maintain Stem Cell Equilibrium and Suppress Brain Tumorigenesis by Attenuating Notch Nuclear Transport." Cancer Res **76**(8): 2443-2452.

Cancer stem cells exert enormous influence on neoplastic behavior, in part by governing asymmetric cell division and the balance between self-renewal and multipotent differentiation. Growth is favored by deregulated stem cell division, which enhances the self-renewing population and diminishes the differentiation program. Mutation of a single gene in Drosophila, Brain Tumor (Brat), leads to disrupted asymmetric cell division resulting in dramatic neoplastic proliferation of neuroblasts and massive larval brain overgrowth. To uncover the mechanisms relevant to deregulated cell division in human glioma stem cells, we first developed a novel adult Drosophila brain tumor model using brat-RNAi driven by the neuroblast-specific promoter inscuteable Suppressing Brat in this population led to the accumulation of actively proliferating neuroblasts and a lethal brain tumor phenotype. brat-RNAi caused upregulation of Notch signaling, a node critical for self-renewal, by increasing protein expression and enhancing nuclear transport of Notch intracellular domain (NICD). In human glioblastoma, we demonstrated that the human ortholog of Drosophila Brat, tripartite motif-containing protein 3 (TRIM3), similarly suppressed NOTCH1 signaling and markedly attenuated the stem cell component. We also found that TRIM3 suppressed nuclear transport of active NOTCH1 (NICD) in glioblastoma and demonstrated that these effects are mediated by direct binding of TRIM3 to the Importin complex. Together, our results support a novel role for Brat/TRIM3 in maintaining stem cell equilibrium and suppressing tumor growth by regulating NICD nuclear transport. Cancer Res; 76(8); 2443-52. (c)2016 AACR.

Muotri, A. R., et al. (2005). "Development of functional human embryonic stem cell-derived neurons in mouse brain." Proc Natl Acad Sci U S A **102**(51): 18644-18648.

Human embryonic stem cells are pluripotent entities, theoretically capable of generating a whole-body spectrum of distinct cell types. However, differentiation of these cells has been observed only in culture or during teratoma formation. Our results show that human embryonic stem cells implanted in the brain ventricles of embryonic mice can differentiate into functional neural lineages and generate mature, active human neurons that successfully integrate into the adult mouse forebrain. Moreover, this study reveals the conservation and recognition of common signals for neural differentiation throughout mammalian evolution. The chimeric model will permit the study of human neural development in a live environment, paving the way for the generation of new models of human neurodegenerative and psychiatric diseases. The model also has the potential to speed up the screening process for therapeutic drugs.

Neal, E. H., et al. (2021). "Influence of basal media composition on barrier fidelity within human pluripotent stem cell-derived blood-brain barrier models." J Neurochem **159**(6): 980-991.

It is increasingly recognized that brain microvascular endothelial cells (BMECs), the principal component of the blood-brain barrier (BBB), are highly sensitive to soluble cues from both the bloodstream and the brain. This concept extends in vitro, where the extracellular milieu can also influence BBB properties in cultured cells. However, the extent to which baseline culture conditions can affect BBB properties in vitro remains unclear, which has implications for model variability and reproducibility, as well as downstream assessments of molecular transport and disease phenotypes. Here, we explore this concept by examining BBB properties within human-induced pluripotent stem cell (iPSC)-derived BMEC-like cells cultured under serum-free conditions in DMEM/F12 and Neurobasal media, which have fully defined compositions. We demonstrate notable differences in both passive and active BBB properties as a function of basal media composition. Further, RNA sequencing and phosphoproteome analyses revealed alterations to various signaling pathways in response to basal media differences. Overall, our results demonstrate that baseline culture conditions can have a profound influence on the performance of in vitro BBB models, and these effects should be considered when designing experiments that utilize such models for basic research and preclinical assays.

Nelander, J., et al. (2013). "Human foetal brain tissue as quality control when developing stem cells towards cell replacement therapy for neurological diseases." Neuroreport **24**(18): 1025-1030.

Human foetal brain tissue has been used in experimental and clinical trials to develop cell replacement therapy in neurodegenerative disorders such as Parkinson's disease and Huntington's disease. These pioneering clinical studies have shown proof of principle that cell replacement therapy can be effective and is worthwhile to develop as a therapeutic strategy for repairing the damaged brain. However, because of the limited availability of foetal brain material, and difficulties in producing standardized and quality-tested cell preparations from this source, there have been extensive efforts in investigating the potential use of alternative cell sources for generating a large number of transplantable, authentic neural progenitors and neurons. In this review, we highlight the value of using human foetal tissue as a reference material for quality control of acquired cell fate of in vitro generated neurons before and after transplantation.

Neuhaus, W. (2017). "Human induced pluripotent stem cell based in vitro models of the blood-brain barrier: the future standard?" Neural Regen Res **12**(10): 1607-1609.

Nishihara, H., et al. (2021). "Differentiation of human pluripotent stem cells to brain microvascular endothelial cell-like cells suitable to study immune cell interactions." STAR Protoc **2**(2): 100563.

We describe the extended endothelial cell culture method (EECM) for the differentiation of human pluripotent stem cells (hPSCs) into brain microvascular endothelial cell (BMEC)-like cells. EECM-BMEC-like cells resemble primary human BMECs in morphology, molecular junctional architecture, and diffusion barrier characteristics. A mature immune phenotype with proper endothelial adhesion molecule expression makes this model distinct from any other hPSC-derived in vitro blood-brain barrier (BBB) model and suitable to study immune cell migration across the BBB in a disease relevant and personalized fashion. For complete details on the use and execution of this protocol, please refer to Lian et al. (2014), Nishihara et al. (2020a).

Nishihara, H., et al. (2020). "Advancing human induced pluripotent stem cell-derived blood-brain barrier models for studying immune cell interactions." FASEB J **34**(12): 16693-16715.

Human induced pluripotent stem cell (hiPSC)-derived blood-brain barrier (BBB) models established to date lack expression of key adhesion molecules involved in immune cell migration across the BBB in vivo. Here, we introduce the extended endothelial cell culture method (EECM), which differentiates hiPSC-derived endothelial progenitor cells to brain microvascular endothelial cell (BMEC)-like cells with good barrier properties and mature tight junctions. Importantly, EECM-BMEC-like cells exhibited constitutive cell surface expression of ICAM-1, ICAM-2, and E-selectin. Pro-inflammatory cytokine stimulation increased the cell surface expression of ICAM-1 and induced cell surface expression of P-selectin and VCAM-1. Co-culture of EECM-BMEC-like cells with hiPSC-derived smooth muscle-like cells or their conditioned medium further increased the induction of VCAM-1. Functional expression of endothelial ICAM-1 and VCAM-1 was confirmed by T-cell interaction with EECM-BMEC-like cells. Taken together, we introduce the first hiPSC-derived BBB model that displays an adhesion molecule phenotype that is suitable for the study of immune cell interactions.

Noakes, Z., et al. (2019). "Human Pluripotent Stem Cell-Derived Striatal Interneurons: Differentiation and Maturation In Vitro and in the Rat Brain." Stem Cell Reports **12**(2): 191-200.

Striatal interneurons are born in the medial and caudal ganglionic eminences (MGE and CGE) and play an important role in human striatal function and dysfunction in Huntington's disease and dystonia.

MGE/CGE-like neural progenitors have been generated from human pluripotent stem cells (hPSCs) for studying cortical interneuron development and cell therapy for epilepsy and other neurodevelopmental disorders. Here, we report the capacity of hPSC-derived MGE/CGE-like progenitors to differentiate into functional striatal interneurons. In vitro, these hPSC neuronal derivatives expressed cortical and striatal interneuron markers at the mRNA and protein level and displayed maturing electrophysiological properties. Following transplantation into neonatal rat striatum, progenitors differentiated into striatal interneuron subtypes and were consistently found in the nearby septum and hippocampus. These findings highlight the potential for hPSC-derived striatal interneurons as an invaluable tool in modeling striatal development and function in vitro or as a source of cells for regenerative medicine.

Ohshima, M., et al. (2019). "Prediction of Drug Permeability Using In Vitro Blood-Brain Barrier Models with Human Induced Pluripotent Stem Cell-Derived Brain Microvascular Endothelial Cells." Biores Open Access **8**(1): 200-209.

The strong barrier function of the blood-brain barrier (BBB) protects the central nervous system (CNS) from xenobiotic substances, while the expression of selective transporters controls the transportation of nutrients between the blood and brain. As a result, the delivery of drugs to the CNS and prediction of the ability of specific drugs to penetrate the BBB can be difficult. Although in vivo pharmacokinetic analysis using rodents is a commonly used method for predicting human BBB permeability, novel in vitro BBB models, such as Transwell models, have been developed recently. Induced pluripotent stem cells (iPSCs) have the potential to differentiate into various types of cells, and protocols for the differentiation of iPSCs to generate brain microvascular endothelial cells (BMECs) have been reported. The use of iPSCs makes it easy to scale-up iPSC-derived BMECs (iBMECs) and enables production of BBB disease models by using iPSCs from multiple donors with disease, which are advantageous properties compared with models that utilize primary BMECs (pBMECs). There has been little research on the value of iBMECs for predicting BBB permeability. This study focused on the similarity of iBMECs to pBMECs and investigated the ability of iPSC-BBB models (monoculture and coculture) to predict in vivo human BBB permeability using iBMECs. iBMECs express BMEC markers (e.g., VE-cadherin and claudin-5) and influx/efflux transporters (e.g., Glut-1, SLC7A5, CD220, P-gp, ABCG2, and MRP-1) and exhibit high barrier function (transendothelial electrical resistance, >1000 Omega x cm(2)) as well as similar transporter expression profiles to pBMECs. We determined that the efflux activity using P-glycoprotein (P-gp) transporter is not sufficient in iBMECs, while in drug permeability tests, iPSC-derived BBB models showed a higher correlation with in vivo human BBB permeability compared with a rat BBB model and the Caco-2 model. In a comparison between monoculture and coculture models, the coculture BBB model showed higher efflux activity for compounds with low CNS permeability (e.g., verapamil and thioridazine). In conclusion, iPSC-BBB models make it possible to predict BBB permeability, and employing coculturing can improve iPSC-BBB function.

Ono, H., et al. (2018). "Prostate stem cell antigen is expressed in normal and malignant human brain tissues." Oncol Lett **15**(3): 3081-3084.

Prostate stem cell antigen (PSCA) is a glycosylphosphatidylinositol (GPI)-anchored cell surface protein and exhibits an organ-dependent expression pattern in cancer. PSCA is upregulated in prostate cancer and downregulated in gastric cancer. PSCA is expressed in a variety of human organs. Although certain studies previously demonstrated its expression in the mammalian and avian brain, its expression in the human brain has not been thoroughly elucidated. Additionally, it was previously reported that PSCA is weakly expressed in the astrocytes of the normal human brain but aberrantly expressed in glioma, suggesting that PSCA is a promising target of glioma therapy and prostate cancer therapy. The current study identified PSCA expression in the neural and choroid plexus cells of the normal human brain by immunohistochemistry. In brain tumors, PSCA was expressed in medulloblastoma and glioma, and its expression was also observed in papilloma and papillary carcinoma of the choroid plexus, ependymoma and meningioma. The results suggest that PSCA may have a tumor-promoting function in brain tumors and is a potential target for their therapy. However, its expression in normal neuronal and choroid plexus cells implies that a PSCA-targeted therapy may lead to certain adverse phenomena.

Onyema, H. N., et al. (2021). "Uptake of polymeric nanoparticles in a human induced pluripotent stem cell-based blood-brain barrier model: Impact of size, material, and protein corona." Biointerphases **16**(2): 021004.

The blood-brain barrier (BBB) maintains the homeostasis of the central nervous system, which is one of the reasons for the treatments of brain disorders being challenging in nature. Nanoparticles (NPs) have been seen as potential drug delivery systems to the brain overcoming the tight barrier of endothelial cells.

Using a BBB model system based on human induced pluripotent stem cells (iPSCs), the impact of polymeric nanoparticles has been studied in relation to nanoparticle size, material, and protein corona. PLGA [poly(lactic-co-glycolic acid)] and PLLA [poly(d,l-lactide)] nanoparticles stabilized with Tween(R) 80 were synthesized (50 and 100 nm). iPSCs were differentiated into human brain microvascular endothelial cells (hBMECs), which express prominent BBB features, and a tight barrier was established with a high transendothelial electrical resistance of up to 4000 Omega cm(2). The selective adsorption of proteins on the PLGA and PLLA nanoparticles resulted in a high percentage of apolipoproteins and complement components. In contrast to the prominently used BBB models based on animal or human cell lines, the present study demonstrates that the iPSC-based model is suited to study interactions with nanoparticles in correlation with their material, size, and protein corona composition. Furthermore, asymmetrical flow field-flow fractionation enables the investigation of size and agglomeration state of NPs in biological relevant media. Even though a similar composition of the protein corona has been detected on NP surfaces by mass spectrometry, and even though similar amounts of NP are interacting with hBMECs, 100 nm-sized PLGA NPs do impact the barrier, forming endothelial cells in an undiscovered manner.

Ostrem, B. E., et al. (2014). "Control of outer radial glial stem cell mitosis in the human brain." Cell Rep **8**(3): 656-664.

Evolutionary expansion of the human neocortex is partially attributed to a relative abundance of neural stem cells in the fetal brain called outer radial glia (oRG). oRG cells display a characteristic division mode, mitotic somal translocation (MST), in which the soma rapidly translocates toward the cortical plate immediately prior to cytokinesis. MST may be essential for progenitor zone expansion, but the mechanism of MST is unknown, hindering exploration of its function in development and disease. Here, we show that MST requires activation of the Rho effector ROCK and nonmuscle myosin II, but not intact microtubules, centrosomal translocation into the leading process, or calcium influx. MST is independent of mitosis and distinct from interkinetic nuclear migration and saltatory migration. Our findings suggest that disrupted MST may underlie neurodevelopmental diseases affecting the Rho-ROCK-myosin pathway and provide a foundation for future exploration of the role of MST in neocortical development, evolution, and disease.

Ou-Yang, C. H., et al. (2021). "Generation of a human induced pluripotent stem cell (iPSC) line (IBMS-iPSC-070-02) from a patient with neurodegeneration with brain iron accumulation (NBIA) having compound heterozygous mutations in PANK2 gene." Stem Cell Res **51**: 102190.

Neurodegeneration with brain iron accumulation (NBIA) is a genetically and phenotypically heterogeneous group of inherited neurodegenerative disorder characterized by basal ganglia iron deposition. Mutations in Pantothenate Kinase 2 (PANK2) are major genetic causes for patients with NBIA. The location of PANK2 in the mitochondria suggests mutant PANK2 causing mitochondrial dysfunction in the pathogenesis of NBIA. Here, we used the Sendai virus delivery system to generate induced pluripotent stem cells (iPSCs) from peripheral blood mononuclear cells of a female patient having compound heterozygous mutations in PANK2. This cellular model could provide a platform for pathophysiological studies of NBIA in the future.

Page, S., et al. (2016). "Cerebral hypoxia/ischemia selectively disrupts tight junctions complexes in stem cell-derived human brain microvascular endothelial cells." Fluids Barriers CNS **13**(1): 16.

BACKGROUND: Cerebral hypoxia/ischemia (H/I) is an important stress factor involved in the disruption of the blood-brain barrier (BBB) following stroke injury, yet the cellular and molecular mechanisms on how the human BBB responds to such injury remains unclear. In this study, we investigated the cellular response of the human BBB to chemical and environmental H/I in vitro. METHODS: In this study, we used immortalized hCMEC/D3 and IMR90 stem-cell derived human brain microvascular endothelial cell lines (IMR90-derived BMECs). Hypoxic stress was achieved by exposure to cobalt chloride (CoCl(2)) or by exposure to 1 % hypoxia and oxygen/glucose deprivation (OGD) was used to model ischemic injury. We assessed barrier function using both transendothelial electrical resistance (TEER) and sodium fluorescein permeability. Changes in cell junction integrity were assessed by immunocytochemistry and cell viability was assessed by trypan-blue exclusion and by MTS assays. Statistical analysis was performed using one-way analysis of variance (ANOVA). RESULTS: CoCl(2) selectively disrupted the barrier function in IMR90-derived BMECs but not in hCMEC/D3 monolayers and cytotoxic effects did not drive such disruption. In addition, hypoxia/OGD stress significantly disrupted the barrier function by selectively disrupting tight junctions (TJs) complexes. In addition, we noted an uncoupling between cell metabolic activity and barrier integrity. CONCLUSIONS: In this study, we demonstrated the ability of IMR90-derived BMECs to respond to hypoxic/ischemic injury triggered by both

chemical and environmental stress by showing a disruption of the barrier function. Such disruption was selectively targeting TJ complexes and was not driven by cellular apoptosis. In conclusion, this study suggests the suitability of stem cell-derived human BMECs monolayers as a model of cerebral hypoxia/ischemia in vitro.

Pansri, P., et al. (2021). "Brain-derived neurotrophic factor increases cell number of neural progenitor cells derived from human induced pluripotent stem cells." PeerJ **9**: e11388.

BACKGROUND: Several pieces of evidence from in vitro studies showed that brain-derived neurotrophic factor (BDNF) promotes proliferation and differentiation of neural stem/progenitor cells (NSCs) into neurons. Moreover, the JAK2 pathway was proposed to be associated with mouse NSC proliferation. BDNF could activate the STAT-3 pathway and induce proliferation in mouse NSCs. However, its effects on proliferation are not fully understood and JAK/STAT pathway was proposed to play a role in this activity. METHODS: In the present study, the effects of BDNF on cell proliferation and neurite outgrowth of Alzheimer's disease (AD) induced pluripotent stem cells (iPSCs)-derived human neural progenitor cells (hNPCs) were examined. Moreover, a specific signal transduction pathway important in cell proliferation was investigated using a JAK2 inhibitor (AG490) to clarify the role of that pathway. RESULTS: The proliferative effect of BDNF was remarkably observed as an increase in Ki-67 positive cells. The cell number of hNPCs was significantly increased after BDNF treatment represented by cellular metabolic activity of the cells measured by MTT assay. This noticeable effect was statistically shown at 20 ng/ml of BDNF treatment. BDNF, however, did not promote neurite outgrowth but increased neuronal cell number. It was found that AG490 suppressed hNPCs proliferation. However, this inhibitor partially decreased BDNF-induced hNPCs proliferation. These results demonstrated the potential role of BDNF for the amelioration of AD through the increase of AD-derived hNPCs number.

Parajuli, B., et al. (2021). "Transnasal transplantation of human induced pluripotent stem cell-derived microglia to the brain of immunocompetent mice." Glia **69**(10): 2332-2348.

Microglia are the resident immune cells of the brain, and play essential roles in neuronal development, homeostatic function, and neurodegenerative disease. Human microglia are relatively different from mouse microglia. However, most research on human microglia is performed in vitro, which does not accurately represent microglia characteristics under in vivo conditions. To elucidate the in vivo characteristics of human microglia, methods have been developed to generate and transplant induced pluripotent or embryonic stem cell-derived human microglia into neonatal or adult mouse brains. However, its widespread use remains limited by the technical difficulties of generating human microglia, as well as the need to use immune-deficient mice and conduct invasive surgeries. To address these issues, we developed a simplified method to generate induced pluripotent stem cell-derived human microglia and transplant them into the brain via a transnasal route in immunocompetent mice, in combination with a colony stimulating factor 1 receptor antagonist. We found that human microglia were able to migrate through the cribriform plate to different regions of the brain, proliferate, and become the dominant microglia in a region-specific manner by occupying the vacant niche when exogenous human cytokine is administered, for at least 60 days.

Parajuli, B., et al. (2022). "Transplantation of Human Induced Pluripotent Stem Cell-Derived Microglia in Immunocompetent Mice Brain via Non-Invasive Transnasal Route." J Vis Exp(183).

Microglia are the specialized population of macrophage-like cells of the brain. They play essential roles in both physiological and pathological brain functions. Most of our current understanding of microglia is based on experiments performed in the mouse. Human microglia differ from mouse microglia, and thus response and characteristics of mouse microglia may not always represent that of human microglia. Further, due to ethical and technical difficulties, research on human microglia is restricted to in vitro culture system, which does not capitulate in vivo characteristics of microglia. To overcome these issues, a simplified method to non-invasively transplant induced pluripotent stem cell-derived human microglia (iPSMG) into the immunocompetent mice brain via a transnasal route in combination with pharmacological depletion of endogenous microglia using a colony-stimulating factor 1 receptor (CSF1R) antagonist is developed. This protocol provides a way to non-invasively transplant cells into the mouse brain and may therefore be valuable for evaluating the in vivo role of human microglia in physiological and pathological brain functions.

Pelkonen, A., et al. (2021). "Functional Characterization of Human Pluripotent Stem Cell-Derived Models of the Brain with Microelectrode Arrays." Cells **11**(1).

Human pluripotent stem cell (hPSC)-derived neuron cultures have emerged as models of electrical activity in the human brain. Microelectrode arrays

(MEAs) measure changes in the extracellular electric potential of cell cultures or tissues and enable the recording of neuronal network activity. MEAs have been applied to both human subjects and hPSC-derived brain models. Here, we review the literature on the functional characterization of hPSC-derived two- and three-dimensional brain models with MEAs and examine their network function in physiological and pathological contexts. We also summarize MEA results from the human brain and compare them to the literature on MEA recordings of hPSC-derived brain models. MEA recordings have shown network activity in two-dimensional hPSC-derived brain models that is comparable to the human brain and revealed pathology-associated changes in disease models. Three-dimensional hPSC-derived models such as brain organoids possess a more relevant microenvironment, tissue architecture and potential for modeling the network activity with more complexity than two-dimensional models. hPSC-derived brain models recapitulate many aspects of network function in the human brain and provide valid disease models, but certain advancements in differentiation methods, bioengineering and available MEA technology are needed for these approaches to reach their full potential.

Peppercorn, K., et al. (2022). "Secreted Amyloid Precursor Protein Alpha, a Neuroprotective Protein in the Brain Has Widespread Effects on the Transcriptome and Proteome of Human Inducible Pluripotent Stem Cell-Derived Glutamatergic Neurons Related to Memory Mechanisms." Front Neurosci **16**: 858524.

Secreted amyloid precursor protein alpha (sAPPalpha) processed from a parent human brain protein, APP, can modulate learning and memory. It has potential for development as a therapy preventing, delaying, or even reversing Alzheimer's disease. In this study a comprehensive analysis to understand how it affects the transcriptome and proteome of the human neuron was undertaken. Human inducible pluripotent stem cell (iPSC)-derived glutamatergic neurons in culture were exposed to 1 nM sAPPalpha over a time course and changes in the transcriptome and proteome were identified with RNA sequencing and Sequential Window Acquisition of All THeoretical Fragment Ion Spectra-Mass Spectrometry (SWATH-MS), respectively. A large subset ( approximately 30%) of differentially expressed transcripts and proteins were functionally involved with the molecular biology of learning and memory, consistent with reported links of sAPPalpha to memory enhancement, as well as neurogenic, neurotrophic, and neuroprotective phenotypes in previous studies. Differentially regulated proteins included those encoded in previously identified Alzheimer's risk genes, APP processing related proteins, proteins involved in synaptogenesis, neurotransmitters, receptors, synaptic vesicle proteins, cytoskeletal proteins, proteins involved in protein and organelle trafficking, and proteins important for cell signalling, transcriptional splicing, and functions of the proteasome and lysosome. We have identified a complex set of genes affected by sAPPalpha, which may aid further investigation into the mechanism of how this neuroprotective protein affects memory formation and how it might be used as an Alzheimer's disease therapy.

Pertuiset, B., et al. (1985). "Stem cell studies of human malignant brain tumors. Part 2: Proliferation kinetics of brain-tumor cells in vitro in early-passage cultures." J Neurosurg **63**(3): 426-432.

The proliferation kinetics were studied in early-passage cultures of cells from 13 human malignant brain tumors and two specimens of normal brain under conditions similar to those used in clonogenic cell-survival studies. Autoradiography was performed in all but four cases to estimate the fraction of cells actively replicating deoxyribonucleic acid (DNA), the approximate cell cycle time, and the effect of low-dose tritiated thymidine on cell proliferation. The mean tumor cell doubling time (TD) was 53 hours for five glioblastomas, 46 hours for two ependymomas, and 83 hours for two medulloblastomas. A gliosarcoma grew fastest (TD = 22 hours) in culture and a pilocytic astrocytoma grew slowest (TD = 144 hours). The approximate cell cycle time ranged from 1 to 2.5 days for all tumors tested. This suggests that chemotherapeutic agents that predominantly kill proliferating cells should be administered in vitro for at least 2 to 2.5 days to achieve maximum cell kill. The approximate growth fraction ranged from 0.65 to 0.96 for all tumors except for the two medulloblastomas and the pilocytic astrocytoma, which had growth fractions of 0.34 and 0.35, respectively. Most laboratories investigating the chemosensitivity of primary or early-passage human tumor cells require that 40% to 70% of cells be killed to consider a drug active in vitro. The results of this study suggest that the cell-cycle-specific agents cannot achieve a high enough cell kill to be considered active for some tumors that grow slowly in culture. An estimate of the in vitro growth rate is necessary to reliably interpret cell-survival results with such agents. Tritiated thymidine appeared to slow cell proliferation in some of the cultures, presumably as a result of radiation-induced DNA damage caused by tritium that had been incorporated into DNA. The degree to which cell growth ws slowed in individual tumors correlated with the patient's clinical response to radiation therapy and postoperative survival time.

Piao, J., et al. (2015). "Human embryonic stem cell-derived oligodendrocyte progenitors remyelinate the brain and rescue behavioral deficits following radiation." Cell Stem Cell **16**(2): 198-210.

Radiation therapy to the brain is a powerful tool in the management of many cancers, but it is associated with significant and irreversible long-term side effects, including cognitive decline and impairment of motor coordination. Depletion of oligodendrocyte progenitors and demyelination are major pathological features that are particularly pronounced in younger individuals and severely limit therapeutic options. Here we tested whether human ESC-derived oligodendrocytes can functionally remyelinate the irradiated brain using a rat model. We demonstrate the efficient derivation and prospective isolation of human oligodendrocyte progenitors, which, upon transplantation, migrate throughout the major white matter tracts resulting in both structural and functional repair. Behavioral testing showed complete recovery of cognitive function while additional recovery from motor deficits required concomitant transplantation into the cerebellum. The ability to repair radiation-induced damage to the brain could dramatically improve the outlook for cancer survivors and enable more effective use of radiation therapies, especially in children.

Pluchino, S. and L. Peruzzotti-Jametti (2013). "Rewiring the ischaemic brain with human-induced pluripotent stem cell-derived cortical neurons." Brain **136**(Pt 12): 3525-3527.

Praca, C., et al. (2019). "Derivation of Brain Capillary-like Endothelial Cells from Human Pluripotent Stem Cell-Derived Endothelial Progenitor Cells." Stem Cell Reports **13**(4): 599-611.

The derivation of human brain capillary endothelial cells is of utmost importance for drug discovery programs focusing on diseases of the central nervous system. Here, we describe a two-step differentiation protocol to derive brain capillary-like endothelial cells from human pluripotent stem cells. The cells were initially differentiated into endothelial progenitor cells followed by specification into a brain capillary-like endothelial cell phenotype using a protocol that combined the induction, in a time-dependent manner, of VEGF, Wnt3a, and retinoic acid signaling pathways and the use of fibronectin as the extracellular matrix. The brain capillary-like endothelial cells displayed a permeability to lucifer yellow of 1 x 10(-3) cm/min, a transendothelial electrical resistance value of 60 Omega cm(2) and were able to generate a continuous monolayer of cells expressing ZO-1 and CLAUDIN-5 but moderate expression of P-glycoprotein. Further maturation of these cells required coculture with pericytes. The study presented here opens a new approach for the study of soluble and non-soluble factors in the specification of endothelial progenitor cells into brain capillary-like endothelial cells.

Raut, S., et al. (2022). "Abeta peptides disrupt the barrier integrity and glucose metabolism of human induced pluripotent stem cell-derived brain microvascular endothelial cells." Neurotoxicology **89**: 110-120.

Amyloid beta (Abeta) peptides are key components of Alzheimer's disease and cerebral amyloid angiopathy and have been associated with detrimental effects at the blood-brain barrier (BBB) in vivo. Yet, the cellular and molecular mechanisms by which such peptides exert their effect on the brain vasculature remain unclear. This study aimed to assess the cellular response of induced pluripotent stem cell (iPSC)-derived brain microvascular endothelial cells (BMECs) to Abeta peptides. Changes in the barrier function, efflux transporters activity, glucose uptake, and metabolism were assessed in such model. Although iPSC-derived BMECs sustained prolonged exposure (<72 h) to a high level of Abeta peptides including Abeta42, such cells also suffered from a loss of barrier integrity, coupled with reduced glucose uptake and impaired bioenergetic activity. Taken together, this study shows the ability of iPSC-derived BMECs to reproduce features observed in other models and suggests that Abeta peptides may compromise the BBB via different targets.

Ribecco-Lutkiewicz, M., et al. (2018). "A novel human induced pluripotent stem cell blood-brain barrier model: Applicability to study antibody-triggered receptor-mediated transcytosis." Sci Rep **8**(1): 1873.

We have developed a renewable, scalable and transgene free human blood-brain barrier model, composed of brain endothelial cells (BECs), generated from human amniotic fluid derived induced pluripotent stem cells (AF-iPSC), which can also give rise to syngeneic neural cells of the neurovascular unit. These AF-iPSC-derived BECs (i-BEC) exhibited high transendothelial electrical resistance (up to 1500 Omega cm(2)) inducible by astrocyte-derived molecular cues and retinoic acid treatment, polarized expression of functional efflux transporters and receptor mediated transcytosis triggered by antibodies against specific receptors. In vitro human BBB models enable pre-clinical screening of central nervous system (CNS)-targeting drugs and are of particular importance for assessing species-specific/selective transport mechanisms. This i-BEC human BBB model discriminates species-selective antibody- mediated transcytosis mechanisms, is predictive of in vivo CNS

exposure of rodent cross-reactive antibodies and can be implemented into pre-clinical CNS drug discovery and development processes.

Rohde, F., et al. (2022). "Electrospun Scaffolds as Cell Culture Substrates for the Cultivation of an In Vitro Blood-Brain Barrier Model Using Human Induced Pluripotent Stem Cells." Pharmaceutics **14**(6).

The human blood-brain barrier (BBB) represents the interface of microvasculature and the central nervous system, regulating the transport of nutrients and protecting the brain from external threats. To gain a deeper understanding of (patho)physiological processes affecting the BBB, sophisticated models mimicking the in vivo situation are required. Currently, most in vitro models are cultivated on stiff, semipermeable, and non-biodegradable Transwell((R)) membrane inserts, not adequately mimicking the complexity of the extracellular environment of the native human BBB. To overcome these disadvantages, we developed three-dimensional electrospun scaffolds resembling the natural structure of the human extracellular matrix. The polymer fibers of the scaffold imitate collagen fibrils of the human basement membrane, exhibiting excellent wettability and biomechanical properties, thus facilitating cell adhesion, proliferation, and migration. Cultivation of human induced pluripotent stem cells (hiPSCs) on these scaffolds enabled the development of a physiological BBB phenotype monitored via the formation of tight junctions and validated by the paracellular permeability of sodium fluorescein, further accentuating the non-linearity of TEER and barrier permeability. The novel in vitro model of the BBB forms a tight endothelial barrier, offering a platform to study barrier functions in a (patho)physiologically relevant context.

Rosenblum, M. L., et al. (1983). "Stem cell studies of human malignant brain tumors. Part 1: Development of the stem cell assay and its potential." J Neurosurg **58**(2): 170-176.

A stem cell assay for human malignant gliomas has been developed. Cells obtained from tumor biopsies grew into colonies composed of malignant glial cells, as documented by histochemical, immunohistochemical, and immunobiological techniques. Studies suggest that the disaggregated cells are representative of the cells within the solid tumor. Clonogenic cells were obtained from 48 tumors and analyzed for their in vitro sensitivity to graded doses of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). The in vitro anti-tumor activity of BCNU at clinically achievable doses was compared to clinical response to the agent based on changes in computerized tomographic scan, radionuclide brain scan, and neurological examinations. Twenty-two patients received nitrosoureas before or after tumor specimen analysis, and were eligible for in vitro-in situ correlations. Clinical tumor sensitivity to nitrosoureas was predicted by culture results in 42% of all evaluable patients, and clinical resistance was predicted in 100%. The capability of the assay can be appreciated best for the 13 patients not treated with BCNU prior to culture; the in vitro prediction of clinical sensitivity and resistance was 71% and 100%, respectively. Preliminary findings show that clinical tumor resistance to BCNU may result from "intrinsic" cell resistance in some patients and from inadequate delivery of drug to tumor cells in other cases. The potential utility of this method to study the reason(s) for tumor cell resistance to drugs, to screen new chemotherapeutic agents, to individualize patient treatment, and to investigate tumor biology is discussed.

Salick, M. R., et al. (2017). "Modelling Zika Virus Infection of the Developing Human Brain In Vitro Using Stem Cell Derived Cerebral Organoids." J Vis Exp(127).

The recent emergence of Zika virus (ZIKV) in susceptible populations has led to an abrupt increase in microcephaly and other neurodevelopmental conditions in newborn infants. While mosquitos are the main route of viral transmission, it has also been shown to spread via sexual contact and vertical mother-to-fetus transmission. In this latter case of transmission, due to the unique viral tropism of ZIKV, the virus is believed to predominantly target the neural progenitor cells (NPCs) of the developing brain. Here a method for modeling ZIKV infection, and the resulting microcephaly, that occur when human cerebral organoids are exposed to live ZIKV is described. The organoids display high levels of virus within their neural progenitor population, and exhibit severe cell death and microcephaly over time. This three-dimensional cerebral organoid model allows researchers to conduct species-matched experiments to observe and potentially intervene with ZIKV infection of the developing human brain. The model provides improved relevance over standard two-dimensional methods, and contains human-specific cellular architecture and protein expression that are not possible in animal models.

Scholz, S., et al. (2022). "Induced pluripotent stem cell-derived brain organoids as potential human model system for chemotherapy induced CNS toxicity." Front Mol Biosci **9**: 1006497.

Neurotoxic phenomena are among the most common side effects of cytotoxic agents. The development of chemotherapy-induced polyneuropathy (CIPN) is a well-recognized adverse reaction in the peripheral nervous system, while changes of cognitive

functions (post-chemotherapy cognitive impairment (PCCI)) are more diffuse and have only recently drawn scientific interest. PCCI in patients most often displays as short-term memory loss, reduced multitasking ability or deficits in language. Not least, due to a lack of preclinical human model systems, the underlying molecular mechanisms are poorly understood, and treatments are missing. We thus investigated whether induced pluripotent stem cell (iPSC)-derived brain organoids can serve as a human model system for the study of chemotherapy induced central nervous system toxicity. We robustly generated mature brain organoids from iPSC-derived neuronal precursor cells (NPC), which showed a typical composition with 1) dividing NPCs forming ventricle like structures 2) matured neurons and 3) supporting glial cells closer to the surface. Furthermore, upon stimulation the brain organoids showed functional signaling. When exposed to increasing concentrations of paclitaxel, a frequently used chemotherapy drug, we observed time dependent neurotoxicity with an EC50 of 153 nM, comparable to a published murine model system. Histological analysis after paclitaxel exposure demonstrated dose dependent apoptosis induction and reduced proliferation in the organoids with further Western blot analyses indicating the degradation of neuronal calcium sensor one protein (NCS-1) and activation of Caspase-3. We could also provide evidence that paclitaxel treatment negatively affects the pool of neuronal and astrocyte precursor cells as well as mature neurons. In summary our data suggests that human iPSC derived brain organoids are a promising preclinical model system to investigate molecular mechanisms underlying PCCI and to develop novel prevention and treatment strategies.

Sheyner, M., et al. (2019). "Enhanced survival of human-induced pluripotent stem cell transplant in parkinsonian rat brain by locally applied cyclosporine." Brain Circ **5**(3): 130-133.

A major limitation with cell transplantation in patients is the unimpressive number of cells survived. The death of grafted cells involves apoptosis and immunorejection. In this review, we encapsulate the recent preclinical development that improves the survival of grafted cells and mitigates the immunorejection of human-induced pluripotent stem cells (iPSCs) through co-grating nanoparticles-containing cyclosporine A (NanoCsA) in hemiparkinsonian rats. The study supported the notion that NanoCsA allows for long-lasting CsA discharge and limits immunorejection of human iPSC xenograft in a 6-hydroxydopamine Parkinson's disease rat model.

Shin, E. S., et al. (2014). "Enhanced efficacy of human brain-derived neural stem cells by transplantation of cell aggregates in a rat model of Parkinson's disease." J Korean Neurosurg Soc **56**(5): 383-389.

OBJECTIVE: Neural tissue transplantation has been a promising strategy for the treatment of Parkinson's disease (PD). However, transplantation has the disadvantages of low-cell survival and/or development of dyskinesia. Transplantation of cell aggregates has the potential to overcome these problems, because the cells can extend their axons into the host brain and establish synaptic connections with host neurons. In this present study, aggregates of human brain-derived neural stem cells (HB-NSC) were transplanted into a PD animal model and compared to previous report on transplantation of single-cell suspensions. METHODS: Rats received an injection of 6-OHDA into the right medial forebrain bundle to generate the PD model and followed by injections of PBS only, or HB-NSC aggregates in PBS into the ipsilateral striatum. Behavioral tests, multitracer (2-deoxy-2-[(18)F]-fluoro-D-glucose ([(18)F]-FDG) and [(18)F]-N-(3-fluoropropyl)-2-carbomethoxy-3-(4-iodophenyl)nortropane ([(18)F]-FP-CIT) microPET scans, as well as immunohistochemical (IHC) and immunofluorescent (IF) staining were conducted to evaluate the results. RESULTS: The stepping test showed significant improvement of contralateral forelimb control in the HB-NSC group from 6-10 weeks compared to the control group ($p<0.05$). [(18)F]-FP-CIT microPET at 10 weeks posttransplantation demonstrated a significant increase in uptake in the HB-NSC group compared to pretransplantation ($p<0.05$). In IHC and IF staining, tyrosine hydroxylase and human beta2 microglobulin (a human cell marker) positive cells were visualized at the transplant site. CONCLUSION: These results suggest that the HB-NSC aggregates can survive in the striatum and exert therapeutic effects in a PD model by secreting dopamine.

Shin, J. C., et al. (2015). "Clinical Trial of Human Fetal Brain-Derived Neural Stem/Progenitor Cell Transplantation in Patients with Traumatic Cervical Spinal Cord Injury." Neural Plast **2015**: 630932.

In a phase I/IIa open-label and nonrandomized controlled clinical trial, we sought to assess the safety and neurological effects of human neural stem/progenitor cells (hNSPCs) transplanted into the injured cord after traumatic cervical spinal cord injury (SCI). Of 19 treated subjects, 17 were sensorimotor complete and 2 were motor complete and sensory incomplete. hNSPCs derived from the fetal telencephalon were grown as neurospheres and transplanted into the cord. In the control group, who did not receive cell implantation but were otherwise closely matched with the transplantation group, 15 patients with traumatic cervical SCI were included. At

1 year after cell transplantation, there was no evidence of cord damage, syrinx or tumor formation, neurological deterioration, and exacerbating neuropathic pain or spasticity. The American Spinal Injury Association Impairment Scale (AIS) grade improved in 5 of 19 transplanted patients, 2 (A --> C), 1 (A --> B), and 2 (B --> D), whereas only one patient in the control group showed improvement (A --> B). Improvements included increased motor scores, recovery of motor levels, and responses to electrophysiological studies in the transplantation group. Therefore, the transplantation of hNSPCs into cervical SCI is safe and well-tolerated and is of modest neurological benefit up to 1 year after transplants. This trial is registered with Clinical Research Information Service (CRIS), Registration Number: KCT0000879.

Shin, Y. J., et al. (2015). "Expression of vascular endothelial growth factor-C (VEGF-C) and its receptor (VEGFR-3) in the glial reaction elicited by human mesenchymal stem cell engraftment in the normal rat brain." J Histochem Cytochem **63**(3): 170-180.

To determine whether vascular endothelial growth factor-C (VEGF-C) and its receptor (VEGFR-3) are involved in the glial reaction elicited by transplanted mesenchymal stem cells (MSCs), we examined the cellular localization of VEGF-C and VEGFR-3 proteins in the striatum of adult normal rats that received bone marrow-derived human MSCs. The MSC grafts were infiltrated with activated microglia/macrophages and astrocytes over a 2-week period post-transplantation, which appeared to parallel the loss of transplanted MSCs. VEGF-C/VEGFR-3 was expressed in activated microglia/macrophages recruited to the graft site, where the induction of VEGF-C protein was rather late compared with that of its receptor. VEGF-C protein was absent or very weak on day 3, whereas VEGFR-3 immunoreactivity was evident within the first three days. Furthermore, within three days, VEGF-C could be detected in the brain macrophages localized immediately adjacent to the needle track. At the same time, almost all the brain macrophages in both regions expressed VEGFR-3. Reactive astrocytes at the graft site expressed VEGFR-3, but not VEGF-C. These data demonstrated the characteristic time- and cell-dependent expression patterns for VEGF-C and VEGFR-3 within the engrafted brain tissue, suggesting that they may contribute to neuroinflammation in MSC transplantation, possibly through the recruitment and/or activation of microglia/macrophages and astrogliosis.

Shreeve, J. (2005). "The other stem-cell debate: to test the potential curative powers of human embryonic stem cells, biologists want to inject them into lab animals. Creating such chimeras makes perfect sense, to a point: a sheep with a human liver? O.K. A mouse brain made up of human cells? Maybe. But a chimp that sobs?" N Y Times Mag: 42-47.

Singh, S. K., et al. (2003). "Identification of a cancer stem cell in human brain tumors." Cancer Res **63**(18): 5821-5828.

Most current research on human brain tumors is focused on the molecular and cellular analysis of the bulk tumor mass. However, there is overwhelming evidence in some malignancies that the tumor clone is heterogeneous with respect to proliferation and differentiation. In human leukemia, the tumor clone is organized as a hierarchy that originates from rare leukemic stem cells that possess extensive proliferative and self-renewal potential, and are responsible for maintaining the tumor clone. We report here the identification and purification of a cancer stem cell from human brain tumors of different phenotypes that possesses a marked capacity for proliferation, self-renewal, and differentiation. The increased self-renewal capacity of the brain tumor stem cell (BTSC) was highest from the most aggressive clinical samples of medulloblastoma compared with low-grade gliomas. The BTSC was exclusively isolated with the cell fraction expressing the neural stem cell surface marker CD133. These CD133+ cells could differentiate in culture into tumor cells that phenotypically resembled the tumor from the patient. The identification of a BTSC provides a powerful tool to investigate the tumorigenic process in the central nervous system and to develop therapies targeted to the BTSC.

Song, L., et al. (2019). "Studying Heterotypic Cell(-)Cell Interactions in the Human Brain Using Pluripotent Stem Cell Models for Neurodegeneration." Cells **8**(4).

Human cerebral organoids derived from induced pluripotent stem cells (iPSCs) provide novel tools for recapitulating the cytoarchitecture of the human brain and for studying biological mechanisms of neurological disorders. However, the heterotypic interactions of neurovascular units, composed of neurons, pericytes (i.e., the tissue resident mesenchymal stromal cells), astrocytes, and brain microvascular endothelial cells, in brain-like tissues are less investigated. In addition, most cortical organoids lack a microglia component, the resident immune cells in the brain. Impairment of the blood-brain barrier caused by improper crosstalk between neural cells and vascular cells is associated with many neurodegenerative disorders. Mesenchymal stem cells (MSCs), with a phenotype overlapping with pericytes, have promotion effects on neurogenesis and angiogenesis, which are mainly attributed to secreted growth factors and extracellular matrices. As the innate

macrophages of the central nervous system, microglia regulate neuronal activities and promote neuronal differentiation by secreting neurotrophic factors and pro-/anti-inflammatory molecules. Neuronal-microglia interactions mediated by chemokines signaling can be modulated in vitro for recapitulating microglial activities during neurodegenerative disease progression. In this review, we discussed the cellular interactions and the physiological roles of neural cells with other cell types including endothelial cells and microglia based on iPSC models. The therapeutic roles of MSCs in treating neural degeneration and pathological roles of microglia in neurodegenerative disease progression were also discussed.

Song, L., et al. (2019). "Assembly of Human Stem Cell-Derived Cortical Spheroids and Vascular Spheroids to Model 3-D Brain-like Tissues." Sci Rep **9**(1): 5977.

Human cerebral organoids derived from induced pluripotent stem cells (iPSCs) provide novel tools for recapitulating the cytoarchitecture of human brain and for studying biological mechanisms of neurological disorders. However, the heterotypic interactions of neurovascular units, composed of neurons, pericytes, astrocytes, and brain microvascular endothelial cells, in brain-like tissues are less investigated. The objective of this study is to investigate the impacts of neural spheroids and vascular spheroids interactions on the regional brain-like tissue patterning in cortical spheroids derived from human iPSCs. Hybrid neurovascular spheroids were constructed by fusion of human iPSC-derived cortical neural progenitor cell (iNPC) spheroids, endothelial cell (iEC) spheroids, and the supporting human mesenchymal stem cells (MSCs). Single hybrid spheroids were constructed at different iNPC: iEC: MSC ratios of 4:2:0, 3:2:1 2:2:2, and 1:2:3 in low-attachment 96-well plates. The incorporation of MSCs upregulated the secretion levels of cytokines VEGF-A, PGE2, and TGF-beta1 in hybrid spheroid system. In addition, tri-cultured spheroids had high levels of TBR1 (deep cortical layer VI) and Nkx2.1 (ventral cells), and matrix remodeling genes, MMP2 and MMP3, as well as Notch-1, indicating the crucial role of matrix remodeling and cell-cell communications on cortical spheroid and organoid patterning. Moreover, tri-culture system elevated blood-brain barrier gene expression (e.g., GLUT-1), CD31, and tight junction protein ZO1 expression. Treatment with AMD3100, a CXCR4 antagonist, showed the immobilization of MSCs during spheroid fusion, indicating a CXCR4-dependent manner of hMSC migration and homing. This forebrain-like model has potential applications in understanding heterotypic cell-cell interactions and novel drug screening in diseased human brain.

Stebbins, M. J., et al. (2019). "Human pluripotent stem cell-derived brain pericyte-like cells induce blood-brain barrier properties." Sci Adv **5**(3): eaau7375.

Brain pericytes play important roles in the formation and maintenance of the neurovascular unit (NVU), and their dysfunction has been implicated in central nervous system disorders. While human pluripotent stem cells (hPSCs) have been used to model other NVU cell types, including brain microvascular endothelial cells (BMECs), astrocytes, and neurons, hPSC-derived brain pericyte-like cells have not been integrated into these models. In this study, we generated neural crest stem cells (NCSCs), the embryonic precursor to forebrain pericytes, from hPSCs and subsequently differentiated NCSCs to brain pericyte-like cells. These cells closely resembled primary human brain pericytes and self-assembled with endothelial cells. The brain pericyte-like cells induced blood-brain barrier properties in BMECs, including barrier enhancement and reduced transcytosis. Last, brain pericyte-like cells were incorporated with iPSC-derived BMECs, astrocytes, and neurons to form an isogenic human model that should prove useful for the study of the NVU.

Stebbins, M. J., et al. (2018). "Activation of RARalpha, RARgamma, or RXRalpha Increases Barrier Tightness in Human Induced Pluripotent Stem Cell-Derived Brain Endothelial Cells." Biotechnol J **13**(2).

The blood-brain barrier (BBB) is critical to central nervous system (CNS) health. Brain microvascular endothelial cells (BMECs) are often used as in vitro BBB models for studying BBB dysfunction and therapeutic screening applications. Human pluripotent stem cells (hPSCs) can be differentiated to cells having key BMEC barrier and transporter properties, offering a renewable, scalable source of human BMECs. hPSC-derived BMECs have previously been shown to respond to all-trans retinoic acid (RA), and the goal of this study was to identify the stages at which differentiating human induced pluripotent stem cells (iPSCs) respond to activation of RA receptors (RARs) to impart BBB phenotypes. Here the authors identified that RA application to iPSC-derived BMECs at days 6-8 of differentiation led to a substantial elevation in transendothelial electrical resistance and induction of VE-cadherin expression. Specific RAR agonists identified RARalpha, RARgamma, and RXRalpha as receptors capable of inducing barrier phenotypes. Moreover, RAR/RXRalpha costimulation elevated VE-cadherin expression and improved barrier fidelity to levels that recapitulated the effects of RA. This study elucidates the roles of RA signaling in iPSC-derived BMEC differentiation, and identifies directed agonist

approaches that can improve BMEC fidelity for drug screening studies while also distinguishing potential nuclear receptor targets to explore in BBB dysfunction and therapy.

Stebbins, M. J., et al. (2016). "Differentiation and characterization of human pluripotent stem cell-derived brain microvascular endothelial cells." Methods **101**: 93-102.

The blood-brain barrier (BBB) is a critical component of the central nervous system (CNS) that regulates the flux of material between the blood and the brain. Because of its barrier properties, the BBB creates a bottleneck to CNS drug delivery. Human in vitro BBB models offer a potential tool to screen pharmaceutical libraries for CNS penetration as well as for BBB modulators in development and disease, yet primary and immortalized models respectively lack scalability and robust phenotypes. Recently, in vitro BBB models derived from human pluripotent stem cells (hPSCs) have helped overcome these challenges by providing a scalable and renewable source of human brain microvascular endothelial cells (BMECs). We have demonstrated that hPSC-derived BMECs exhibit robust structural and functional characteristics reminiscent of the in vivo BBB. Here, we provide a detailed description of the methods required to differentiate and functionally characterize hPSC-derived BMECs to facilitate their widespread use in downstream applications.

Steinbeck, J. A., et al. (2012). "Human embryonic stem cell-derived neurons establish region-specific, long-range projections in the adult brain." Cell Mol Life Sci **69**(3): 461-470.

While the availability of pluripotent stem cells has opened new prospects for generating neural donor cells for nervous system repair, their capability to integrate with adult brain tissue in a structurally relevant way is still largely unresolved. We addressed the potential of human embryonic stem cell-derived long-term self-renewing neuroepithelial stem cells (lt-NES cells) to establish axonal projections after transplantation into the adult rodent brain. Transgenic and species-specific markers were used to trace the innervation pattern established by transplants in the hippocampus and motor cortex. In vitro, lt-NES cells formed a complex axonal network within several weeks after the initiation of differentiation and expressed a composition of surface receptors known to be instrumental in axonal growth and pathfinding. In vivo, these donor cells adopted projection patterns closely mimicking endogenous projections in two different regions of the adult rodent brain. Hippocampal grafts placed in the dentate gyrus projected to both the ipsilateral and contralateral pyramidal cell layers, while axons of donor neurons placed in the motor cortex extended via the external and internal capsule into the cervical spinal cord and via the corpus callosum into the contralateral cortex. Interestingly, acquisition of these region-specific projection profiles was not correlated with the adoption of a regional phenotype. Upon reaching their destination, human axons established ultrastructural correlates of synaptic connections with host neurons. Together, these data indicate that neurons derived from human pluripotent stem cells are endowed with a remarkable potential to establish orthotopic long-range projections in the adult mammalian brain.

Stevanato, L., et al. (2009). "c-MycERTAM transgene silencing in a genetically modified human neural stem cell line implanted into MCAo rodent brain." BMC Neurosci **10**: 86.

BACKGROUND: The human neural stem cell line CTX0E03 was developed for the cell based treatment of chronic stroke disability. Derived from fetal cortical brain tissue, CTX0E03 is a clonal cell line that contains a single copy of the c-mycERTAM transgene delivered by retroviral infection. Under the conditional regulation by 4-hydroxytamoxifen (4-OHT), c-mycERTAM enabled large-scale stable banking of the CTX0E03 cells. In this study, we investigated the fate of this transgene following growth arrest (EGF, bFGF and 4-OHT withdrawal) in vitro and following intracerebral implantation into a mid-cerebral artery occluded (MCAo) rat brain. In vitro, 4-weeks after removing growth factors and 4-OHT from the culture medium, c-mycERTAM transgene transcription is reduced by ~75%. Furthermore, immunocytochemistry and western blotting demonstrated a concurrent decrease in the c-MycERTAM protein. To examine the transcription of the transgene in vivo, CTX0E03 cells (450,000) were implanted 4-weeks post MCAo lesion and analysed for human cell survival and c-mycERTAM transcription by qPCR and qRT-PCR, respectively. RESULTS: The results show that CTX0E03 cells were present in all grafted animal brains ranging from 6.3% to 39.8% of the total cells injected. Prior to implantation, the CTX0E03 cell suspension contained 215.7 (SEM = 13.2) copies of the c-mycERTAM transcript per cell. After implantation the c-mycERTAM transcript copy number per CTX0E03 cell had reduced to 6.9 (SEM = 3.4) at 1-week and 7.7 (SEM = 2.5) at 4-weeks. Bisulfite genomic DNA sequencing of the in vivo samples confirmed c-mycERTAM silencing occurred through methylation of the transgene promoter sequence. CONCLUSION: In conclusion the results confirm that CTX0E03 cells downregulated c-mycERTAM transgene expression both in vitro following EGF, bFGF and 4-OHT withdrawal and in vivo following

implantation in MCAo rat brain. The silencing of the c-mycERTAM transgene in vivo provides an additional safety feature of CTX0E03 cells for potential clinical application.

Sun, J., et al. (2022). "Comparative studies between the murine immortalized brain endothelial cell line (bEnd.3) and induced pluripotent stem cell-derived human brain endothelial cells for paracellular transport." PLoS One **17**(5): e0268860.

Brain microvascular endothelial cells, forming the anatomical site of the blood-brain barrier (BBB), are widely used as in vitro complements to in vivo BBB studies. Among the immortalized cells used as in vitro BBB models, the murine-derived bEnd.3 cells offer culturing consistency and low cost and are well characterized for functional and transport assays, but result in low transendothelial electrical resistance (TEER). Human-induced pluripotent stem cells differentiated into brain microvascular endothelial cells (ihBMECs) have superior barrier properties, but the process of differentiation is time-consuming and can result in mixed endothelial-epithelial gene expression. Here we performed a side-by-side comparison of the ihBMECs and bEnd.3 cells for key paracellular diffusional transport characteristics. The TEER across the ihBMECs was 45- to 68-fold higher than the bEnd.3 monolayer. The ihBMECs had significantly lower tracer permeability than the bEnd.3 cells. Both, however, could discriminate between the paracellular permeabilities of two tracers: sodium fluorescein (MW: 376 Da) and fluorescein isothiocyanate (FITC)-dextran (MW: 70 kDa). FITC-dextran permeability was a strong inverse-correlate of TEER in the bEnd.3 cells, whereas sodium fluorescein permeability was a strong inverse-correlate of TEER in the ihBMECs. Both bEnd.3 cells and ihBMECs showed the typical cobblestone morphology with robust uptake of acetylated LDL and strong immuno-positivity for vWF. Both models showed strong claudin-5 expression, albeit with differences in expression location. We further confirmed the vascular endothelial- (CD31 and tube-like formation) and erythrophagocytic-phenotypes and the response to inflammatory stimuli of ihBMECs. Overall, both bEnd.3 cells and ihBMECs express key brain endothelial phenotypic markers, and despite differential TEER measurements, these in vitro models can discriminate between the passage of different molecular weight tracers. Our results highlight the need to corroborate TEER measurements with different molecular weight tracers and that the bEnd.3 cells may be suitable for large molecule transport studies despite their low TEER.

Tajiri, N., et al. (2014). "Intravenous transplants of human adipose-derived stem cell protect the brain from traumatic brain injury-induced neurodegeneration and motor and cognitive impairments: cell graft biodistribution and soluble factors in young and aged rats." J Neurosci **34**(1): 313-326.

Traumatic brain injury (TBI) survivors exhibit motor and cognitive symptoms from the primary injury that can become aggravated over time because of secondary cell death. In the present in vivo study, we examined the beneficial effects of human adipose-derived stem cells (hADSCs) in a controlled cortical impact model of mild TBI using young (6 months) and aged (20 months) F344 rats. Animals were transplanted intravenously with 4 x 10(6) hADSCs (Tx), conditioned media (CM), or vehicle (unconditioned media) at 3 h after TBI. Significant amelioration of motor and cognitive functions was revealed in young, but not aged, Tx and CM groups. Fluorescent imaging in vivo and ex vivo revealed 1,1' dioactadecyl-3-3-3',3'-tetramethylindotricarbocyanine iodide-labeled hADSCs in peripheral organs and brain after TBI. Spatiotemporal deposition of hADSCs differed between young and aged rats, most notably reduced migration to the aged spleen. Significant reduction in cortical damage and hippocampal cell loss was observed in both Tx and CM groups in young rats, whereas less neuroprotection was detected in the aged rats and mainly in the Tx group but not the CM group. CM harvested from hADSCs with silencing of either NEAT1 (nuclear enriched abundant transcript 1) or MALAT1 (metastasis associated lung adenocarcinoma transcript 1), long noncoding RNAs (lncRNAs) known to play a role in gene expression, lost the efficacy in our model. Altogether, hADSCs are promising therapeutic cells for TBI, and lncRNAs in the secretome is an important mechanism of cell therapy. Furthermore, hADSCs showed reduced efficacy in aged rats, which may in part result from decreased homing of the cells to the spleen.

Thelin, E. P., et al. (2018). "Elucidating Pro-Inflammatory Cytokine Responses after Traumatic Brain Injury in a Human Stem Cell Model." J Neurotrauma **35**(2): 341-352.

Cytokine mediated inflammation likely plays an important role in secondary pathology after traumatic brain injury (TBI). The aim of this study was to elucidate secondary cytokine responses in an in vitro enriched (>80%) human stem cell-derived neuronal model. We exposed neuronal cultures to pre-determined and clinically relevant pathophysiological levels of tumor necrosis factor-alpha (TNF), interleukin-6 (IL-6) and interleukin-1beta (IL-1beta), shown to be present in the inflammatory aftermath of TBI. Data from this reductionist human model were then compared with our in vivo data. Human embryonic stem cell (hESC)-derived neurons were

exposed to recombinant TNF (1-10,000 pg/mL), IL-1beta (1-10,000 pg/mL), and IL-6 (0.1-1000 ng/mL). After 1, 24, and 72 h, culture supernatant was sampled and analyzed using a human cytokine/chemokine 42-plex Milliplex kit on the Luminex platform. The culture secretome revealed both a dose- and/or time-dependent release of cytokines. The IL-6 and TNF exposure each resulted in significantly increased levels of >10 cytokines over time, while IL-1beta increased the level of C-X-C motif chemokine 10 (CXCL10/IP10) alone. Importantly, these patterns are consistent with our in vivo (human) TBI data, thus validating our human stem cell-derived neuronal platform as a clinically useful reductionist model. Our data cumulatively suggest that IL-6 and TNF have direct actions, while the action of IL-1beta on human neurons likely occurs indirectly through inflammatory cells. The hESC-derived neurons provide a valuable platform to model cytokine mediated inflammation and can provide important insights into the mechanisms of neuroinflammation after TBI.

Toriya, M., et al. (2006). "Distinct functions of human numb isoforms revealed by misexpression in the neural stem cell lineage in the Drosophila larval brain." Dev Neurosci **28**(1-2): 142-155.

Mammalian Numb (mNumb) has multiple functions and plays important roles in the regulation of neural development, including maintenance of neural progenitor cells and promotion of neuronal differentiation in the central nervous system (CNS). However, the molecular bases underlying the distinct functions of Numb have not yet been elucidated. mNumb, which has four splicing isoforms, can be divided into two types based on the presence or absence of an amino acid insert in the proline-rich region (PRR) in the C-terminus. It has been proposed that the distinct functions of mNumb may be attributable to these two different types of isoforms. In this study, we used the outer optic anlage (OOA) of the Drosophila larval brain as an assay system to analyze the functions of these two types of isoforms in the neural stem cells, since the proliferation pattern of neuroepithelial (NE) stem cells in the OOA closely resembles that of the vertebrate neural stem/progenitor cells. They divide to expand the progenitor cell pool during early neurogenesis and to produce neural precursors/neurons during late neurogenesis. Clonal analysis in the OOA allows one to discriminate between the NE stem cells, which divide symmetrically to expand the progenitor pool, and the postembryonic neuroblasts (pNBs), which divide asymmetrically to produce neural precursors (ganglion mother cells), each of which divides once to produce two neurons. We found that in the OOA, the human Numb isoform with a long PRR domain (hNumb-PRRL), which is mainly expressed during early neurogenesis in the mouse CNS, promotes proliferation of both NE cells and pNBs without affecting neuronal differentiation, while the other type of hNumb isoform with a short PRR domain (hNumb-PRRS), which is expressed throughout neurogenesis in the mouse embryonic CNS, inhibits proliferation of the stem cells and promotes neuronal differentiation. We also found that hNumb-PRRS, a functional homologue of Drosophila Numb, more strongly decreases the amount of nuclear Notch than hNumb-PRRL, and could antagonize Notch functions probably through endocytic degradation, suggesting that the two distinct types of hNumb isoforms could contribute to different phases of neurogenesis in the mouse embryonic CNS.

Tornero, D., et al. (2017). "Synaptic inputs from stroke-injured brain to grafted human stem cell-derived neurons activated by sensory stimuli." Brain **140**(3): 692-706.

Transplanted neurons derived from stem cells have been proposed to improve function in animal models of human disease by various mechanisms such as neuronal replacement. However, whether the grafted neurons receive functional synaptic inputs from the recipient's brain and integrate into host neural circuitry is unknown. Here we studied the synaptic inputs from the host brain to grafted cortical neurons derived from human induced pluripotent stem cells after transplantation into stroke-injured rat cerebral cortex. Using the rabies virus-based trans-synaptic tracing method and immunoelectron microscopy, we demonstrate that the grafted neurons receive direct synaptic inputs from neurons in different host brain areas located in a pattern similar to that of neurons projecting to the corresponding endogenous cortical neurons in the intact brain. Electrophysiological in vivo recordings from the cortical implants show that physiological sensory stimuli, i.e. cutaneous stimulation of nose and paw, can activate or inhibit spontaneous activity in grafted neurons, indicating that at least some of the afferent inputs are functional. In agreement, we find using patch-clamp recordings that a portion of grafted neurons respond to photostimulation of virally transfected, channelrhodopsin-2-expressing thalamo-cortical axons in acute brain slices. The present study demonstrates, for the first time, that the host brain regulates the activity of grafted neurons, providing strong evidence that transplanted human induced pluripotent stem cell-derived cortical neurons can become incorporated into injured cortical circuitry. Our findings support the idea that these neurons could contribute to functional recovery in stroke and other conditions causing neuronal loss in cerebral cortex.

van den Hurk, M. and C. Bardy (2019). "Single-cell multimodal transcriptomics to study neuronal diversity in human stem cell-derived brain tissue and organoid models." J Neurosci Methods **325**: 108350.

Advances in human cell reprogramming and induced pluripotent stem cell technologies generate tremendous potential for neuroscience studies in health and disease, while the neuroscientist toolbox for engineering a range of brain tissues and neuronal cell types is rapidly expanding. Here, we discuss how the emergence of new single-cell genomics methods may help benchmarking and optimizing the tissue engineering process. The inherent heterogeneity and variability of reprogrammed brain tissue may conceal important disease mechanisms if not accounted for by rigorous experimental design. Single-cell genomics methods may address this technical challenge and ultimately improve the development of new therapeutics for neurological and psychiatric disorders.

Vangipuram, S. D. and W. D. Lyman (2010). "Ethanol alters cell fate of fetal human brain-derived stem and progenitor cells." Alcohol Clin Exp Res **34**(9): 1574-1583.

BACKGROUND: Prenatal ethanol (ETOH) exposure can lead to fetal alcohol spectrum disorder (FASD). We previously showed that ETOH alters cell adhesion molecule gene expression and increases neurosphere size in fetal brain-derived neural stem cells (NSC). Here, our aim was to determine the effect of ETOH on the cell fate of NSC, premature glial-committed precursor cells (GCP), and premature neuron-committed progenitor cells (NCP). METHODS: NSC, GCP, and NCP were isolated from normal second-trimester fetal human brains (n = 3) by positive selection using magnetic microbeads labeled with antibodies to CD133 (NSC), A2B5 (GCP), or PSA-NCAM (NCP). As a result of the small percentage in each brain, NSC were cultured in mitogenic media for 72 hours to produce neurospheres. The neurospheres from NSC and primary isolates of GCP and NCP were used for all experiments. Equal numbers of the 3 cell types were treated either with mitogenic media or with differentiating media, each containing 0 or 100 mM ETOH, for 120 hours. Expression of Map2a, GFAP, and O4 was determined by immunoflourescence microscopy and western blot analysis. Fluorescence intensities were quantified using Metamorph software by Molecular Devices, and the bands of western blots were quantified using densitometry. RESULTS: ETOH in mitogenic media promoted formation of neurospheres by NSC, GCP, and NCP. Under control conditions, GCP attached and differentiated, NSC and NCP formed neurospheres that were significantly smaller in size than those in ETOH. Under differentiating conditions, Map2a expression increased significantly in NSC and GCP and reduced significantly in NCP, and GFAP expression reduced significantly in GCP and NCP, and Gal-C expression reduced significantly in all 3 cell types in the presence of ETOH compared to controls. CONCLUSIONS: This study shows that ETOH alters the cell fate of neuronal stem and progenitor cells. These alterations could contribute to the mechanism for the abnormal brain development in FASD.

Wang, G., et al. (2022). "Pre-clinical study of human umbilical cord mesenchymal stem cell transplantation for the treatment of traumatic brain injury: safety evaluation from immunogenic and oncogenic perspectives." Neural Regen Res **17**(2): 354-361.

Stem cell therapy is a promising strategy for the treatment of traumatic brain injury (TBI). However, animal experiments are needed to evaluate safety; in particular, to examine the immunogenicity and tumorigenicity of human umbilical cord mesenchymal stem cells (huMSCs) before clinical application. In this study, huMSCs were harvested from human amniotic membrane and umbilical cord vascular tissue. A rat model of TBI was established using the controlled cortical impact method. Starting from the third day after injury, the rats were injected with 10 muL of 5 x 10(6)/mL huMSCs by cerebral stereotaxis or with 500 muL of 1 x 10(6)/mL huMSCs via the tail vein for 3 successive days. huMSC transplantation decreased the serum levels of proinflammatory cytokines in rats with TBI and increased the serum levels of anti-inflammatory cytokines, thereby exhibiting good immunoregulatory function. The transplanted huMSCs were distributed in the liver, lung and brain injury sites. No abnormal proliferation or tumorigenesis was found in these organs up to 12 months after transplantation. The transplanted huMSCs negligibly proliferated in vivo, and apoptosis was gradually observed at later stages. These findings suggest that huMSC transplantation for the treatment of traumatic brain injury displays good safety. In addition, huMSCs exhibit good immunoregulatory function, which can help prevent and reduce secondary brain injury caused by the rapid release of inflammatory factors after TBI. This study was approved by the Ethics Committee of Wuhan General Hospital of PLA (approval No. 20160054) on November 1, 2016.

Wilson, H. K., et al. (2015). "Exploring the effects of cell seeding density on the differentiation of human pluripotent stem cells to brain microvascular endothelial cells." Fluids Barriers CNS **12**: 13.

BACKGROUND: Brain microvascular-like endothelial cells (BMECs) derived from human pluripotent stem cells (hPSCs) have significant promise as tools for drug screening and studying the structure

and function of the BBB in health and disease. The density of hPSCs is a key factor in regulating cell fate and yield during differentiation. Prior reports of hPSC differentiation to BMECs have seeded hPSCs in aggregates, leading to non-uniform cell densities that may result in differentiation heterogeneity. Here we report a singularized-cell seeding approach compatible with hPSC-derived BMEC differentiation protocols and evaluate the effects of initial hPSC seeding density on the subsequent differentiation, yield, and blood-brain barrier (BBB) phenotype. METHODS: A range of densities of hPSCs was seeded and differentiated, with the resultant endothelial cell yield quantified via VE-cadherin flow cytometry. Barrier phenotype of purified hPSC-derived BMECs was measured via transendothelial electrical resistance (TEER), and purification protocols were subsequently optimized to maximize TEER. Expression of characteristic vascular markers, tight junction proteins, and transporters was confirmed by immunocytochemistry and quantified by flow cytometry. P-glycoprotein and MRP-family transporter activity was assessed by intracellular accumulation assay. RESULTS: The initial hPSC seeding density of approximately 30,000 cells/cm(2) served to maximize the yield of VE-cadherin+ BMECs per input hPSC. BMECs displayed the highest TEER (>2,000 Omega x cm(2)) within this same range of initial seeding densities, although optimization of the BMEC purification method could minimize the seeding density dependence for some lines. Localization and expression levels of tight junction proteins as well as efflux transporter activity were largely independent of hPSC seeding density. Finally, the utility of the singularized-cell seeding approach was demonstrated by scaling the differentiation and purification process down from 6-well to 96-well culture without impacting BBB phenotype. CONCLUSIONS: Given the yield and barrier dependence on initial seeding density, the singularized-cell seeding approach reported here should enhance the reproducibility and scalability of hPSC-derived BBB models, particularly for the application to new pluripotent stem cell lines.

Xiaoqin, Z., et al. (2021). "Dedifferentiated human umbilical cord mesenchymal stem cell reprogramming of endogenous hSDF-1alpha expression participates in neural restoration in hypoxic-ischemic brain damage rats." Genes Dis **8**(3): 331-343.

The transplantation of human umbilical cord mesenchymal stem cells (hUC-MSCs) can promote hypoxic-ischemic brain damage (HIBD) nerve repair, but finding suitable seed cells to optimize transplantation and improve treatment efficiency is an urgent problem to be solved. In this study, we induced hUC-MSCs into dedifferentiated hUC-MSCs (De-hUC-MSCs), and the morphology, stem cell surface markers, proliferation and tri-directional differentiation ability of the De-hUC-MSCs and hUC-MSCs were detected. A whole-gene chip was utilized for genome cluster, gene ontology and KEGG pathway analyses of differentially expressed genes. De-hUC-MSCs were transplanted into HIBD rats, and behavioral experiments and immunofluorescence assays were used to assess the therapeutic effect. A lentivirus vector for human stromal cell-derived factor-1 (hSDF-1alpha) was constructed, and the role of hSDF-1alpha in the neuroprotective effect and mechanism of De-hUC-MSCs was verified. De-hUC-MSCs displayed similar cell morphology, stem cell surface marker expression, cell proliferation and even three-dimensional differentiation ability as hUC-MSCs but exhibited greater treatment potential in vivo. The reprogramming mechanism of hSDF-1alpha participated in the dedifferentiation process. By successfully constructing a stable hSDF-1alpha cell line, we found that De-hUC-MSCs might participate in nerve repair through the hSDF-1alpha/CXCR4/PI3K/Akt pathway. De-hUC-MSCs reprogramming of endogenous hSDF-1alpha expression may mediate the hSDF-1alpha/CXCR4/PI3K/Akt pathway involved in nerve repair in HIBD rats.

Xu, R., et al. (2021). "Developing human pluripotent stem cell-based cerebral organoids with a controllable microglia ratio for modeling brain development and pathology." Stem Cell Reports **16**(8): 1923-1937.

Microglia play critical roles in brain development, homeostasis, and disease. Microglia in animal models cannot accurately model human microglia due to notable transcriptomic and functional differences between human and other animal microglia. Incorporating human pluripotent stem cell (hPSC)-derived microglia into brain organoids provides unprecedented opportunities to study human microglia. However, an optimized method that integrates appropriate amounts of microglia into brain organoids at a proper time point, resembling in vivo brain development, is still lacking. Here, we report a new brain region-specific, microglia-containing organoid model by co-culturing hPSC-derived primitive neural progenitor cells and primitive macrophage progenitors. In the organoids, the number of human microglia can be controlled, and microglia exhibit phagocytic activity and synaptic pruning function. Furthermore, human microglia respond to Zika virus infection of the organoids. Our findings establish a new microglia-containing brain organoid model that will serve to study human microglial function in a variety of neurological disorders.

Yamashita, M., et al. (2020). "Inhibition of transforming growth factor beta signaling pathway

promotes differentiation of human induced pluripotent stem cell-derived brain microvascular endothelial-like cells." Fluids Barriers CNS **17**(1): 36.

BACKGROUND: The blood-brain barrier (BBB) plays an important role as a biological barrier by regulating molecular transport between circulating blood and the brain parenchyma. In drug development, the accurate evaluation of BBB permeability is essential to predict not only the efficacy but also the safety of drugs. Recently, brain microvascular endothelial-like cells derived from human induced pluripotent stem cells (iPSCs) have attracted much attention. However, the differentiation protocol has not been optimized, and the enhancement of iPSC-derived brain microvascular endothelial-like cells (iBMELCs) function is required to develop highly functional BBB models for pharmaceutical research. Thus, we attempted to improve the functions of differentiated iBMELCs and develop a versatile BBB model by modulating TGF-beta signaling pathway without implementing complex techniques such as co-culture systems. METHODS: iPSCs were differentiated into iBMELCs, and TGF-beta inhibitor was used in the late stage of differentiation. To investigate the effect of TGF-beta on freezing-thawing, iBMELCs were frozen for 60-90 min or 1 month. The barrier integrity of iBMELCs was evaluated by transendothelial electrical resistance (TEER) values and permeability of Lucifer yellow. Characterization of iBMELCs was conducted by RT-qPCR, immunofluorescence analysis, vascular tube formation assay, and acetylated LDL uptake assay. Functions of efflux transporters were defined by intracellular accumulation of the substrates. RESULTS: When we added a TGF-beta inhibitor during iBMELCs differentiation, expression of the vascular endothelial cell marker was increased and blood vessel-like structure formation was enhanced. Furthermore, TEER values were remarkably increased in three iPSC lines. Additionally, it was revealed that TGF-beta pathway inhibition suppressed the damage caused by the freezing-thawing of iBMELCs. CONCLUSION: We succeeded in significantly enhancing the function and endothelial characteristics of iBMELCs by adding a small molecular compound, a TGF-beta inhibitor. Moreover, the iBMELCs could maintain high barrier function even after freezing-thawing. Taken together, these results suggest that TGF-beta pathway inhibition may be useful for developing iPSC-derived in vitro BBB models for further pharmaceutical research.

Yan, L., et al. (2021). "Recent progress and new challenges in modeling of human pluripotent stem cell-derived blood-brain barrier." Theranostics **11**(20): 10148-10170.

The blood-brain barrier (BBB) is a semipermeable unit that serves to vascularize the central nervous system (CNS) while tightly regulating the movement of molecules, ions, and cells between the blood and the brain. The BBB precisely controls brain homeostasis and protects the neural tissue from toxins and pathogens. The BBB is coordinated by a tight monolayer of brain microvascular endothelial cells, which is subsequently supported by mural cells, astrocytes, and surrounding neuronal cells that regulate the barrier function with a series of specialized properties. Dysfunction of barrier properties is an important pathological feature in the progression of various neurological diseases. In vitro BBB models recapitulating the physiological and diseased states are important tools to understand the pathological mechanism and to serve as a platform to screen potential drugs. Recent advances in this field have stemmed from the use of pluripotent stem cells (PSCs). Various cell types of the BBB such as brain microvascular endothelial cells (BMECs), pericytes, and astrocytes have been derived from PSCs and synergistically incorporated to model the complex BBB structure in vitro. In this review, we summarize the most recent protocols and techniques for the differentiation of major cell types of the BBB. We also discuss the progress of BBB modeling by using PSC-derived cells and perspectives on how to reproduce more natural BBBs in vitro.

Yin, X., et al. (2020). "Integration of Human Induced Pluripotent Stem Cell (hiPSC)-Derived Neurons into Rat Brain Circuits." Bio Protoc **10**(17): e3746.

Human neuron transplantation offers novel opportunities for modeling human neurologic diseases and potentially replacement therapies. However, the complex structure of the human cerebral cortex, which is organized in six layers with tightly interconnected excitatory and inhibitory neuronal networks, presents significant challenges for in vivo transplantation techniques to obtain a balanced, functional and homeostatically stable neuronal network. Here, we present a protocol to introduce human induced pluripotent stem cell (hiPSC)-derived neural progenitors to rat brains. Using this approach, hiPSC-derived neurons structurally integrate into the rat forebrain, exhibit electrophysiological characteristics, including firing, excitatory and inhibitory synaptic activity, and establish neuronal connectivity with the host circuitry.

Yin, X. J., et al. (2006). "[Experimental study on growth, proliferation and differentiation of neural stem cell from subventricular zone of human fetal brain at different gestational age]." Zhonghua Er Ke Za Zhi **44**(7): 500-504.

OBJECTIVE: To study growth characteristics of neural stem cells (NSCs) from subventricular zone

(SVZ) of the different human fetal brain at different gestational age and to provide experimental and theoretical evidences for clinical application of NSCs for treatment of certain diseases. METHODS: Ninety human embryos at gestational age 16 - 36 weeks were collected and were divided into six groups according to gestational age: 16 w, 20 w, 24 w, 28 w, 32 w and 36 w. Each group had 15 embryos and brain tissues were taken from each embryo's SVZ. All subjects had congenital heart disease or digestive tract abnormity diagnosed with B ultrasound at antepartum, but none had abnormal development of brain. Pregnant mother and her husband desire termination of pregnancy. The morphology, existing mode and the number of neural stem cells in subventricular zone were examined with immunohistochemical method. The NSCs in subventricular zone were cultured, passaged and differentiated with cell culture technique, then were identified with immunohistochemical method. RESULTS: NSCs in SVZ from the different human fetal brain existed in a scattered manner in the network formed by stellate cells, NSCs had round, ellipse and fusiform shape, especially in stellate shape. NSCs had larger and smaller size and distributed in dense or scattered forms, each having zero to two enations, most had one or two. NSCs had less cytoplasm. The nucli of the NSCs had a round shape with loose chromatin and 1 - 4 nucleoli. Most of NSCs existed in singular scattered form, some of them showed symmetrical or asymmetrical division, some of them showed synaptic connection with other NSCs. The number of NSCs in SVZ from groups with different fetal age decreased with increasing gestational age (chi(2) = 4644.602, P < 0.01). NSCs in SVZ from the different human fetal brain cultured with serum-free medium formed typical neurospheres in suspension. The cells could be passaged continuously, and could express nestin antigen. Serum-contained medium induced neural stem cells to differentiate and express specific antigens of neuron, astrocyte and oligodendrocyte. CONCLUSIONS: NSCs existed in SVZ of human embryos at different gestational age. There are differences in morphology, existing pattern and the number of NSCs in SVZ at different gestational age. NSCs in SVZ at different gestational age may be cultured in vitro.

Yoon, H. H., et al. (2013). "Are human dental papilla-derived stem cell and human brain-derived neural stem cell transplantations suitable for treatment of Parkinson's disease?" Neural Regen Res **8**(13): 1190-1200.

Transplantation of neural stem cells has been reported as a possible approach for replacing impaired dopaminergic neurons. In this study, we tested the efficacy of early-stage human dental papilla-derived stem cells and human brain-derived neural stem cells in rat models of 6-hydroxydopamine-induced Parkinson's disease. Rats received a unilateral injection of 6-hydroxydopamine into right medial forebrain bundle, followed 3 weeks later by injections of PBS, early-stage human dental papilla-derived stem cells, or human brain-derived neural stem cells into the ipsilateral striatum. All of the rats in the human dental papilla-derived stem cell group died from tumor formation at around 2 weeks following cell transplantation. Postmortem examinations revealed homogeneous malignant tumors in the striatum of the human dental papilla-derived stem cell group. Stepping tests revealed that human brain-derived neural stem cell transplantation did not improve motor dysfunction. In apomorphine-induced rotation tests, neither the human brain-derived neural stem cell group nor the control groups (PBS injection) demonstrated significant changes. Glucose metabolism in the lesioned side of striatum was reduced by human brain-derived neural stem cell transplantation. [(18)F]-FP-CIT PET scans in the striatum did not demonstrate a significant increase in the human brain-derived neural stem cell group. Tyrosine hydroxylase (dopaminergic neuronal marker) staining and G protein-activated inward rectifier potassium channel 2 (A9 dopaminergic neuronal marker) were positive in the lesioned side of striatum in the human brain-derived neural stem cell group. The use of early-stage human dental papilla-derived stem cells confirmed its tendency to form tumors. Human brain-derived neural stem cells could be partially differentiated into dopaminergic neurons, but they did not secrete dopamine.

Zhang, J., et al. (2013). "Recombinant human brain natriuretic peptide therapy combined with bone mesenchymal stem cell transplantation for treating heart failure in rats." Mol Med Rep **7**(2): 628-632.

This study aimed to investigate the effects of combined recombinant human brain natriuretic peptide (rhBNP) therapy and bone mesenchymal stem cell (BMSC) transplantation on cell survival in myocardial tissues and on heart function in a rat model of heart failure (HF). Rat BMSCs were isolated, amplified and adherent cultured in vitro. A rat model of HF was established via the intraperitoneal injection of doxorubicin (Adriamycin). The rats were randomly divided into normal, HF, BMSC, rhBNP and BMSC plus rhBNP groups. The BMSCs were administered once via tail vein injection and rhBNP was infused via the jugular vein. Echocardiography and polygraphy were used to evaluate heart function. An enzyme-linked immunosorbent assay was used to detect the changes in brain natriuretic peptide (BNP) concentration prior to and following intervention. Western blot analysis was used to detect the expression

of the myocardium-specific proteins GATA-binding protein 4 (GATA-4), connexin 43 (Cx43) and cardiac troponin I (cTnI). The results of cardiac echocardiography and the hemodynamic data show that various indicators of left ventricular systolic function in the BMSC plus rhBNP group were significantly improved compared with those in the other groups (P<0.05). No significant differences in the improvement of cardiac function were observed between the BMSC and rhBNP groups (P>0.05). Following treatment, a significant difference in BNP levels was observed between the BMSC plus rhBNP and the BMSC groups (P<0.05). The GATA-4, Cx43 and cTnI expression levels in the BMSC plus rhBNP group were higher than those in the BMSC group. Compared with rhBNP treatment, BMSC transplantation alone does not significantly improve heart function. However, combining rhBNP therapy and BMSC transplantation increases the expression levels of GATA-4 and other proteins to improve cardiac systolic and diastolic function.

Zhang, L., et al. (2023). "Human umbilical cord mesenchymal stem cell-derived exosome suppresses programmed cell death in traumatic brain injury via PINK1/Parkin-mediated mitophagy." CNS Neurosci Ther.

AIMS: Recently, human umbilical cord mesenchymal stem cell (HucMSC)-derived exosome is a new focus of research in neurological diseases. The present study was aimed to investigate the protective effects of HucMSC-derived exosome in both in vivo and in vitro TBI models. METHODS: We established both mouse and neuron TBI models in our study. After treatment with HucMSC-derived exosome, the neuroprotection of exosome was investigated by the neurologic severity score (NSS), grip test score, neurological score, brain water content, and cortical lesion volume. Moreover, we determined the biochemical and morphological changes associated with apoptosis, pyroptosis, and ferroptosis after TBI. RESULTS: We revealed that treatment of exosome could improve neurological function, decrease cerebral edema, and attenuate brain lesion after TBI. Furthermore, administration of exosome suppressed TBI-induced cell death, apoptosis, pyroptosis, and ferroptosis. In addition, exosome-activated phosphatase and tensin homolog-induced putative kinase protein 1/Parkinson protein 2 E3 ubiquitin-protein ligase (PINK1/Parkin) pathway-mediated mitophagy after TBI. However, the neuroprotection of exosome was attenuated when mitophagy was inhibited, and PINK1 was knockdown. Importantly, exosome treatment also decreased neuron cell death, suppressed apoptosis, pyroptosis, and ferroptosis and activated the PINK1/Parkin pathway-mediated mitophagy after TBI in vitro. CONCLUSION: Our results provided the first evidence that exosome treatment played a key role in neuroprotection after TBI through the PINK1/Parkin pathway-mediated mitophagy.

Zhang, Y., et al. (2017). "Systemic administration of cell-free exosomes generated by human bone marrow derived mesenchymal stem cells cultured under 2D and 3D conditions improves functional recovery in rats after traumatic brain injury." Neurochem Int **111**: 69-81.

Multipotent human bone marrow derived mesenchymal stem cells (hMSCs) improve functional outcome after experimental traumatic brain injury (TBI). The present study was designed to investigate whether systemic administration of cell-free exosomes generated from hMSCs cultured in 2-dimensional (2D) conventional conditions or in 3-dimensional (3D) collagen scaffolds promote functional recovery and neurovascular remodeling in rats after TBI. Wistar rats were subjected to TBI induced by controlled cortical impact; 24 h later tail vein injection of exosomes derived from hMSCs cultured under 2D or 3D conditions or an equal number of liposomes as a treatment control were performed. The modified Morris water maze, neurological severity score and footfault tests were employed to evaluate cognitive and sensorimotor functional recovery. Animals were sacrificed at 35 days after TBI. Histological and immunohistochemical analyses were performed for measurements of lesion volume, neurovascular remodeling (angiogenesis and neurogenesis), and neuroinflammation. Compared with liposome-treated control, exosome-treatments did not reduce lesion size but significantly improved spatial learning at 33-35 days measured by the Morris water maze test, and sensorimotor functional recovery, i.e., reduced neurological deficits and footfault frequency, observed at 14-35 days post injury ($p < 0.05$). Exosome treatments significantly increased the number of newborn endothelial cells in the lesion boundary zone and dentate gyrus, and significantly increased the number of newborn mature neurons in the dentate gyrus as well as reduced neuroinflammation. Exosomes derived from hMSCs cultured in 3D scaffolds provided better outcome in spatial learning than exosomes from hMSCs cultured in the 2D condition. In conclusion, hMSC-generated exosomes significantly improve functional recovery in rats after TBI, at least in part, by promoting endogenous angiogenesis and neurogenesis and reducing neuroinflammation. Thus, exosomes derived from hMSCs may be a novel cell-free therapy for TBI, and hMSC-scaffold generated exosomes may selectively enhance spatial learning.

Zhou, S. (2011). "From bone to brain: human skeletal stem cell therapy for stroke." Cent Nerv Syst Agents Med Chem **11**(2): 157-163.

Human adult skeletal stem cells, a.k.a. mesenchymal stem cells or marrow stromal cells (MSCs), have been identified as precursors of several different mesenchymal cellular lineages, including osteoblasts, chondrocytes, myoblasts, adipocytes, and fibroblasts, as well as non-mesenchymal lineages including neurons and glial cells. Adult stem cell transplantation is a promising strategy for the treatment of stroke. MSCs are also used as a platform for gene therapies and therapeutic agents. In this review, we discuss recent progress of human skeletal stem cell biology, in vitro differentiation of MSCs into neural stem cells and neurons, MSC therapy for stroke, MSC aging and the challenge of autologous cell therapy for stroke in elderly patients.

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