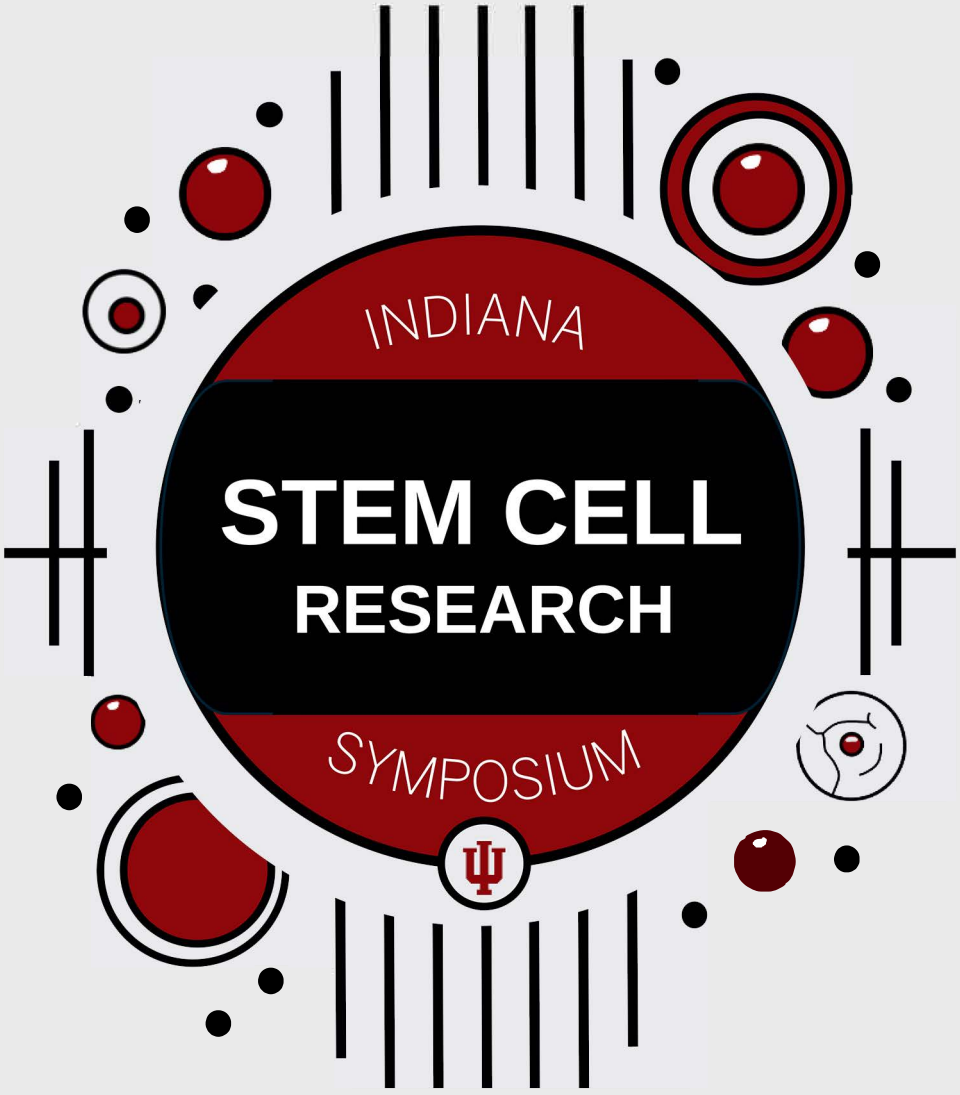

Supported by IU Research Conference Grant
and Stark Neurosciences Research Institute

Monday, November 11

9 a.m. to 5 p.m.

Goodman Hall Auditorium

SCHOOL OF MEDICINE
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Special Thanks To:

Funding:
IU Research Conference Grant
Stark Neurosciences Research Institute

Conference Co-Organizers:
Jason Meyer, Director of SNRI Stem Cell Research Group
Scott Canfield, Associate Professor of Anatomy, Cell Biology, and Physiology
Tasneem Sharma, Assistant Professor of Ophthalmology

Poster Judges:
Scott Canfield, Arupratan Das, Tasneem Sharma, Cátia Gomes, Nur Jury Garfe, Hande Karahan, Joshua Kulas, Abdul Syed

Administrative Assistance:
Angela Bryant, Kylee Sutton, Sarah Castle, Randy Talley

Symposium Logo:
Rylee Anderson

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AGENDA

Events held in Goodman Hall Auditorium unless otherwise specified

9:00	Registration and Coffee
10:00	Introductory Comments
10:15	Oral Presentation Session #1
	Dr. Tasneem Sharma, IUSM - "Stem Cell-Based Therapeutic Strategies for Glaucoma Treatment"
	Dr. Ranjie Xu, Purdue University - "Develop a Vascularized Neuroimmune Organoid Model for Studying Sporadic Alzheimer's Disease and Drug Discovery"
	Dr. Stephanie Bissel, IUSM - "Cellular mechanisms of human microglia bearing genetic variants associated with risk and resilience to Alzheimer's Disease"
	Kaiyuan Yang, IU-Bloomington - "Biological neuromorphic computing using multiple brain organoids"
11:15	Poster Teaser Presentations
11:30	Lunch (NB-101)
12:30	Poster Presentations (GH1353A/B)
2:00	Group Photo (Goodman Hall Lobby)
2:15	Oral Presentation Session #2
	Dr. Scott Canfield, IUSM-Terre Haute - "Modelling Pathological States of the Blood- Brain Barrier Derived from Human Pluripotent Stem Cells"
	Dr. Feng Guo, IU-Bloomington - "Intersection of AI and Organoids"
	Dr. Cátia Gomes, IUSM - "Reactive astrocytes promote retinal ganglion cell degeneration in human pluripotent stem cell models through complement C3 activation"
	Dr. Rebecca Schmitt, IUSM - "Investigation of GNE mutation classes and aberrant autophagy in GNE myopathy"
3:15	Keynote Presentation
	Dr. Sally Temple, Neural Stem Cell Institute
	"Using Human Brain Organoids to Explore the Progression of Neurodegenerative Disease"
4:15	Concluding Remarks and Award Announcements

List of Poster Presentations

Poster #	Last Name	First Name	Poster Title
1	Abhyankar	Surabhi	Investigating Hematopoietic Stem Cells Transcriptome to Identify RNA-based Biomarkers for Diabetic Retinopathy
2	Cantor	Erica	Passage number affects differentiation of sensory neurons from human induced pluripotent stem cells
3	Cowger	Kate	Generation of novel lines of human induced pluripotent stem cells from elderly rural Indiana neurodegenerative patients
4	Dutta	Sayanta	Targeting 5HT1A Serotonin Receptor Provides Glaucoma Neuroprotection by Restoring Mitochondria
5	Shahul Hameed	Shahna	Assessing therapeutic potential of human neuritin-1 in glaucoma patient stem cell derived retinal ganglion cells.
6	Tian	Chunhui	Exploring Pathogenic Role of Monocytes in Alzheimer's Disease through engineered human brain organoid chips
7	Tutrow	Kaylee	Alzheimer's Disease-related fractalkine receptor polymorphism confers distinct phenotypes in iPSC-derived human microglia-like cells
8	Wu	Wei	Spinal cord injury (SCI) often leads to neuronal loss, axonal degeneration, and behavioral dysfunction.
9	Yanru	Ji	Developing a Vascularized Neuroimmune Organoid Model for Studying Sporadic Alzheimer's Disease and Drug Screening
10	Afify	Reham	Harnessing the Potential of Human iPSC-derived Microglia-like Cells as Antigen Presenting Cells for MAIT Cells to Study AD Pathology
11	Anderson	Rylee	The Effects of Aluminum on the Blood Brain Barrier
12	Baker	Aaron	Generation of novel APBB2 SNP iPSC lines to study increased risk for glaucoma unique to individuals of African ancestry.
13	Burket	Noah	NF2 Loss Disrupts Differentiation of Neuroepithelial Stem Cells
14	Carino	Pia	The Effects of Propofol on a Blood Brain Barrier Model Derived from Human iPSCs
15	Estaki	Zohreh	Intramuscular Administration of Adipose Stem Cell-Derived Secretome in a mouse model of Amyotrophic Lateral Sclerosis
16	Hanquier	Jocelyne	Regulation of Neuronal Differentiation by Lysine Methylation
17	Haskell	Angela	Functional analysis of induced pluripotent stem cell derived microglia-like cells
18	Hennigan	Andrew	The Utilization of Developmental Muscle Precursors in Volumetric Muscle Loss
19	Hetzer	Shelby	Establishing an in vitro model of retino-thalamic projections to study optic nerve injury in a dish.
20	Jung	Sunghan	Small molecule APP degrader reduces Aβ42 through lysosomal pathway in Alzheimer's patient-derived iPSC differentiated neurons and 3D organoids.
21	Kim	Hyunjin	THE IMPACT OF CHRONIC MANGANESE ON GLUTAMATE EXCITOTOXICITY IN HUMAN IPSC-DERIVED CORTICAL MODEL OF ALZHEIMER'S DISEASE
22	Patil	Shruti	Development of a human stem cell model of NeurofibromatosisType 1 for assessment of optic pathway phenotypes
23	Pena	Kiersten	A Highly Reproducible and Efficient Method for Retinal Organoid Differentiation From Human Pluripotent Stem Cells
24	Prosser	Carson	Induction of aging-related features in human pluripotent stem cell-derived retinal ganglion cells to study neurodegeneration in glaucoma
25	Shields	Priya	The Effects of Stress on ALS Patient iPSC-derived Motor Neurons
26	Sisil	Addison	The Effects of PFAS on the Blood-Brain Barrier
27	Smiley	Jake	Comparison of Patient and Wild Type hiPSC derived Schwann Cells for understanding disease mechanisms of NF2-related schwannomatosis
28	Strom	Madeleine	Developmental Exposure of hiPSC-derived Cortical Cultures to Methylmercury Induces Persistent Functional Effects
29	Tilston-Lunel	Andrew	Cell fate decisions: Can we toy with them?
30	Varghese	Laurna	Rapid induction of functional neurons using NGN2-based directed differentiation in human induced pluripotent stem cells

ORAL PRESENTATION ABSTRACTS

Stem Cell-Based Therapeutic Strategies for Glaucoma Treatment

Shahna S. Hameed1, Tasneem P. Sharma1, *

1Department of Ophthalmology, Indiana University School of Medicine, Indiana University, Indianapolis, 1160 W. Michigan St, Indianapolis, IN, 46202 *Presenting author

Purpose: Glaucoma is a progressive optic neuropathy characterized by retinal ganglion cell (RGC) death and gradual visual field loss. RGC transplantation presents a promising therapeutic approach for end-stage glaucoma and can be investigated using preclinical models, such as stem cell technologies and *ex vivo* model systems. Previous studies suggest certain RGC subtypes show varying susceptibility to glaucomatous neurodegeneration, indicating subtype-specific response to apoptosis. Human induced pluripotent stem cells (iPSCs) emerge as a robust tool for studying RGC subtype loss in glaucoma and offer a viable source for therapeutic RGC transplantation. This study aimed to assess both the selective loss of RGC subtypes in glaucoma and viability of hiPSC derived RGC transplantation in a preclinical human glaucoma model.
Methods: Human iPSCs were reprogrammed from non-glaucomatous and glaucomatous donor keratocytes (N=3). Characterization of keratocytes (Keratocan, α-SMA) and iPSCs (TRA-1-60, OCT4, SOX2, C-MYC, KLF4) were confirmed by protein and gene markers. The iPSCs were differentiated into retinal organoids (ROs) with downstream RGC generation. Day-30 ROs were profiled for RGC subtype markers (*CDH6, MMP17, RBPMS2, FABP4, SPP1, OPN4, SDK2, JAM2, PVALB, FOXP1)* and cleaved CASP-3 immunostaining for apoptosis. The iPSC derived RGCs were AAV2-GFP transduced and seeded in human control eyes (N=4). Eyes were pressurized for 5–7 days at normal (left eye) and elevated intraocular pressure (IOP) (right eye). RGC survival, apoptosis, retinal activity, and optic nerve head extracellular matrix (ECM) deposition was assessed.
Results: Keratocytes were successfully reprogrammed into iPSCs, differentiated into ROs and RGCs generated. Three major iPSC derived RGC subtypes were identified in ROs, with a significant increase in α-RGCs (SPP1) observed in glaucomatous ROs. Cleaved CASP-3 correlated with RGC loss in both ROs and cultured RGCs. Downstream GFP-transduced RGCs transplanted into donor eyes were successfully maintained at normal and elevated IOPs for 5-7 days (left eye: 9.5 ± 1.45, right eye: 23.1 ± 1.5, N=4, $p<0.05$). Under normal IOP, transplanted RGCs showed higher survival rate (*THY1*, $p<0.05$) with less ECM deposition ($p<0.05$) and improved retinal activity.
Conclusions: This study confirmed glaucoma-specific RGC subtype loss and demonstrated iPSC derived RGCs as a reliable option for advanced glaucoma therapy.

Develop a Vascularized Neuroimmune Organoid Model for Studying Sporadic Alzheimer's Disease and Drug Discovery

Ranjie Xu
Purdue University

Alzheimer's Disease (AD) is the most common cause of dementia afflicting 55 million individuals worldwide, with limited treatment available. Current AD models mainly focus on familial AD (fAD), which is due to genetic mutations. However, models for studying sporadic AD (sAD), which represents over 95% of AD cases without specific genetic mutations, are severely limited. Moreover, the fundamental species differences between humans and animals might significantly contribute to clinical failures for AD therapeutics that have shown success in animal models, highlighting the urgency to develop more translational human models for studying AD, particularly sAD. In this study, we developed a complex human pluripotent stem cell (hPSC)-based vascularized neuroimmune organoid model, which contains multiple cell types affected in human AD brains, including human neurons, microglia, astrocytes, and blood vessels. Importantly, we demonstrated that brain extracts from individuals with sAD can effectively induce multiple AD pathologies in organoids four weeks post-exposure, including amyloid beta (Aβ) plaques-like aggregates, tau tangles-like aggregates, neuroinflammation, elevated microglial synaptic pruning, synapse/neuronal loss, and impaired neural network. Furthermore, after treatment with Lecanemab, an FDA-approved drug targeting Aβ, AD brain extract exposed organoids showed a significant reduction of amyloid burden. Thus, the neuroimmune organoid model provides a unique opportunity to study AD, particularly sAD under a pathophysiological relevant three-dimensional (3D) human cell environment. It also holds great promise to facilitate AD drug development, particularly for immunotherapies.

Cellular mechanisms of human microglia bearing genetic variants associated with risk and resilience to Alzheimer's Disease

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Recent genetic studies have highlighted microglia as critical mediators of Alzheimer's disease (AD) pathogenesis. Genetic variants of phospholipase C gamma-2 (PLCG2), selectively expressed by microglia, have been identified that modulate AD risk. Notably, the PLCG2P522R variant, a functional hypermorph, is associated with reduced AD risk, whereas the PLCG2M28L variant, a loss-of-function mutation, is linked to increased AD risk. PLCG2 is an enzyme that functions as an immune signaling hub downstream of surface receptors such TREM2.

Our previous research has demonstrated bidirectional modulation of plaque load, behavior, electrophysiology, and microglial responses in amyloidogenic mice bearing these PLCG2 mutations. However, the cellular signaling alterations mediated by these PLCG2 variants remain unknown. To address this knowledge gap, we cultured iPSC-derived microglia-like cells harboring the variants, as well as a PLCG2 knock-out line, and analyzed basal-level differences in microglial function. Transcriptomic pathway analysis revealed differentially expressed genes associated with cell survival, mitochondrial function, and immune sensor proteins. Follow up functional analysis indicated that the protective and risk variants of PLCG2 show altered proliferation, survival, and microglial phenotypic expression.

These results provide new insights into the distinct roles of PLCG2 variants in microglial function and AD pathogenesis. Understanding these basal-level differences is crucial for elucidating how PLCG2 variants modulate AD risk.

This work was supported by NIA grant RF1 AG074566 and U54 AG065181.

Biological neuromorphic computing using multiple brain organoids

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Abstract

Neuromorphic hardware inspired by biological systems holds significant promise for advancing large-scale artificial intelligence, thanks to benefits like lower energy consumption, rapid learning capabilities, and environmental friendliness. However, current neuromorphic systems face challenges in processing large volumes of sequential and temporal data. This study introduces a parallel biocomputing architecture that harnesses biological neural networks within brain organoids for real-world tasks. The system involves multiple cortical organoids derived from stem cells, each interacting independently through information-encoded stimulation and multi-well electrode array recordings. These organoids exhibit nonlinear dynamics and fading memory capabilities, allowing them to autonomously process temporal and sequential information. Our novel approach integrates this system within a reservoir computing framework to effectively classify letters and words, demonstrating its potential for practical applications.

Modelling Pathological States of the Blood-Brain Barrier Derived from Human Pluripotent Stem Cells

Scott G. Canfield

Indiana University School of Medicine – Terre Haute

Abstract: The Canfield laboratory focuses on the development and utilization of a human induced pluripotent stem cell-derived blood-brain barrier (iPSC-BBB) model. The BBB is comprised of barrier forming brain microvascular endothelial cells supported by astrocytes, pericytes, and neurons. The BBB is critical in maintaining homeostasis between the brain parenchyma and the surrounding vasculature. BBB integrity is maintained by both active and passive barrier components that ensure barrier health. Countless pathological states affect the integrity of the BBB and can exacerbate and/or initiate disease pathologies. The Canfield lab utilizes human iPSCs to differentiate and maintain a robust human-derived BBB model with in vivo-like properties. Specifically, they utilize the iPSC-derived BBB model in understanding pathological states of the developing human blood brain barrier including Alzheimer's disease, post-operative delirium, alcohol use disorder, environmental toxins and diabetes.

Intersection of AI and Organoids

Feng Guo
IU-Bloomington

Human organoids are three-dimensional organ-like cultures derived from human stem cells and tissues. They can recapitulate key physiology of specific organs or tissues found in the human body, holding promising potential in studying organ development, disease modeling, drug screening, and regenerative medicine applications. For example, human brain organoids can recreate certain brain cell diversity and brain structure to mimic the development of embryonic brain tissues and inspire new artificial intelligence (AI) technology. Our group is dedicated to developing and integrating "Intelligent Organoid Systems" by leveraging innovative device design, sensor fusion, and AI-guided system integration to interface organoids to address the challenges in AI computing and precision medicine. In this talk, I will introduce the development of organoid computing and AI-powered organoid systems and showcase their translational applications in disease diagnosis, personalized therapy, and drug screening for translational oncology, neurology, and immunology. By building the intersection between organoids and AI, we hope to create innovative intelligent tools that are highly efficient, accurate, and convenient, ultimately revolutionizing medicine.

Reactive astrocytes promote retinal ganglion cell degeneration in human pluripotent stem cell models through complement C3 activation

Cátia Gomes
Indiana University School of Medicine

Astrocytes closely associate with retinal ganglion cells (RGCs) in the nerve fiber layer of the retina and optic nerve, where they provide support for RGCs but can contribute to RGC degeneration in a glaucomatous state. Complement cascade activation is a consistent feature observed in glaucoma, and neurotoxic reactive astrocytes express increased levels of complement C3. However, our understanding of how astrocytes promote neurotoxicity, and how complement activation is involved in astrocyte-mediated neurodegeneration remain incomplete. Using human pluripotent stem cell (hPSC)-derived RGCs and astrocytes, we explored how reactive astrocytes contribute to RGC degeneration, and how complement C3 activation is involved in astrocyte reactivity. hPSC-derived astrocytes were induced to a reactive phenotype through incubation with C1q, TNFα and IL1α, and their neurotoxic effect was determined through co-cultures with RGCs. Furthermore, shRNA approaches were used to modulate complement C3 in astrocytes. Reactive astrocytes exhibited a pronounced hypertrophic profile, upregulation of genes associated with inflammatory pathways as well as increased secretion of several cytokines. Reactive astrocytes promoted marked morphological alterations to RGCs, including neurite retraction and reduced neurite complexity, through direct contact and via secreted factors in transwell co-cultures. Moreover, to better mimic the compartmentalized nature of RGCs and location of reactive astrocytes in the optic nerve head, microfluidic devices were developed to properly orient astrocytes along the proximal axonal compartment. Results showed a detrimental effect of reactive astrocytes over RGC axonal compartment. Importantly, complement activation contributed to the acquisition of the reactive phenotype by hPSC-derived astrocytes, as shRNA-mediated reduction of complement C3 resulted in a less pronounced hypertrophic profile and attenuated reactivity-associated features in astrocytes. These results demonstrate that reactive astrocytes promote RGC neurodegeneration in a microfluidic platform that mimics reactive astrocyte orientation along the proximal axon. Results of this study demonstrate a role for the complement cascade in this neuroinflammatory response, and that the modulation of the complement cascade in reactive astrocytes could represent a novel therapeutic strategy for neuroinflammatory aspects of glaucoma.

Investigation of GNE mutation classes and aberrant autophagy in GNE myopathy

Rebecca Schmitt
Indiana University School of Medicine

Diseases that cause dysfunction of nerves or muscles are classified as neuromuscular diseases (NMDs). To date, most NMDs do not have cures and subsequently lead to pain, morbidity, and decreased quality of life. A subtype of NMD is termed myopathy, which is muscle weakness that manifests due to the abnormal function of the skeletal muscles. One such myopathy is UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE) myopathy (GNEM). It is classically categorized as a distal myopathy where over time muscle weakness from the distal leg progresses proximally and then to the upper body/arms, causing severely impaired mobility, and eventual dependence on caregivers occurs. GNE is primarily known to function as the rate limiting step in sialic acid (SA) biosynthesis. Clinical trials have employed SA or its precursors for attempted treatment, but most have fallen short, especially as evidence linking SA to GNEM is deficient. Creation of murine models of GNEM has also proven challenging, thus limiting the ability to elucidate the role of GNE in GNEM. As such, our lab has established an in vitro GNEM model employing human induced pluripotent stem cells (iPSCs), derived from fibroblasts, from GNEM and healthy patients. When GNEM-derived iPSCs are differentiated down the muscle lineage, they demonstrate decreased myogenic differentiation, characteristics of the human disease, and altered autophagy. Subsequent pharmacological activation of autophagy resulted in improved myogenic outcomes of GNEM-derived iPSCs. Our ongoing studies utilize this human cellular model while applying isogenic controls and including additional relevant GNEM patient mutations to systematically assess GNE mutation classes for determining genotype/phenotype correlations, query the possibility of undiscovered molecular functions of GNE, and employ pharmacological interventions for identifying/targeting the autophagy disruption in GNEM; towards the end goal of improving patient outcomes.

POSTER PRESENTATION ABSTRACTS

Harnessing the Potential of Human iPSC-derived Microglia-like Cells as Antigen Presenting Cells for MAIT Cells to Study AD Pathology

Reham Afify
Indiana University School of Medicine

Background: Microglia are dominant immune cells residing in the brain that regulate brain homeostasis and T-cell responses. An important immune function of microglia involves presenting microbial antigens to mucosal-associated invariant T (MAIT) cells; MAIT cells recognize microbial vitamin B-derived metabolites presented by the MHC class I-like molecule, MR1. Our recent findings highlighted a detrimental role for the MR1/MAIT cell axis in Alzheimer's disease (AD) using the 5XFAD mouse model. Here, our work is focused on how the MR1/MAIT cell innate immune axis in humans contributes to AD, using iPSC-derived human microglia-like cells (iMG) to provide potential mechanistic insights underlying the MR1/MAIT cell axis in AD. As an initial evaluation, we have assessed the activation of iMG and their potential as functional antigen presenting cells (APC) to MAIT cells when exposed to a microbe that can provide a MR1-presented, MAIT cell-specific antigen, such as from E. coli.

Methods: Initially, we will generate iMG from healthy donors that will be incubated with E. coli for the assessment TNF-α levels using ELISA as a readout of microglial activation. Subsequently, coculture experiments with human MAIT cells and E. coli-stimulated iMG will provide an initial evaluation of microglial MR1-dependent MAIT cell activation via MR1-dependent antigen presentation, monitored by MAIT cell production of various cytokines measured by ELISA.

Results: We expect that iMG will produce increased levels of TNF-α in response to E. coli compared to the control group. Similarly, we anticipate elevated cytokine levels secreted by MAIT cells via an MR1-dependent pathway.

Conclusions: These experiments will allow us to apply our human iPSC-based model to studying human microglia as APC and pave the way for a broader understanding of the contributions of the MR1/MAIT cell axis in AD. To our knowledge, our proposed study will be the first of its kind to utilize iMG as APC for human MAIT cells.

The Effects of Aluminum on the Blood Brain Barrier

Rylee Anderson
Indiana University School of Medicine

The blood brain barrier (BBB) is a selectively permeable membrane that is designed to limit the molecules that can reach the brain. It is made up of astrocytes, neurons, and endothelial cells held together by tight junctions and other stabilizing proteins. Aluminum is a metal that is found in many consumer products across the world and is considered safe for humans, as the body effectively clears it. However, recent studies have shown that an increased amount of aluminum in the body, either through accumulation over time or through over-exposure due to environmental waste, can alter the BBB. We used human induced pluripotent stem cells that were selectively differentiated into brain membrane endothelial cells (BMECs) to test how different concentrations of aluminum effect the barrier. To test the barrier integrity, trans-endothelial electrical resistance (TEER) was monitored over the course of 72 hours following exposure. To confirm barrier changes, a sodium fluorescein permeability assay was performed, as well as tight junction localization visualized by immunofluorescence. Efflux activity was assessed utilizing efflux-specific substrates and inhibitors. We found that while tight junction expression and efflux activity was not affected by aluminum, barrier integrity was diminished. To further elucidate the aluminum-induced diminished barrier we measured reactive oxygen species (ROS) following aluminum exposure. We found that aluminum increases ROS in our iPSC-derived BBB model. A further understanding of how aluminum induces barrier integrity may lead to improved safety precautions when handling environmental toxins.

Generation of novel APBB2 SNP iPSC lines to study increased risk for glaucoma unique to individuals of African ancestry

Aaron Baker
Indiana University School of Medicine

Glaucoma has been found to occur more frequently and with higher severity in populations of African ancestry compared to other races, yet research in this population is lacking. A recent GWAS study identified a SNP in the gene APBB2 linked to increased risk for glaucoma uniquely within individuals of African ancestry. The discovery of this SNP provides an exciting opportunity to study why those of African ancestry populations are at increased risk for glaucoma. Human induced pluripotent stem cells (iPSCs) can model genetic diseases through the differentiation of stem cells into cell types associated with the disease state. For the study of APBB2 and how a SNP at this locus may confer increased risk for glaucoma, we generated SNP mutant and isogenic control iPSCs from lines derived from donors of African ancestry using CRISPR/Cas9 gene editing approaches. These iPSC lines were then differentiated into retinal organoids followed by the isolation and maturation of retinal ganglion cells (RGCs), with assays to characterize neurodegenerative features including morphological analyses by neurite tracing, functional assays by multielectrode array, and protein expression assays. As a result of these studies, we have successfully generated lines of human iPSCs with the APBB2 risk variant for glaucoma via CRISPR/Cas9 gene editing, along with paired isogenic controls. These cell lines were also engineered to express a BRN3b-mNeonGreen-Thy1.2 reporter for identification and purification of RGCs. Each of these cell lines was capable of robust differentiation into retinal organoids, which were then used for the purification and maturation of RGCs for downstream analyses. Measurements of neurite length and complexity suggest that the APBB2 SNP mutant line exhibits deficits in morphological features and possesses a decreased maturation rate when compared to the isogenic control. Further MEA analysis exhibits proper RGC functional properties, such as spontaneous activity and synchronous network bursting. Continuing experiments are focused on analyzing gene expression data and protein pathologies to decipher differences in functional properties between both the APBB2 SNP mutant lines and isogenic controls. These studies represent the first known attempt to develop iPSC-based models from individuals of African ancestry for the study of increased risk for glaucoma among this population. Ongoing studies with these cell lines will likely elucidate important cellular and molecular aspects that uniquely lead to this increased risk for glaucoma among the African ancestry population.

Investigating Hematopoietic Stem Cells Transcriptome to Identify RNA-based Biomarkers for Diabetic Retinopathy

Surabhi Abhrankyar
Indiana University School of Medicine

Purpose: Given the increasing prevalence of diabetic retinopathy (DR), it is of utmost importance to identify newer biomarkers that can help with early diagnosis and prevent the progression to severe DR. In recent years, hematopoietic stem cells (HSCs) have gained substantial attention for playing a significant role in the pathophysiology of DR; however, there is a gap in the knowledge about transcriptional changes in HSCs due to diabetes and DR.

Methods: We recruited individuals with 1) no diabetes 2) diabetes, and 3) varying severity of DR. Blood samples were collected from all the individuals to isolate HSCs, which were then a) analyzed for the angiogenic and inflammatory markers and b) sorted for the lin-CD34+ CD45mid cells and subsequently sequenced for the miRNA and mRNA.

Results: We observed decreased circulatory CD34+ HSCs in the proliferative DR state, suggesting a downregulation of protective HSCs in the later stages of DR. The cell surface expression analysis showed increased monocytes (Lin-CD14+) in DR individuals. Interestingly, we found a shift towards inflammatory phenotype in CD34+ cells, as evident by flow cytometric analysis. There was an increase in Lin-CD34+CD45midTLR8+ cells in the proliferative DR state. The miRNA sequencing reflected 8 miRNAs with significant differences ($p<0.05$) in clinical samples, 7 being upregulated and 1 being downregulated in diabetes and DR when compared to the control/healthy individuals. Further analysis of the downstream mRNA targets of these miRNAs and differential mRNAs from the sequencing data revealed the involvement of pro-inflammatory, pro-angiogenic, integrin signaling, Wnt signaling, VEGF signaling, and macrophage activation pathways.

Conclusion: The present study offers an excellent opportunity to identify an easily assessable peripheral blood-derived HSCs-based miRNA biomarker that can ease the identification of DR at early stages. Furthermore, the sequencing data have revealed unique miRNAs and mRNAs, which can be studied for their applicability.

NF2 Loss Disrupts Differentiation of Neuroepithelial Stem Cells

Noah Burket
Indiana University School of Medicine

NF2-related schwannomatosis is a tumor predisposition syndrome caused by mutations in the NF2 gene and associated with spinal ependymomas (SP-EPN). These tumors are suspected to originate from mutations in the radial glia (RG) cell lineage. They are only effectively treated through high-risk surgical resection, emphasizing the critical need for identification of targets for medical therapy. Yet, the role of NF2-dependent disruption in RG cell development is poorly understood. We hypothesize that a loss of the NF2 gene in NES cells will prevent normal differentiation and promote a RG-like progenitor state. An NF2-knockout was generated in neuroepithelial stem (NES) cells using CRISPR/Cas9. Knockouts were validated using Western blot and Sanger sequencing. In vitro differentiation was induced with removal of growth factors. NF2-knockout phenotypes were assessed with polymerase chain reaction and compared with wildtype NES cells. Preliminary data has shown that NF2-knockout cells express similar levels of early pan-neural and neural stem cell genes compared to wildtype after CRISPR editing. The NF2-knockodown cells retain this stem cell-like gene expression following attempted differentiation, whereas wildtype cells take on a primarily neural phenotype. The knockout cells also form what appears to be pre-neoplastic spheres when allowed to differentiate. Two clones that were identified as having NF2 mutations on each allele still retain NF2 protein expression. NF2-knockout NES cells fail to differentiate normally compared to wildtype NES cells. They retain early stem cell-like markers, including DACH1, HES1, and PLZF. Furthermore, formation of spheres when growth factors were removed hints at NF2 loss being relevant to formation of pre-neoplastic growths. Future work will include investigating downstream effects of NF2 loss in this model. The long-term goal of this project is to use this model to study potential therapies for medical treatment of SP-EPN.

Passage number affects differentiation of sensory neurons from human induced pluripotent stem cells

Erica Cantor
Indiana University School of Medicine

Induced pluripotent stem cells (iPSCs) can be utilized in neurological disease-modeling and drug discovery due to their capability to differentiate into neurons which reflect the genetics of the patient from which they are derived. These cells demonstrate significant variability in culture, however, due to heterogeneity in culture conditions. In this study, we investigated the effect of passage number on the differentiation of iPSCs into peripheral sensory neurons (iPSC-dSNs) in order to optimize differentiation. Three iPSC lines were reprogrammed from the peripheral blood of three donors and differentiated into iPSC-dSNs at passage numbers within each of three ranges: low (5-10 passages), intermediate (20-26 passages), and high (30-38 passages). Prior to differentiation, the morphology and pluripotency of the parent iPSC lines were assessed. Differentiated iPSC-dSNs were evaluated based on electrophysiological properties and the expression of key neuronal marker genes.
Across passage number groups, all iPSC lines displayed a similar morphology and level of pluripotency. However, iPSC-dSNs differentiated from low-passage iPSCs exhibited expression levels of neuronal markers and sodium channel function most similar to the desired sensory neuron phenotype compared to those differentiated from intermediate or high passage numbers. These results indicate that lower passage numbers (5-10) may be more optimal for the differentiation of iPSCs into peripheral sensory neurons.

The Effects of Propofol on a Blood Brain Barrier Model Derived from Human iPSCs

Pia Carino
Indiana University School of Medicine

The Blood Brain Barrier (BBB) is responsible for regulating and maintaining the physical and metabolic barrier between the CNS and the surrounding vasculature. The BBB consists of barrier forming brain microvascular endothelial cells (BMECs) supported by neurons, astrocytes, and pericytes that form the neurovascular unit. Barrier breakdown can be linked to exposure to various sources such as anesthetics. Prolonged exposure to anesthetics can have negative cognitive and neurological effects, primarily through neuron toxicity. Propofol is an inductive anesthetic commonly used in surgeries and other medical practices. Our lab has previously shown that propofol can induce barrier breakdown in a monoculture system composed of human induced pluripotent stem cell (iPSC) derived BMECs. To create a co-culture system, the same iPSCs were differentiated to neurons and astrocytes. To study the effects of propofol on tight junction proteins, BMECs were plated on a 96-well plate and cultured with neuron and astrocyte conditioned media. Our co-culture model was exposed to clinically relevant concentrations of propofol and directly compared to a control group. Tight junction proteins, a critical component of barrier tightness, were visualized by immunocytochemistry. We evaluated barrier integrity following propofol exposure by trans endothelial electrical resistance (TEER) and sodium fluorescein permeability by placing BMECs onto a Transwell insert in co-culture with iPSC-derived neurons and astrocytes. Our lab has previously shown that Matrix Metalloproteinase-2 concentrations (MMP-2), which are known to cause tight junction delocalization, are increased in propofol treated BMECs. We evaluated MMP-2 concentrations in a co-culture model with a Sensolyte 520 MMP-2 Assay kit. Similarly to monoculture, our co-culture BBB model preliminarily displays reduced barrier integrity and altered tight junction expression following propofol exposure. The model also displays heightened MMP-2 activity. A further understanding of the effects of anesthetics on the BBB will unveil novel therapeutic approaches and may improve anesthesia safety.

Generation of novel lines of human induced pluripotent stem cells from elderly rural Indiana neurodegenerative patients

Kate Cowger1,2, Bingying Han3,4, Melody Hernandez1,2, Kiersten Peña1,2, Karly Hooper1,2, Aaron Bowman3,4, Jason Meyer1,2

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Human induced pluripotent stem cells (hiPSCs) are a novel in vitro modeling system which can be analyzed to understand genetic features and cellular mechanisms of neurodegenerative diseases (NDs). Recent discoveries of genetic risk factors for NDs, including Alzheimer's Disease and Parkinson's Disease have been identified, but these genetic risk factors only account for a small percentage of patients with NDs. Additionally, not all patients with risk factors develop the disorder, leading us to explore other contributions to the onset of neurodegenerative diseases. Strong associations exist between pathogenic gene-environmental interactions (GxE) and the development of NDs from exposure to environmental chemicals and pathogens. To investigate effects of persistent exposure to environmental neurotoxicants on the onset of NDs, blood specimens have been obtained from 20 same-sex sibling pairs from northern Indiana that have relative phenotypic differences in ND cognitive and motor function. By isolating peripheral blood mononuclear cells (PBMCs), the samples are reprogrammed into hiPSCs that can thus be differentiated into an array of neural cells including dopaminergic and glutamatergic lineages. To generate hiPSCs, PBMCs collected from patient blood samples are reprogrammed using the Sendai virus as a vessel for transcription factors (Oct3/4, Sox2, hc-Myc, & hKlf4) that function in the genome to revert the cells to a pluripotent state. Stem cell colonies begin to form between two to four weeks after transduction and are maintained until passage 10 to ensure complete reprogramming and removal of the Sendai virus. Immunohistochemistry confirms expression of pluripotency markers and karyotyping is completed to verify genomic stability. Inducing an persistent state of neurotoxicity in these lineages will allow for the characterization of specific sensitivities to environmental toxins related to the rural Indiana region. Exploring genetic/epigenetic pathways connected to age-related signaling networks may elucidate the effects of GxE interactions on homeostatic processes that allow for a persistent neurotoxic state observed in NDs.

Targeting the 5-HT1A Serotonin Receptor Provides Glaucoma Neuroprotection by Restoring Mitochondrial Homeostasis

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Mitochondrial dysfunction is an early hallmark of central nervous system (CNS) disorders, including glaucoma. Retinal ganglion cells (RGCs), the projection neurons of the retina that degenerate in glaucoma, are highly vulnerable to mitochondrial abnormalities due to their high ATP demand for spontaneous action potential propagation along their long unmyelinated axons. Therefore, a therapeutic strategy that restores mitochondrial health in RGCs represents a promising neuroprotective approach, potentially complementing intraocular pressure (IOP) management for long-term glaucoma treatment.

Here, we utilized human stem cell-derived RGCs (hRGCs) and conducted small-molecule screening to enhance mitochondrial health, identifying ADC1, a Gi/o-coupled 5-HT1A receptor antagonist, as a neuroprotective agent. In hRGCs, ADC1 transiently activated mitochondrial biogenesis and cAMP levels, a key second messenger that supports multiple neuroprotective pathways. Additionally, ADC1 treatment improved mitochondrial health, reduced excitotoxicity, and decreased apoptosis in hRGCs carrying the E50K mutation in the Optineurin (OPTNE50K) gene, which is associated with severe normal-tension glaucoma.

In vivo, ADC1 demonstrated robust neuroprotective effects; systemic administration preserved RGC bodies and promoted axon regeneration following acute optic nerve crush injury in mice, while also protecting RGC bodies and preserving vision in a microbead-induced high-IOP glaucoma model. This research establishes a foundation for targeted neuroprotective strategies that restore mitochondrial health in specific neurons, such as RGCs in glaucoma, with potential applications across diverse CNS disorders.

Intramuscular Administration of Adipose Stem Cell-Derived Secretome in a mouse model of Amyotrophic Lateral Sclerosis

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Objective: Amyotrophic lateral sclerosis (ALS) is a devastating motor neuron (MN) disease with no cure. The involvement of numerous cellular and physiological processes and the complexity of the disease are significant obstacles to developing effective therapies. Thus, a multifactorial approach like stem cell-based therapeutics is likely to be the most appropriate as it can target multiple mechanisms simultaneously. Adipose-derived stem cells (ASCs) are multipotent mesenchymal stem cells that can be obtained easily from adipose tissue. Stem cell secretomes contain various beneficial trophic factors and cytokines. Previous data has shown the therapeutic benefits of the systemic ASC secretome in ALS mouse model. In the present study, we hypothesized that local intramuscular (IM) administration of hydrogel containing ASC secretome at late pre-symptomatic stages of disease (P63-P70) would improve neuromuscular junction (NMJ) innervation as well as physiological (muscle torque) and electrophysiological landmarks (e.g. CMAP, MUNE).

Method: Sixteen mSOD1G93A mice (8 female, 8 male) received IM injection in right gastrocnemius muscle with either hydrogel containing ASC-secretome or hydrogel containing normal saline at P63 . Mice were sacrificed at P84, right and left gastrocnemius were harvested, weighed, and prepared for NMJ innervation analysis.

Twelve mSOD1G93A mice (6 female, 6 male) received IM injection in left and right gastrocnemius muscle with hydrogel-ASC secretome or hydrogel-saline respectively at P69. In vivo plantarflexion torque, CMAP and MUNE were measured at P89. Then, mice were sacrificed at P90, and right and left gastrocnemius were harvested for NMJ innervation analysis.

Results: There was no significant difference in NMJ innervation and CMAP measures between hydrogel-saline and hydrogel-ASC secretome treated legs in both females and males. MUNE and max torque were significantly increased in males in the leg receiving hydrogel-ASC secretome.

Conclusion: Gradual release of ASC secretome from hydrogel into the muscle has a positive impact on muscle force and number of motor units in males. Further studies are needed to investigate therapeutic effects of hydrogel- ASC secretome at different time points and with different dosages in this model.

Regulation of Neuronal Differentiation by Lysine Methylation

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Aberration in neuronal differentiation has emerged as a major convergence point for neurodevelopmental disorders (NDDs). Neuronal differentiation is regulated in part by lysine methylation on histone and non-histone proteins; in fact, our quantitative mass spectrometry (TMT LC-MS/MS) analysis reveals lysine methylation events on non-histone proteins across distinct stages of neuronal differentiation. Moreover, lysine methyltransferases (KMTs) and demethylases (KDMs) are critical for proper brain development, though the mechanisms are not defined. Dysregulation of KMTs/KDMs has critical developmental and cellular consequences, and ~30% of these enzymes are associated with NDDs. The KMT ASH1L is associated with NDDs in human patients and mouse models, and haploinsufficiency of ASH1L results in developmental and differentiation defects. Studies have shown that ASH1L regulates neuronal differentiation, arborization, and synaptic pruning; however, mechanistic understanding of how ASH1L regulates these processes is not known. While a role for ASH1L KMT activity has been proposed, it has not been directly tested, and there are conflicting reports in the literature on the physiologically relevant substrates of ASH1L. To address this gap in knowledge, we characterized differentiation of Lund human mesencephalic (LUHMES) neural progenitor cells (isolated from the mesencephalon of an 8-week old female; immortalized via v-myc overexpression) into dopaminergic-like neurons while inhibiting ASH1L KMT activity with a small molecule compound, AS-99. Disruption of ASH1L KMT activity resulted in a decrease in neurite branching, supporting a direct role for ASH1L KMT activity in the regulation of neuronal differentiation. To gain mechanistic insight into ASH1L regulation of differentiation, we have performed an exhaustive biochemical characterization of ASH1L methyltransferase activity using in vitro peptide arrays, revealing its substrate selectivity. From this selectivity profile, we have identified putative non-histone substrates of ASH1L, and future work will be geared towards delineating ASH1L substrates and determining the consequences of histone and/or non-histone substrate methylation on neuronal differentiation progression.

Functional analysis of induced pluripotent stem cell derived microglia-like cells

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Microglia are the residential immune cells of central nervous system (CNS) that play a critical role in CNS development and protection of the brain from injury by surveying the brain microenvironment. Microglia are highly phagocytic cells that maintain brain homeostasis through dedicated sensory mechanisms that recognize neurodegeneration-associated molecular patterns (NAMPs), such as molecules on the surface of dying neurons, extracellular protein aggregates, myelin debris, and other known products of CNS damage. Recent discoveries have revealed microglia are important regulators of neurodegenerative pathogenesis. Several factors such as the sensitivity of microglia to changes in their environment and low yields of primary human microglia pose significant challenges in the research of these cells. Moreover, there are several fundamental differences between human and murine microglia, positing a clear need for human-relevant disease models to study the role of microglia in disease progression. In this study, we report the development and validation an induced pluripotent stem (iPS) cell line using Epi5™ episomal reprogramming vectors that deliver five reprogramming factors (Oct4, Sox2, Klf4, L-Myc, and Lin28) without integration. These cells were then differentiated into microglia-like cells (iMG) which we characterize using a variety of methods. We find that iMG express known microglia protein markers including PU.1, IBA1, P2RY12, and TMEM119. Using RT-qPCR, we found that iMG rapidly change gene expression in response to LPS and IFN-γ, with significant increases in CXCL10, IRF8, IL-1β, and TNF-α mRNA expression. To assess microglial phagocytosis, we treated cells with pHrodo-conjugated S. Aureus bioparticles or myelin debris and measured the fluorescent signal over time using high content imaging techniques. Both types of stimulation led to robust phagocytosis. Stimulation with S. Aureus bioparticles also led to rapid changes in iMG morphology, while the iMG stimulated with myelin debris maintained more ramified morphology similar to unstimulated controls. We conclude that the iMG responses to NAMPs and cytokines suggests the significant potential of iMG as a human disease model to study neuroimmune biology.

The Utilization of Developmental Muscle Precursors in Volumetric Muscle Loss

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Volumetric Muscle Loss (VML) is a form of muscle injury in which a significant amount of muscle is lost from one area of the body. In US military service members, VML injuries are responsible for more than 90% of muscle conditions that result in long-term disability. VML is distinct from other muscle injuries in that long after the wound has closed, there maintains a permanent effect on muscle function as well as scarring. Despite the prolonged impact these injuries have, there are little to no treatment methods to mitigate the effects experienced by VML victims. The current focus of the VML field centers on the use of scaffolds to fill the gap of a VML injury, and reports have demonstrated that a combination of cells and scaffold is more effective than scaffold alone at curbing the effects of VML. Current cell-based approaches are focused on the application of adult muscle stem cells, also known as Satellite Cells (SCs), to the VML injury via implantation in a scaffold. However, these adult SC transplantations have low engraftment rates, limited cell mobility, and poor stem cell differentiation and renewal capacity. While adult SCs appear to lack feasibility, there are a class of developmentally significant muscle precursor cells that have the potential to provide greater benefits during the healing of VML injuries. We hypothesize that these muscle precursors of interest will have increased functional capacities and result in increased healing outcomes when implanted via scaffold in a VML mouse model when compared to adult satellite cells. To this end, we show SCs isolated from neonatal mice have increased proliferative and differentiative abilities when compared to adult SCs. We have also shown that these cells are capable of proliferating and differentiating when implanted into a degradable polymer in vitro. This research highlights the potential these developmental cell types may have in VML healing as well as functional restoration and may lead to potentially superior treatment options for VML treatment.

Establishing an in vitro model of retino-thalamic projections to study optic nerve injury in a dish.

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Glaucoma and other optic neuropathies affect millions of people each year, leading to a wide range of visual deficits and blindness. Optic neuropathies are termed as such because the primary injury to cells occurs to the axons of the optic nerve. This induces progressive axon dieback and eventual death of retinal ganglion cells (RGC). There is currently no known method to completely regenerate these long optic nerve axons making restoration of the eye-to-brain connection one of the largest barriers to regeneration of the visual system. Importantly, there is also no established in vitro model of RGC to brain-cell (i.e., thalamic neurons) connectivity. Thus, we aim to create a functional eye-to-brain model on a chip (i.e., a three-chamber microfluidic platform) using human induced pluripotent stem cells (hiPSCs) differentiated into RGCs and thalamic neurons. Growing cells in a microfluidic platform allows us to keep the cell bodies of RGCs (chamber 1) separate from their axons (chamber 2) and the cell bodies of thalamic neurons (chamber 3) as would be the case in their natural environment. Further, this allows us unique access to RGC axons where we can induce injury and study axon dieback and regrowth in vitro. Thus far, experiments include axon tracing using the retro/anterograde dye, DiO; establishment of an axon injury paradigm using the microtubule destabilizer, colchicine; and co-culture of RGCs with thalamic neurons. Future experiments will assess how co-culture affects axon outgrowth and synaptic connectivity (using multi-electrode array and immunohistochemistry for synaptic proteins) and will attempt to induce regeneration using a DREADD-based stimulation approach to induce RGC activity and provoke RGC axon growth toward their thalamic targets.

Small molecule APP degrader reduces Aβ42 through lysosomal pathway in Alzheimer's patient-derived iPSC differentiated neurons and 3D organoids.

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Alzheimer's disease (AD) is the cause of 60–80% of dementia cases. The main reason is the accumulation of amyloid-β (Aβ) outside neurons, resulting in the following cleavage of amyloid precursor protein (APP). Through the process of screening our library of small molecules, we have identified compounds that degrade APP and reduce Aβ42 release from neurons produced by AD patients' induced pluripotent stem cells (iPSC). We demonstrated that the small molecules 0043 and 0152 bind with cytoplasmic activation/proliferation-associated protein 1 (CAPRIN1) and APP in AD patients' iPSC differentiated neurons (AD-iPSC-Ns). The results showed that CAPRIN1 binds to APP, degrading APP by CAPRIN1-mediated lysosomal degradation and reducing both APP and Aβ42 in AD-iPSC-Ns and 3D organoids which are derived from AD-iPSC. Using recombinant APP and CAPRIN1, interactions between proteins are increased by small molecular APP degraders. In conclusion, targeted protein degradation of APP may provide a novel therapeutic approach for AD and the small molecule APP degraders are currently under development as anti-aging drugs for AD therapy.

THE IMPACT OF CHRONIC MANGANESE ON GLUTAMATE EXCITOTOXICITY IN HUMAN IPSC-DERIVED CORTICAL MODEL OF ALZHEIMER'S DISEASE

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Manganese (Mn) is an essential metal widespread in the environment, but in excess can cause neurotoxicity. Alzheimer's disease (AD) is a chronic multifactorial neurodegenerative disorder. Most AD cases cannot solely be attributed to familial inheritance and involve contributions from environmental risk factors. Perturbed glutamate neurobiology is an important overlapping pathology shared by Mn neurotoxicity and AD. Neuronal hyperexcitability has been reported in AD patients. Studies have shown that acute exposure to high Mn levels inhibits synaptic glutamate uptake. However, the effects of chronic exposure to pathophysiologically relevant Mn levels and its implication in AD etiology remains unknown. Hence, we hypothesized that chronic Mn exposure increases susceptibility to glutamate excitotoxicity depending on AD genetic risk. We utilize cortical neurons and astrocytes generated from induced pluripotent stem cells derived from neurotypical and AD patients. Cells were cultured for ~100 days and subsequently exposed to Mn (vehicle, 0.5 or 5μM) for up to 40 days. Alterations in glutamate uptake were quantified using 14C-glutamate. We observed a significant 30~40% decrease in uptake in AD neurons/astrocytes, an effect not seen in neurotypical controls. Gene expression and immunocytochemical analyses indicated an absence of inflammatory mediators and astrocyte reactivity, suggesting impaired glutamate uptake is likely a direct effect of Mn rather than a secondary effect caused by neuroinflammation. scRNA-sequencing revealed alterations in biochemical pathways implicated in Mn toxicity, glutamate neurotransmission, and/or AD. In summary, we provide insight into discerning transcriptomic/functional alterations caused by chronic Mn and how an individual's genetic predisposition to AD may alter this pathophysiology.

Development of a human stem cell model of Neurofibromatosis Type 1 for assessment of optic pathway phenotypes

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Neurofibromatosis type 1 (NF) is a pediatric neoplasia of the nervous system, caused by germline mutations of the neurofibromin gene. Among characteristic pathological phenotypes, the development of optic pathway gliomas (OPGs) affects visual acuity or cause blindness among other ophthalmic features in symptomatic patients. Although transgenic rodent models of NF1 OPGs have helped elucidate disease pathogenesis, significant differences exist in the retina and optic nerves of rodents and primates, including differences in NF1 disease pathology. To address this shortcoming, we have developed a human induced pluripotent stem cell (iPSC) model that mimics many features of the optic nerve and supporting glial cells as a novel tool to characterize OPG-associated clinical heterogeneity and prognostic markers at cellular level. We introduced NF1-relevant single nucleotide polymorphisms (SNPs) into lines of iPSCs using CRISPR/Cas9 editing, resulting in paired disease models and isogenic controls. These cell lines were then differentiated to yield retinal organoids and astrospheres following established procedures to acquire retinal ganglion cells (RGCs) and astrocytes cultures, respectively. These RGCs and astrocytes were characterized for changes associated with the NF1 gene variants via gene and protein expression. The iPSC lines with NF1-associated gene variants were successfully generated and further analyses of these cell lines assess for the possibility of off-target effects of gene editing. These cell lines were also edited to express an RGC-specific mNeonGreen reporter for RGC identification, as well as the mThy1.2 cell surface antigen for subsequent RGC purification. RGC purification from retinal organoids results in highly enriched population of RGCs from all cell lines tested. Likewise, astrocytes were differentiated from both cell lines at high purity, which after maturation expressed variety of astrocyte specific markers. The NF1 variant astrocytes were found to be more proliferative compared to their isogenic controls. Initial experiments were then pursued to combine cell types in a novel, in vitro microfluidic platform mimicking some aspects of the optic pathway for the analysis of NF1 phenotypes. Experiments in progress will further assess changes in RGC morphological features such as neurite complexity as well as functional properties. Taken together, these results demonstrate the generation of human iPS cell lines with patient relevant NF1 gene variants as a disease model for neurofibromatosis type 1, as well as the use of these cells to begin to explore features associated with the OPG phenotype.

A Highly Reproducible and Efficient Method for Retinal Organoid Differentiation From Human Pluripotent Stem Cells

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Human pluripotent stem cell (hPSC)-derived retinal organoids are 3D aggregates of cells that recapitulate both the spatial and temporal patterning of the human retina. These organoids are valuable tools for studying human retinogenesis and retinal diseases. However, limitations in the efficiency and reproducibility of current retinal organoid differentiation protocols have hindered their use for more high throughput applications. This necessitates the development of more highly reproducible 3D retinal organoid models for studying retinal development and disease.

In the current study, quick reaggregation methods at various cell densities were tested to generate highly reproducible 3D retinal organoids from human pluripotent stem cells (hPSCs). Additionally, a role for BMP signaling in retinal specification was tested by treatment with either BMP4 or the BMP inhibitor LDN-193189 at early time points. Retinal organoid differentiation efficiency was assessed at various time points based on morphological analyses and the expression of retinal-specific markers. To assess transcriptional changes that occur at the early stages of retinal specification, we also conducted mRNA-seq analyses.

Results indicated that differentiating retinal organoids using quick reaggregation methods generated organoids that were highly reproducible and consistent in both their size and shape compared to more traditional methods. Additionally, after treatment with BMP4, pure populations of retinal organoids were generated at 100% efficiency from multiple widely utilized cell lines. Conversely, after treatment with LDN-193189, retinal differentiation was completely inhibited and forebrain cortical organoids were derived. Subsequently, mRNA-seq analyses identified distinct transcriptional profiles of the earliest stages of retinal vs forebrain specification. These optimized methods also yielded retinal organoids with expedited retinal neuron differentiation timelines when compared to traditional methods.

Taken together, this study provides a novel and highly reproducible method for generating retinal organoids suitable for high-throughput applications. The ability to control the retinal/forebrain cell fate determination at 100% efficiency allows for the study of transcriptional changes occurring at the earliest stages of human retinal development."

Induction of aging-related features in human pluripotent stem cell-derived retinal ganglion cells to study neurodegeneration in glaucoma

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Glaucoma is a progressive neurodegenerative disease involving the degeneration of retinal ganglion cells (RGCs), which project visual information from the eye to the brain. Human pluripotent stem cells (hPSCs) have been established as a model of studying glaucoma that is more physiologically relevant to the disease presentation in humans compared to previously used rodent models. However, hPSCs have a “young” phenotype due to the reprogramming that allows them to assume a pluripotent state. While this is essential for the use of hPSC models, it does not create an accurate disease model for glaucoma, in which one of the largest risk factors is aging. Therefore, it is essential to create an aged hPSC model to study glaucoma in a more physiologically relevant human system. To establish an aged phenotype in RGCs, we utilized two small molecules with different mechanisms of action, BIBR1532 and MLN4924. BIBR1532 is an inhibitor of telomerase and stress inducer while MLN4924 is an inhibitor of neddylation, but both have been previously associated with aging-related processes such as DNA damage and cellular senescence. hPSCs were differentiated into 3D retinal organoids for the isolation of RGCs. RGCs were then incubated with BIBR1532 or MLN4924 for two weeks and aging-related phenotypes in the treated cells were compared to untreated controls. Morphological alterations, including a reduction in the number of primary neurites and overall neurite complexity was observed in treated RGCs compared to the respective untreated controls. Moreover, increased focal nuclear expression of γH2AX was also exhibited by RGCs treated with BIBR1532 and MLN4924, which suggests DNA damage in aging related RGCs. Interestingly, increased expression of several aging/senescence-related genes was observed in treated RGCs compared to untreated controls, suggesting that both compounds are capable of producing aging-related characteristics in hPSC derived RGCs. Taken together, our results suggest that BIBR1532 and MLN4924 are able to induce an aging-related phenotype in RGCs derived from hPSCs, which more accurately reflects glaucoma in an in vitro human model, furthering the knowledge of this disease and opening new avenues for glaucoma research.

Assessing therapeutic potential of human neuritin-1 in glaucoma patient stem cell derived retinal ganglion cells

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Purpose

Glaucoma is a progressive neurodegenerative disease, and even with therapeutically targeting elevated intraocular pressure (IOP), many patients still lose vision due to retinal ganglion cell (RGC) loss. Therefore, it is crucial to target RGC loss by a prophylactic neuroprotection strategy. Previously, RGC degeneration has been linked to neurotrophic factor deprivation. Our previous studies have shown that secreted human neuritin1 (hNRN1) protects RGCs after axonal injury in acute glaucoma models and ex-vivo human pressurized eyes. Thus, we aim to evaluate hNRN1's therapeutic effects in RGCs from glaucomatous and non-glaucomatous donors by treating them with hNRN1. Additionally, we aim to assess induced pluripotent stem cell (iPSC) derived RGC transplantation potential in donor eyes under normal and elevated IOP.

Methods

Human iPSCs were generated from non-glaucomatous and glaucomatous donor keratocytes by CytoTune Sendai reprogramming and differentiated into retinal organoids (RO) to obtain RGCs. The iPSC-derived RGCs were seeded onto collagen gels, and hNRN1(200 ng) was applied to their soma and axonal regions. RGC survival and neurite outgrowth were assessed through RBPMS, CASP3, and NEFL staining. Further, iPSC-derived RGCs were transduced with AAVs to overexpress hNRN1 (IRES-hNRN1-RFP), scramble (scrmb-shRNA-eGFP), or silence hNRN1 (hNRN1-shRNA-eGFP). After 13 days, vector expression and RGC growth were analyzed. The iPSC-derived RGC transplantation potential was analyzed by seeding AAV2-GFP transduced non-glaucomatous RGCs into control eyes (N=4). Eyes were pressurized for 5–7 days under normal IOP (left eye) and elevated IOP (right eye). RGC survival, apoptosis, retinal activity, and optic nerve head extracellular matrix (ECM) deposition were assessed downstream.

Results

Keratocytes were successfully reprogrammed into iPSCs, differentiated into ROs and RGCs generated. The hNRN1 treatment reduced RGC apoptosis and promoted neurite outgrowth in RGCs cultured within collagen gels. The hNRN1 overexpression exhibited enhanced RGC survival highlighting target-specific protection of hNRN1. Non-glaucomatous iPSC-derived RGCs were successfully transplanted into human donor eyes. Under normal IOP, the transplanted RGCs showed a higher survival rate with less ECM deposition ($p<0.05$) and improved retinal activity.

Conclusion

The study confirmed hNRN1 as a novel therapeutic target for glaucomatous RGC degeneration. iPSC-derived RGCs present a viable cell therapy solution for advanced glaucoma.

The Effects of Stress on ALS Patient iPSC-derived Motor Neurons

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Amyotrophic Lateral Sclerosis (ALS) is a fatal disease characterized by upper and lower motor neuron degeneration. This progressive disease presents as muscle atrophy, dismantling of the neuromuscular junction (NMJ), and ultimately death via respiratory failure. The NMJ is a chemical synapse that normally functions to join the presynaptic motor axon terminal with postsynaptic skeletal muscle fibers. Curiously, distal axon loss has been shown to precede soma death within mouse models such as the Sod1G93A ALS model. We hypothesized that transcripts normally present within the MN axon terminal under physiologic conditions are dysregulated in ALS. Our lab utilized a ribosomal profiling method adapted from Sanz et al to unveil a distinct MN axonal translatome, raising the possibility that deficits in local gene translation could contribute to neurodegenerative etiology. To evaluate this possibility, ALS patient iPSC-derived MNs were exposed to stress conditions and found to contain stress granules, translationally-inactive particulates composed of RNA binding proteins, mRNAs, and ribosomal proteins. Further investigation of the transcripts sequestered will be used to identify novel transcript impairments throughout ALS disease progression, within axons as well as cell bodies. Understanding translational deficits in ALS may serve as a therapeutic avenue to rescue MN axonal degeneration.

The Effects of PFAS on the Blood-Brain Barrier

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Per- and polyfluoroalkyl substances, PFAS, are a group of chemicals that have been known to accumulate in the brain, leading to adverse effects on central nervous system functioning. Following the ingestion of PFAS, the chemicals must cross the blood-brain barrier (BBB) to then accumulate in brain tissue. The BBB is highly selective and consists of endothelial cells, bound together by tight junction proteins, surrounding the microvasculature of the brain. Efflux transporters located in the membrane of the endothelial cells pump specific substances across the barrier, which allows for the regulation of substances that can enter and exit the brain. The mechanism by which PFAS cross the BBB is unknown; however, we hypothesize that PFAS at physiologically relevant concentrations disrupt critical barrier properties in a human-derived BBB model. We utilize a human stem cell-derived BBB model to investigate mechanisms that PFAS may use to cross the BBB. Specifically, we utilize trans-endothelial electrical resistance and fluorescein permeability to determine barrier integrity following PFAS exposure. To further assess tight junction localization we visualize tight junction proteins with immunocytochemistry. Finally, to determine the effects of PFAS on efflux activity we utilize efflux specific substrates and inhibitors. Our preliminary data implicates that, various PFAS at a concentration of 100 ppb diminished barrier integrity and altered efflux activity. Further investigation and understanding of the effects of PFAS on the BBB unveils novel approaches to limiting toxicity of PFAS and creating a better understanding of how these chemicals impacts those who are exposed.

Comparison of Patient and Wild Type hiPSC derived Schwann Cells for understanding disease mechanisms of NF2-related schwannomatosis

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NF2-related schwannomatosis is an autosomal-dominant multiple neoplasia caused by a loss-of-function mutation in the Neurofibromatosis type 2 (NF2) gene. The NF2 gene has tumor-suppressive functions, and pathogenic variants in its protein product, merlin, cause NF2-related schwannomatosis. Schwann cells are vital to the development and maintenance of the peripheral nervous system and their dysfunction has been implicated in a range of neurological and neoplastic disorders, including NF2-related schwannomatosis. Our understanding of the role of merlin in Schwann cell differentiation, proliferation, tumor development and progression remains limited. In the present study, we investigated the role of different NF2 mutations in Schwann cell differentiation and proliferation using NF2 patient derived hiPSCs. First, we generated and characterized multiple hiPSC lines from wild type donors (WT) and three different NF2-related schwannoma patients. Differentiation of these hiPSC lines into the three embryonic germ layers was confirmed using embryoid body formation and immunofluorescence staining for ectoderm, mesoderm, and endoderm markers. For all hiPSCs, karyotyping was performed, and pluripotency was validated by immunofluorescence. Further, we present a protocol for the derivation of Schwann cell progenitors (SCPs) and Schwann like (SLC) cells from hiPSCs in vitro, including immunostaining for SOX10, GAP43, Nestin, and S100β to validate the success of the differentiation. Analysis by qPCR showed expression of SCP and Schwann cell specific genes including SOX10, NGFR, CDH19, GAP43, GFAP, MPZ and S100β in all differentiated cells. Additionally, merlin protein expression was reduced in NF2 patient derived SCPs as compared with WT. These patient derived cells are a beneficial pre-clinical resource to study the molecular mechanisms underlying NF2-related schwannomatosis. Using our newly developed hiPSC techniques, we aim to elucidate the role of merlin in Schwann cell differentiation, tumorigenesis, and therapeutic responses.

Developmental Exposure of hiPSC-derived Cortical Cultures to Methylmercury Induces Persistent Functional Effects

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Neurodegenerative diseases (NDs) arise from complex interplay between genetic and environmental factors. Exposure to heavy metals like methylmercury (MeHg) during early development has been linked to an increased risk of NDs. This study investigated the hypothesis that MeHg exposure can persistently alter neuronal network activity patterns, rendering individuals more susceptible to developing NDs. This study modeled early developmental exposures utilizing male hiPSC-derived cortical glutamatergic neuron models. Developing cultures were exposed to environmentally relevant MeHg concentrations of 0.1 µM and 1.0 µM from days 4-10 of differentiation, a period characterized by the presence of neuroepithelial (NE) and radial glial (RG) cells. Methylmercury was removed, and differentiation continued until cultures were functionally mature (around day 80). Around day 110, cells were plated onto microelectrode arrays and spontaneous neuronal network activity was recorded every three days for approximately six weeks. Statistical significance was analyzed using a linear mixed-effects model fit by maximum likelihood. Developmental MeHg exposure led to persistent alterations in neuronal activity, including changes in spontaneous firing patterns and responses to stimuli. Mean spike firing rate and mean burst rate was significantly increased in cultures exposed to 0.1 µM MeHg, and significantly decreased in the 1 µM MeHg exposed group from DoM 30-39. The number of network bursts was significantly decreased in 1 µM cultures on DoM 36-39. At 30, 33, and 39 DoM, synchronicity and complexity was significantly increased in the 0.1 µM group, but was significantly decreased in the 1 µM group in comparison to the control group. These effects were observed even after removal of MeHg exposure, suggesting a persistently altered state in mature neuronal network function. This study demonstrates that early-life MeHg exposure can create a persistent neurotoxic state in regard to neuronal network function. This state may increase vulnerability to NDs by altering neuronal function and responsiveness to subsequent insults.

Exploring Pathogenic Role of Monocytes in Alzheimer's Disease through engineered human brain organoid chips

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Recent evidence increasingly links Alzheimer’s disease (AD) pathogenesis to neuroinflammation. Despite their role as key mediators of inflammation in the human immune system, peripheral monocytes remain understudied in AD pathogenesis, largely due to the lack of suitable human models. To bridge this gap, we developed engineered human brain organoid (hBO) chips derived from human embryonic stem cells (hESCs) to model dynamic interactions of monocytes with key brain components in AD neuroinflammation. By incorporating 3D-printed scaffolds into hBO cultures within standard 96-well plates, we produced hBO chips with tubular structures, significantly reducing necrosis and improving organoid viability. Using these engineered hBO chips, we observed notable alterations AD monocytes, including increased infiltration rates and reduced amyloid-beta (Aβ) clearance ability. Additionally, AD monocytes triggered increased astrocyte activation and neuronal apoptosis, further contributing to AD neuroinflammation. Importantly, we observed significantly elevated expression of IL1B and CCL3 at both transcriptional and protein levels, emphasizing the central role of these cytokines in monocyte-mediated neuroinflammation in AD. These findings illuminate the role of monocytes in AD pathogenesis and present engineered hBO chips as a versatile platform for studying neuroinflammation and developing therapeutics for neuroinflammatory diseases.

Cell fate decisions: Can we toy with them?

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In the nasal cavity, cell fate decisions critically shape the functional composition of both the respiratory and olfactory epithelia, enabling these tissues to respond to environmental challenges such as pathogens, pollutants, and injury. These decisions are governed by complex signaling pathways that integrate mechanical cues, genetic regulation, and cellular interactions to guide epithelial cell differentiation, tissue remodeling, and repair. The respiratory epithelium consists of specialized cell types, including basal, ciliated, and secretory cells, whose development and maintenance are regulated by the coordinated integration of extracellular signals and intrinsic genetic programs. In contrast, cell fate decisions in the olfactory epithelium are crucial for maintaining the balance between the regeneration of sensory neurons, supporting cells, and basal progenitor cells. While these epithelial systems are anatomically adjacent and share some common features, they remain functionally distinct. Inflammatory processes in the respiratory epithelium can also impact the olfactory epithelium, as inflammation and cytokine signaling may disrupt cell fate decisions and alter the regeneration of sensory neurons, leading to olfactory dysfunction. A key question arises: could stem cells from one compartment be reprogrammed to compensate for the loss or dysfunction in the other? Exploring the plasticity of stem cells within these two epithelial tissues may provide new insights into their regenerative capacities and therapeutic potential for treating both olfactory dysfunction and respiratory diseases.

Alzheimer's Disease-related fractalkine receptor polymorphism confers distinct phenotypes in iPSC-derived human microglia-like cells

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Dysfunctional microglial activity has been identified as a potential mechanism leading to accumulation of amyloid beta and pTau and subsequent neurodegeneration in Alzheimer's Disease (AD). The CX3CR1/fractalkine axis serves as a mechanism for bi-directional communication between microglia and neurons, respectively, to promote an anti-inflammatory microglial state. Previous studies have demonstrated that deficiency in CX3CR1 signaling leads microglia to develop a more pro-inflammatory phenotype, induces phagocytic deficits, and increases susceptibility of neurons to cell death. The CX3CR1-V249I polymorphism was recently identified as a potential risk allele for AD. However, the role of fractalkine dysfunction in human cells and the mechanisms by which microglia with the CX3CR1-V249I SNP contribute to neurodegeneration remain unclear. To address this shortcoming, we utilized human induced pluripotent stem cells and CRISPR/Cas9 technology to elucidate the effects of the V249I polymorphism on human microglia-like cells (hMGLs) compared to an isogenic control cell line. We demonstrate effective differentiation from isogenic control and CX3CR1-V249I backgrounds into hMGLs. Transcriptional profiling via RNA-seq analyses demonstrated alterations in pathways such as apoptosis, toll-like receptor signaling, and the inflammasome due to the CX3CR1-V249I SNP. Both heterozygous and homozygous microglia bearing the V249I allele demonstrated decreased phagocytosis of amyloid beta in vitro compared to controls, with this effect modulated by the presence of fractalkine in heterozygous but not homozygous V249I hMGLs. Both heterozygous and homozygous V249I microglia exhibited increased stress-induced cell death compared to controls, with homozygous hMGLs demonstrating increased cell death at earlier time points. These findings suggest that the CX3CR1-V249I polymorphism may confer a dysfunctional microglia phenotype, which may subsequently contribute to neuronal dysfunction. Ongoing work will elucidate how this polymorphism contributes to AD-related neurodegeneration in 2D and 3D co-culture models. Collectively, the results of this study highlight the importance of understanding CX3CR1 function in AD pathology to identify targetable mechanisms for intervention.

Rapid induction of functional neurons using NGN2-based directed differentiation in human induced pluripotent stem cells

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Studies involving human neurological disease are limited by availability of patient-derived neurons. The directed differentiation of human induced pluripotent stem cells (iPSCs) can provide a continuous source of patient-derived neurons as opposed to direct neuron induction from fibroblasts or other somatic cells. However, current growth factor-based neuronal differentiation protocols from iPSCs are cumbersome and requires weeks to isolate mature neurons and are currently incapable of providing purity of differentiated neurons apart from other differentiating cell types. Thus, the manual isolation of desired neurons reduces their yield and specificity. Moreover, growth factor-induced neuronal differentiation tends to be highly variable, further compromising reproducibility. To overcome these shortcomings, we have explored the use of a rapid single-step induced neuron (iN) methodology from iPSCs. Using a lentiviral delivery system, we induced constitutive tetracycline expression to overexpress exogeneous neurogenin-2 (NGN2) driven by the tetO promoter. The forced NGN2 expression aids in the direct lineage conversion of iPSCs into neuronal cells. Co-expression of eGFP and puromycin resistance in these constructs further aided in the selection and survival of transduced cells. Cells transduced with these lentiviral vectors exhibited neuronal morphologies in surviving cells within one week. In ongoing experiments, we aim to confirm the neuronal properties of these cells via immunostaining of key neuronal markers. Standardization of iPSCs-derived induced-neurons protocol in our lab will contribute to increasing the yield and specificity of isolated neurons with low cell-to-cell variability, which is necessary for determining disease pathogenesis and drug targets.

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Spinal cord injury (SCI) often leads to neuronal loss, axonal degeneration, and behavioral dysfunction. We recently show that in vivo reprogramming of NG2 glia produces new neurons, reduces glial scaring, and ultimately leads to improved function after SCI. By examining endogenous neurons, we here unexpect- edly uncover that NG2 glia reprogramming also induces robust axonal regeneration of the corticospinal tract and serotonergic neurons. Such reprogramming-induced axonal regeneration may contribute to the reconstruction of neural networks essential for behavioral recovery.

Developing a Vascularized Neuroimmune Organoid Model for Studying Sporadic Alzheimer's Disease and Drug Screening

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Alzheimer's Disease (AD) is a progressive neurodegenerative disease afflicting 50 million individuals worldwide. It is characterized by multiple pathological hallmarks, including synaptic and neuronal loss, brain atrophy, and substantial cognitive decline. Current animal models mainly focus on familial AD (fAD), which is caused by genetic mutations. However, models for studying sporadic AD (sAD), which represents over 95% of AD cases without specific genetic cause, are still lacking. Notably, the significant species differences between humans and animals might be the leading cause of clinical fails for most AD therapeutics that have shown success in animal models, highlighting the importance to develop more translational human-centric models for studying AD, particularly sAD. In this study, we developed a novel human induced pluripotent stem cells (hiPSCs)-based vascularized neuroimmune organoid model. These organoids contain multiple cell types in the human brain, including human neurons, microglia, astrocytes, and blood vessels. We further induced AD pathologies by challenging them with AD patient-derived brain homogenate. Our results suggested that AD brain homogenate treated organoids successfully recapitulated multiple AD pathological hallmarks including amyloid plaques, tau tangles, neuroinflammation, elevated microglial synaptic pruning, and synaptic and neuronal loss, as compared to control group. Remarkbly, after 2 weeks of treatment with Lacanemab, an FDA-approved drug targeting amyloid beta, we observed a significant reduction of amyloid burden. In summary, the neuroimmune organoid model provides a unique opportunity to study sAD in a human cell setting. This innovative model also facilitates AD drug development, particularly for testing AD immunotherapy, such as antibody-based AD treatment.