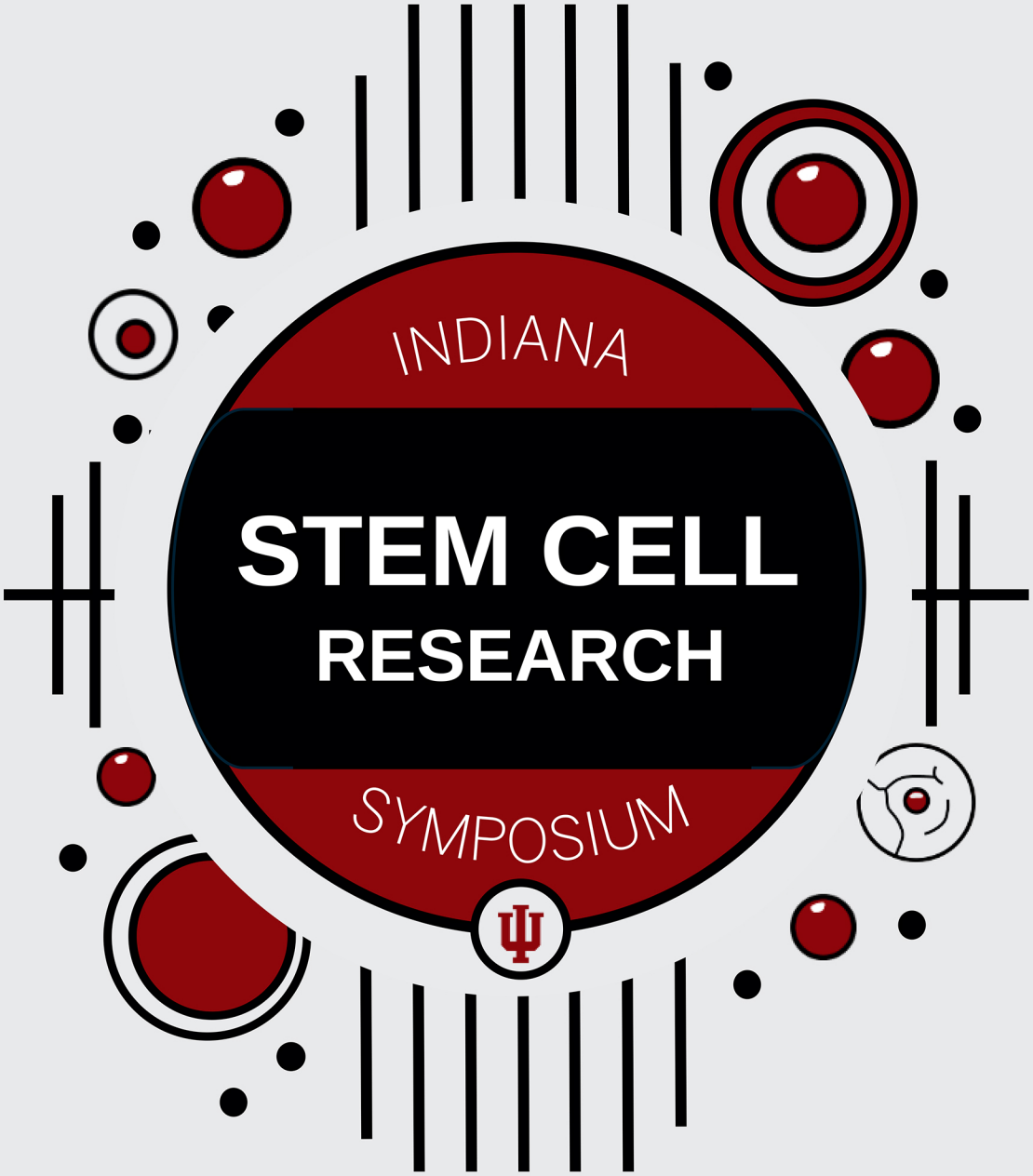

Monday, Nov. 10

8 a.m. to 5 p.m.

Medical Education and Research Building

SCHOOL OF MEDICINE

STARK NEUROSCIENCES RESEARCH INSTITUTE

Special Thanks To:

Funding:
Stark Neurosciences Research Institute

Conference Organization:
Jason Meyer, Scott Canfield, Angela Bryant, Jennifer Shepherd, Julie Landaw

Abstract Judging:
Scott Canfield, Tasneem Sharma, Feng Guo, Ranjie Xie

Poster Judging:
Scott Canfield, Jignesh Tailor, Xiaoping Bao, Arup Das, Aaron Bowman, Cátia Gomes, Hande Karahan, Miguel Moutinho

Registration:
Shruti Patil, Laurna Varghese, Zuber Sayyad

Event Escorts:
Aaron Baker, Shelby Hetzer, Kevin Quist

Symposium Logo:
Rylee Anderson

Agenda	
8:00 am	*Registration and Coffee*
8:45	*Introductory Comments – HA 1019*
9:00	*Oral Presentation Session #1* • **Jignesh Tailor**, Indiana University School of Medicine "Recapitulating cancer predisposition in the central nervous system with neuroepithelial stem (NES) cells" • **Xufeng Xue**, Cincinnati Children's Hospital "A patterned neural organoid via microfluidic morphogen gradients" • **Xiaoping Bao**, Purdue University "Engineer CAR-neutrophils from human pluripotent stem cells for targeted chemoimmunotherapy against glioblastoma" • **Lei Yang**, Indiana University School of Medicine “Long non-coding RNAs regulat human cardiac development and disease • **Jason Tchieu**, Cincinnati Children’s Hospital “ZMYND11 safeguards brain-specific splicing by repressing poised developmental genes” • **Shruti Patil**, Indiana University School of Medicine “Modeling optic nerve glioma formation and axonal degeneration using a human iPSC-derived compartmentalized microfluidic platform”
10:30	*Coffee Break*
10:45	*Oral Presentation Session #2* • **Erica Cai**, Indiana Biosciences Research Institute "Designing Stronger Beta Cells: From Mechanistic Insight to Immune Evasion" • **Arup Das**, Indiana University School of Medicine "Pharmacologic Reprogramming of Mitochondrial Metabolism Links Human Stem Cell Differentiation to Neuroprotection in Retinal Ganglion Cells" • **Priya Shields**, Indiana University School of Medicine “Pursuing the translatome of motor neurons in ALS” • **Yanru Ji**, Purdue University “Develop a human tauopathy model using human-mouse neuronal chimeras”
12:00 pm	*Lunch – HA 1024*
1:00	*Poster Session – NB 101*
2:30	*Group Photo – HA 1019*
2:45	*Oral Presentation Session #3* • **Aaron Bowman**, Purdue University • "Developmental exposure to methylmercury causes persistent effects in human stem cell derived cortical glutamatergic cultures" • **Kyle McCracken**, Cincinnati Children's Hospital "Engineering tissue interactions enhances structural organization of human kidney organoids" • **Cátia Gomes**, Indiana University School of Medicine “Relay neurons bridge axotomized retinal ganglion cells and thalamic targets after optic pathway injury in an hPSC model” • **Holly Poling**, Cincinnati Children’s Hospital “Functional innervated gut tissue generation via a confined culture scaffold system”

	• **Gyuhyung Jin**, Purdue University “Engineered chimeric antigen receptor neutrophils derived from human pluripotent stem cells for the treatment of cardiac fibrosis”
4:00	*Keynote Presentation* **Tracy Young-Pearse,** Harvard University "Moving beyond amyloid and tau to capture the biological heterogeneity of Alzheimer’s disease using human induced pluripotent stem cell models"
5:00	*Concluding Remarks and Awards*

Oral Presentation Abstracts – Session #1

Recapitulating cancer predisposition in the central nervous system with neuroepithelial stem (NES) cells	
Jignesh Tailor	Indiana University School of Medicine

A patterned neural organoid via microfluidic morphogen gradients	
Xufeng Xue	Cincinnati Children's Hospital
Glioblastoma (GBM), the most common type of primary brain tumor, is characterized by high mortality rate, short lifespan, and poor prognosis with a high tendency of recurrence. Functional therapeutics, including PRMT5 inhibitors, radiosensitizers, and emerging chimeric antigen receptor (CAR)-T immunotherapy, have been developed to treat GBM. However, the existence of physiological blood-brain barrier (BBB) or blood-brain-tumor barrier has impeded the efficient delivery of such promising therapeutics into the brain and limited their therapeutic efficacy. Given the native ability of neutrophils to cross BBB and penetrate the brain parenchyma, here we tested the therapeutic concept that neutrophils could be engineered with synthetic CARs to specifically target GBM and effectively deliver chemo-drugs to brain tumor as a novel dual chemoimmunotherapy for the first time. Primary neutrophils are short-lived and resistant to genetic modification. Therefore, we genetically engineered human pluripotent stem cells with different chlorotoxin (CLTX) CARs and differentiated them into functional CAR-neutrophils. As compared to CAR-natural killer (NK) cells, systemically administered hPSC-derived CLTX CAR-neutrophils significantly reduced tumor burden in xenograft mouse models and extended their lifespan, suggesting superior abilities of neutrophils in crossing BBB and penetrating GBM xenograft in mice. We also loaded hypoxia-activated prodrug tirapazamine (TPZ) into CAR-neutrophils using silica nanoparticles with rough surfaces (R-SiO2-TPZ) and demonstrated their enhanced antitumor activities in xenograft mouse models, serving as a novel dual chemoimmunotherapy against GBM. Our results established that CAR neutrophil-mediated drug delivery may provide an effective and universal strategy for specific targeting of solid tumors.	

Engineer CAR-neutrophils from human pluripotent stem cells for targeted chemoimmunotherapy against glioblastoma	
Xiaoping Bao	Purdue University
Glioblastoma (GBM), the most common type of primary brain tumor, is characterized by high mortality rate, short lifespan, and poor prognosis with a high tendency of recurrence. Functional therapeutics, including PRMT5 inhibitors, radiosensitizers, and emerging chimeric antigen receptor (CAR)-T immunotherapy, have been developed to treat GBM. However, the existence of physiological blood-brain barrier (BBB) or blood-brain-tumor barrier has impeded the efficient delivery of such promising therapeutics into the brain and limited their therapeutic efficacy. Given the native ability of neutrophils to cross BBB and penetrate the brain parenchyma, here we tested the therapeutic concept that neutrophils could be engineered with synthetic CARs to specifically target GBM and effectively deliver chemo-drugs to brain tumor as a novel dual chemoimmunotherapy for the first time. Primary neutrophils are short-lived and resistant to genetic modification. Therefore, we genetically engineered human pluripotent stem cells with different chlorotoxin (CLTX) CARs and differentiated them into functional CAR-neutrophils. As compared to CAR-natural killer (NK) cells, systemically administered hPSC-derived CLTX CAR-neutrophils significantly reduced tumor burden in xenograft mouse models and extended their lifespan, suggesting superior abilities of neutrophils in crossing BBB and penetrating GBM xenograft in mice. We also loaded hypoxia-activated prodrug tirapazamine (TPZ) into CAR-neutrophils using silica nanoparticles with rough surfaces (R-SiO2-TPZ) and demonstrated their enhanced antitumor activities in xenograft mouse models, serving as a novel dual chemoimmunotherapy against GBM. Our results established that CAR neutrophil-mediated drug delivery may provide an effective and universal strategy for specific targeting of solid tumors.	

Long Non-coding RNAs Regulate Human Cardiac Development and Disease	
Lei Yang	Indiana University School of Medicine

The human heart exhibits unique features in morphogenesis, metabolism, function, and disease compared to the rodent heart, suggesting the presence of species-specific molecular mechanisms that remain largely unexplored. Our primary research interest is to elucidate the human-specific molecular mechanisms that regulate human heart development and disease, focusing on human long non-coding RNAs.

ZMYND11 Safeguards Brain-specific Splicing by Repressing Poised Developmental Genes	
Jason Tchieu	Cincinnati Children's Hospital Medical Center

Neurodevelopmental disorders (NDDs) encompass diverse phenotypes, ranging from social communication deficits to cognitive disability, and involve disruptions across multiple brain regions. Advances in genomics have identified hundreds of NDD risk variants, many in genes expressed during critical developmental stages; however, the mechanisms by which these variants confer risk remain poorly understood. Here, we leverage human pluripotent stem cell–derived cortical models to investigate the neurodevelopmental consequences of mutations in ZMYND11, a newly implicated NDD risk gene. ZMYND11 encodes a histone reader and transcriptional co-repressor previously linked to tumor suppression. We find that ZMYND11-deficient cortical neural stem cells aberrantly activate latent developmental pathways, impairing progenitor maintenance and neuron production. Beyond chromatin regulation, ZMYND11 controls a brain-specific isoform switch involving the splicing regulator RBFOX2. Extending these findings, we show that other chromatin-related autism risk genes converge on similar developmental pathway activation and splicing dysregulation, phenotypes that can be partially rescued by restoring ZMYND11’s regulatory functions.

Modeling Optic Nerve Glioma Formation and Axonal Degeneration Using a Human iPSC-Derived Compartmentalized Microfluidic Platform	
Shruti Patil	Indiana University School of Medicine

Optic nerve degeneration is a hallmark of various retinal pathologies, yet the underlying mechanisms remain poorly understood. To interrogate the role of neuron-glia interactions in axonal vulnerability, we developed a human stem cell-based, compartmentalized microfluidic platform that isolates retinal ganglion cell (RGC) somas from their axons, enabling precise spatial control over cellular environments. In this three-chamber system, human induced pluripotent stem cell (iPSC)-derived RGCs extend axons from a soma-containing chamber through microgrooves into adjacent chambers, where they can be selectively co-cultured with astrocytes or exposed to engineered extracellular conditions. This architecture facilitates targeted studies of glial modulation of axonal integrity. When RGCs were cultured with patient-derived astrocytes carrying a pathogenic mutation associated with neurofibromatosis type 1 (NF1), we observed significant reductions in neurite complexity and electrophysiological activity as measured by multi-electrode array (MEA). The NF1 mutations causes loss of neurofibromin function that leads to accumulation of GTP-bound (active) KRAS and overactivation of RAS-MAPK pathway. The NF1 mutant-astrocytes exhibited glioma like properties with increased KRAS levels and proliferative rate. The introduction of these patient-derived astrocytes into the axonal compartment induced distinct axonal morphological changes and signs of axonal stress, suggesting deleterious glial signaling in a disease-relevant context. Moreso, the RGCs carrying NF1-mutation presented more susceptibility towards glioma-induced degeneration. Bulk RNA sequencing revealed transcriptional signatures consistent with disrupted neuronal and glial homeostasis and axonal degeneration, which further serve as a foundation for comprehending NF1 glioma-associated alterations at cellular level. Overall, this modular platform provides a physiologically relevant system to dissect disease mechanisms underlying optic nerve degeneration and to enable future therapeutic testing.

Oral Presentation Abstracts – Session #2

Designing stronger beta cells: from mechanistic insight to immune evasion	
Erica Cai	Indiana Biosciences Research Institute

Type 1 diabetes (T1D) results from immune-mediated destruction of insulin-producing beta cells, leaving patients dependent on lifelong insulin therapy. While the advent of induced pluripotent stem cell (iPSC)-derived beta cells offers a promising avenue for cell replacement, persistent autoimmunity remains a major barrier to long-term graft survival. In the absence of immunosuppression, autoreactive T cells rapidly eliminate transplanted beta cells. Although encapsulation devices provide partial immune protection, they are hindered by poor oxygenation and limited nutrient exchange, leading to graft hypoxia, beta cell dysfunction, and eventual failure. Enhancing beta cell resilience to immune-mediated attack is thus critical for realizing the full potential of beta cell replacement therapies in T1D. We performed a genome-scale *in vivo* CRISPR screen in non-obese diabetic (NOD) mice, which serve as a model for T1D, using autoimmunity as a selection pressure. This approach aimed to discover gene edits that could confer protection to beta cells. Among the genes identified, we focused on ZBED3 due to its associations with human diabetes reported in several genome-wide association studies (GWAS). Our study revealed that ZBED3 functions as a high-level transcriptional activator for multiple mediators in the type I and type II interferon signaling pathways, including STAT1, STAT2, and IRF1. Loss of *ZBED3* in beta cells resulted in decreased surface expression of MHC-I / HLA-I, reducing beta cell immunogenicity to T cells, and enhancing resistance to autoreactive immune cell-mediated beta cell killing. By delineating ZBED3's impact on beta cell stress response and immune recognition, this study provides mechanistic insights into how intrinsic transcriptional programs shape beta cell vulnerability in type 1 diabetes. These results highlight ZBED3 as a promising target for improving beta cell replacement therapy in T1D, with the potential to extend beta cell autoimmune tolerance and support long-term graft survival. Furthermore, this work establishes a foundation for future efforts aimed at modulating immune evasion pathways in islet transplantation and beta cell engineering.

Pharmacologic Reprogramming of Mitochondrial Metabolism Links Human Stem Cell Differentiation to Neuroprotection in Retinal Ganglion Cells	
Arupratan Das	Indiana University School of Medicine

Metabolic collapse with mitochondrial damage presents the early pathology for retinal ganglion cell (RGC) degeneration in optic neuropathies such as glaucoma, yet no RGC-targeted therapies exist. Here, we identify a clinically tested small molecule that reprograms mitochondrial maturation and metabolic state to both promote differentiation and protect mature human RGCs.

We performed a high-content live-cell mitochondrial screen in human pluripotent stem cell-derived RGCs (hRGCs), using the mitochondrial membrane potential reporter Mito-Tracker Deep Red (MTDR), to identify compounds that restore mitochondrial health. Healthy mitochondria maintain the membrane potential through active electron transport and proton motive force that is sensed by the MTDR probe to get inside the organelle. Hits were subsequently evaluated for neuroprotection by their ability to reduce cellular apoptosis in the differentiated hRGC culture. This led to the discovery of the 5-HT1A G protein-coupled receptor antagonist WAY-100635 (WAY). In wild-type hRGCs, WAY induced a reversible increase in cAMP via 5-HT1A engagement, followed by reversible activation of mitochondrial biogenesis through the master regulator PGC-1a. In hRGCs containing the glaucoma-associated Optineurin E50K mutation, which impair mitophagy, WAY improved mitochondrial health and conferred neuroprotection while shifting metabolism toward aerobic glycolysis, a metabolic reprogramming known to support neuronal survival in humans. Importantly, in mouse models of acute optic nerve injury and elevated intraocular pressure (a glaucoma risk factor), systemic WAY treatment robustly protected RGC axons, preserved cell bodies, and maintained functional circuitry between eye and brain. Strikingly, when applied at the progenitor stage, WAY accelerated

mitochondrial maturation (increased cristae complexity) and promoted a metabolic switch toward oxidative phosphorylation (OXPHOS), thereby driving early cell-cycle exit and enhanced differentiation of RGCs. Together, our findings establish a first-in-class pharmacologic paradigm for simultaneously protecting and maturing RGCs via reversible metabolic and mitochondrial reprogramming, advantages that avoid the delivery challenges and constitutive effects of gene therapy. Given the central role of mitochondrial dysfunction across neurodegenerative disease, this approach may extend beyond glaucoma and applicable to Parkinson's disease and ALS, facilitating translation via repurposing of a drug with established safety.

Pursuing the Translatome of Motor Neurons in ALS

Priya Shields | Indiana University School of Medicine

Amyotrophic Lateral Sclerosis (ALS) is a fatal disease characterized by upper and lower motor neuron (MN) degeneration. ALS 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 in models such as Sod1G93A mice. We hypothesized that translation within MN axons will be distinct from MN somas located within the ventral spinal cord, and 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 discover 581 differentially expressed genes enriched in axons/NMJs, revealing the existence of an axonal translatome distinct from the cell body. Subsequent Gene Ontology and KEGG GSEA analyses revealed that mRNA processing, synaptic neurotransmitter regulation, and cytoskeletal organization- associated transcripts are enriched within MN axons. These findings raise the possibility that deficits in local gene translation could contribute to neurodegenerative etiology. To further evaluate this, ALS patient iPSC-derived MNs were exposed to stress conditions, which triggered an increase in somal ratios of phosphorylated TDP43 to non-phosphorylated TDP43. Additionally, stressed ALS MNs contain non-nuclear, TDP43+ stress granules, translationally-inactive particulates composed of RNA binding proteins, mRNAs, and ribosomal proteins. Importantly, transcripts normally localized in the MN axon were found to be mis-localized by toxicant exposure. Further investigation of these sequestered transcripts will identify transport and splicing impairments throughout ALS disease progression, within axons as well as cell bodies. Ultimately, understanding translational deficits in ALS may serve as a therapeutic avenue to rescue MN axonal degeneration.

Develop a Human Tauopathy Model Using Human-Mouse Neuronal Chimeras

Yanru Ji | Purdue University

Intraneuronal aggregation of tau protein is one of the hallmarks of Alzheimer's disease (AD) and strongly correlates with cognitive decline. Under normal conditions, tau binds to microtubules, regulating axonal transport and microtubule dynamics. Hyperphosphorylation causes tau to detach from microtubules and self-assemble into toxic aggregates, leading to synaptic loss and neuronal death. Conventional tauopathy models, such as hiPSCs-derived in vitro models, transgenic rodents, and seed-dependent transgenic mice models, significantly advance our knowledge of disease mechanisms. However, several limitations persist: (1) in vitro cultured neurons do not resemble the aged phenotype of AD brains; (2) most rodent models rely on frontal temporal dementia (FTD) mutations, producing tauopathies that diverge from those in AD; and (3) human and rodent tau differ in splice form composition at adulthood. Thus, a more pathophysiologically relevant human model is needed to elucidate AD-specific tau pathology and to facilitate therapeutic discovery. To this end, we generated a tauopathy model by engrafting human neurons into mice brains and stereotaxically injecting AD patient-derived tau seeds into the hippocampus. The transplanted neurons preserved the physiological 1:1 ratio of 3R/4R tau isoforms seen in mature human neurons by 6 months of age. Seed inoculation induced misfolding of endogenous tau, producing pre-tangles and mature neurofibrillary tangles over time. The number of pathology-bearing neurons increased progressively, and

lesions disseminated across connected brain regions, mirroring the spreading in AD. Notably, human neurons represented a disproportionate fraction of tangle-positive cells, indicating higher vulnerability to human-derived seeds. Chimeric mice also exhibited progressive memory deficits, linking tau accumulation to functional impairment. In summary, we have established a human–mouse chimera that faithfully models the initiation, propagation, and behavioral consequences of AD-related tauopathy in mature human neurons. This platform offers a powerful tool for mechanistic studies in tau-mediated neurodegeneration and may be adaptable to other seed-related neurological disorders, as well as for therapeutic testing.

Oral Presentation Abstracts – Session #3

Developmental exposure to methylmercury causes persistent effects in human stem cell derived cortical glutamatergic cultures	
Aaron Bowman	Purdue University
Persistent neurotoxicity can contribute to the development and progression of chronic neurodegenerative diseases and disorders. Environmental factors such as pesticides, heavy metals, and air pollution have been identified as significant contributors to the occurrence and progression of chronic neurological diseases and disorders. Exposure to these substances can lead to neurotoxic effects that persist over time. This talk will focus on the potential for persistent neurotoxic outcomes resulting from exposure to methylmercury (MeHg), a neurotoxin which indiscriminately disrupts multiple homeostatic pathways. Specifically the work presented was performed to evaluate the continued risk of such outcomes even after exposure has ceased. The mechanisms of persistent neurotoxicity in the pathophysiology of neurological diseases may be different than acute toxicity, and data examining this question will be presented from a hiPSC-derived neural cell model system.	

Engineering tissue interactions enhances structural organization of human kidney organoids	
Kyle McCracken	Cincinnati Children's Hospital
Kidney organoids generated from human pluripotent stem cells (hPSCs) comprise nephron-like structures that lack collecting ducts (CDs) and instead terminate as blind-ended tubules. To address this limitation, we have developed a developmentally inspired assembloid approach to integrate ureteric bud (UB) progenitor tissues, the embryologic building blocks of the kidney's collecting system. In this model, the UB progenitors grew into extensive tubular networks that interacted with and connected to the organoid nephrons. The epithelial fusion process in the organoids recapitulated the developmental process that occurs *in vivo* and was specific to only the distal-most segments of the nephrons, establishing potential mechanism to drain fluid out of nephrons into CDs for the first time. Further, we showed that the nephron-UB anastomoses could be established *in vivo* following transplantation, and this led to fluid accumulation over time in the engrafted CDs. This innovative system provides a powerful platform to interrogate the mechanisms of this important fusion event, and this approach is an important advance in kidney tissue engineering since generating a continuous drainage pathway from nephrons is an obligatory step toward production of functional renal tissue.	

Relay Neurons Bridge Axotomized Retinal Ganglion Cells and Thalamic Targets After Optic Pathway Injury in a hPSC Model	
Catia Gomes	Indiana University School of Medicine
Glaucoma is a progressive neurodegenerative disease characterized by the loss of retinal ganglion cells (RGCs) and their axonal projections in the optic nerve, leading to irreversible visual impairment. The degeneration of RGC axons disrupts connectivity between the retina and central visual targets such as the thalamic lateral geniculate nucleus (LGN) in the brain. Despite extensive research, effective strategies to restore this eye-brain connectivity remain limited. To address these shortcomings, we investigated whether excitatory neurons could serve as relay cells to facilitate the reconnection of axotomized RGCs with their thalamic targets following injury, therefore modeling the chronic axonal degeneration observed in glaucoma. RGCs were cultured on 3-chamber microfluidic platforms that enable physical separation of axons from somas, with thalamic cells seeded in the third chamber to replicate the retina-brain connection. Axotomy was induced in the middle chamber to mimic optic nerve injury, after which excitatory neurons were introduced into this compartment to assess their ability to promote RGC axonal regrowth and reconnection with thalamic targets. Our results demonstrate that the presence of excitatory neurons within the axotomized pathway enhances RGC axonal regeneration and supports synaptic reformation. Moreover,	

increased connectivity was observed between RGCs and local excitatory neurons, as well as between these excitatory neurons and thalamic targets, suggesting the establishment of a relay mechanism that bridges the disrupted visual circuit. Together our results suggest that excitatory neurons can act as functional relay cells to re-establish disrupted eye-brain connectivity following axonal injury. By promoting RGC axonal regrowth and facilitating synaptic reconnection with thalamic targets, these neurons help reconstruct the visual signaling pathway disrupted in the optic nerve of glaucoma patients. This relay-based approach offers a promising strategy for restoring neural circuits damaged by neurodegenerative diseases and may inform the development of novel regenerative therapies for vision loss associated with glaucoma and related optic neuropathies.

Functional Innervated Gut Tissue Generation Via a Confined Culture Scaffold System

Holly Poling | Cincinnati Children's Hospital

Human pluripotent stem cell-derived organoids offer a powerful model for studying gastrointestinal tissue development, yet conventional methods are constrained by scalability, incomplete maturation, and limited functional complexity. Here, we present a novel and user-friendly confined culture system (CCS) that employs a custom-fabricated scaffolding tray to direct the fusion and elongation of thousands of spheroids into centimeter-scale, tubular gastrointestinal organoids. This platform is broadly adaptable to spheroid-based organoid systems and supports the robust generation of small intestinal, colonic, and gastric tissues with improved size, structural organization, and physiological relevance. Compared to standard protocols, CCS-derived tissues reach transplantation maturity in half the time and demonstrate enhanced engraftment. Uniquely, CCS organoids also co-develop a functional de novo enteric nervous system without the need for exogenous neural crest cells or additional neurotrophic cues. Electrophysiological and transcriptomic profiling confirm the emergence of diverse neuronal subtypes, including excitatory and inhibitory populations, and supporting glial cells. Together, this results in functional neuromuscular coordination with isometric force contractions comparable to that of native human tissue. This modular and scalable platform redefines gastrointestinal tissue engineering by combining simplicity, reproducibility, and versatility, advancing organoid models for developmental studies, disease modeling, and translational applications in regenerative medicine.

Engineered Chimeric Antigen Receptor Neutrophils Derived from Human Pluripotent Stem Cells for the Treatment of Cardiac Fibrosis

Gyuhyung Jin | Purdue University

Cardiac fibrosis represents a common pathological endpoint of many cardiac disorders, marked by the persistent activation of fibroblasts and accumulation of extracellular matrix (ECM), which disrupts normal tissue architecture and leads to hypertrophy, arrhythmias, and progressive heart failure. While current pharmacologic therapies attempt to suppress fibrotic signaling, they are nonspecific and often limited by systemic toxicity. More selective strategies that directly eliminate activated fibroblasts are urgently needed. Recent studies have demonstrated the potential of chimeric antigen receptor (CAR) T cells targeting fibroblast activation protein (FAP) to reverse fibrosis and restore cardiac function in preclinical models. However, CAR T-cell approaches are constrained by patient-to-patient variability, manufacturing complexity, and cost. To overcome these barriers, we developed an alternative, universal platform using neutrophils generated from human pluripotent stem cells (hPSCs) engineered to express an anti-FAP CAR. Through CRISPR/Cas9-mediated integration of a FAP-specific CAR construct, we produced stable hPSC lines and differentiated them into neutrophils exhibiting characteristic molecular and phenotypic markers. Using an in vitro fibrosis model derived from hPSC-based epicardial fibroblasts, we observed that anti-FAP CAR neutrophils displayed selective cytotoxicity toward activated fibroblasts, whereas unmodified neutrophils did not. These findings establish a proof-of-principle for an off-the-shelf, neutrophil-based immunotherapy targeting cardiac fibrosis. The approach harnesses the natural infiltration and cytotoxic properties of

neutrophils and can be extended to fibrotic diseases in other organs. This work provides a foundation for developing next-generation, stem cell–derived immune therapeutics for regenerative cardiology and beyond.

Poster Abstracts:

Poster 1	
Investigating Polygenic Contributions to Alzheimer's Disease Pathology Using a Human NGN2-Based iPSC-Derived Neuronal Model	
Reham Afify	Indiana University School of Medicine
Alzheimer's disease (AD) is a polygenic neurodegenerative disorder in which the combined impact of genomic variants contributes to disease susceptibility and progression. Although genome-wide association studies (GWAS) have significantly advanced our understanding of the genetic architecture underlying AD, the functional impact of polygenic risk on human neurons remains largely unclear. This highlights the need for physiologically relevant human neuronal models that capture the genetic complexity of AD pathology. To more properly elucidate polygenic contributions to AD pathology, we generated multiple lines of human induced pluripotent stem cells (iPSCs) from patient samples from the ADNI study based upon varied polygenic risk scores (PRS), including patients from the uppermost and lowermost quartiles. With these iPSCs, we then aimed to optimize an NGN2-induced neuronal differentiation protocol to enable robust, efficient, and reproducible generation of excitatory cortical neurons from iPSCs derived from AD patients with variable PRS. iPSC lines were successfully differentiated into neurons across all groups, with neuronal identity and morphology validated using immunocytochemistry (ICC), confirming the robustness of the protocol across genetically diverse backgrounds. Ongoing experiments aim to functionally and molecularly characterize these neurons to uncover genotype-associated differences. Neuronal activity is being assessed using multi-electrode array (MEA) technology, while Western blotting, ELISA, and ICC are used to quantify AD-relevant protein burden, including amyloid-beta (Aβ) and phosphorylated tau. We anticipate that neurons, particularly those derived from individuals with high PRS, will exhibit altered electrophysiological properties, disrupted synaptic integrity, and dysregulated expression of AD-related proteins compared to neurons from low-PRS or healthy control individuals. Ultimately, this study utilizing NGN2-based iPSC-derived neuronal platform offers a promising tool to unravel how polygenic risk shapes AD pathophysiology. It holds potential to uncover polygenic contributions to disease pathology, support genotype-guided therapeutic development, and advance personalized risk prediction in AD.	

Poster 2

The Nuclear Factor I Gene Family Dictates the Temporal Progression of Cortical Development by Establishing Radial Glia Cell Identity

Elena Albizzati | Cincinnati Children's Hospital

Cortical development depends on precise temporal control of progenitor competence to ensure the orderly generation of neuronal and glial lineages. Radial glial cells (RGCs), which are the primary neural stem cells of the embryonic forebrain, proliferate early in embryogenesis, undergo a sequential series of fate switches that produce the canonical layered architecture of the neocortex. This temporal progression is driven by intrinsic transcriptional programs in coordination with extrinsic signals, yet the molecular mechanisms that regulate these intrinsic timers remain poorly understood. Here, we explore the role of Nuclear Factor I (NFI) genes, a family of transcription factors essential for brain development implicated in distinct neurodevelopmental disorders. Using conditional mouse models, we determined the independent and combinatorial functions in RGCs and their effect on cortical development. While single and double knockout (KO) mice (Nfia, Nfib, Nfix) lead to mild delays in corticogenesis, triple KO (cTKO-ABX) causes severe cortical defects, marked by a significant accumulation of progenitors that fail to exit the proliferative state and are unable to commit to cell fates beyond early neurogenesis. To extend these findings to humans, we derived human pluripotent stem cell (hPSCs) lines endogenously FLAG-tagged for each NFI gene. Using ChIP-seq and epigenomic analyses, we found extensive, overlapping, and stage-dependent binding in critical regulatory regions, including numerous enhancer sites within opening chromatin regions. These results underscore the role of NFIs as core regulators of RGCs maturation in chromatin remodeling and transcriptional regulation. Together, this work suggests that NFIs drive RGCs maturation through developmental states and highlights how redundant, but specialized transcription factors coordinate intrinsic timing mechanisms crucial for the developing neocortex.

Poster 3

Generation and Characterization of APBB2 SNP iPSC Lines to Study Glaucoma Risk 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 of African ancestry, yet research into the biological basis for these differences in susceptibility are lacking. A recent GWAS study identified a SNP in the APBB2 gene that was linked to increased risk for glaucoma uniquely within populations of African ancestry, and the discovery of this SNP provides an exciting opportunity to study why this population is at increased risk for glaucoma. To examine how a SNP at the APBB2 locus may confer increased risk for glaucoma, we leveraged CRISPR/Cas9 gene editing approaches to generate isogenic human induced pluripotent stem cell (iPSC) lines with paired control and ABPP2 variant genetic backgrounds. Each of these cell lines were capable of robust differentiation into retinal organoids, which were then used for the purification and maturation of RGCs for downstream analyses. Upon differentiation and maturation of RGCs, results indicated that those with the APBB2 SNP exhibited significantly decreased neurite complexity and increased neuronal excitability, suggestive of disease-related features. Ongoing experiments are focusing upon assessing transcriptional changes as a consequence of this APBB2 variant, as well as how this variant may alter amyloid processing. These studies represent the first known attempt to develop iPSC-based models for the study of increased risk for glaucoma due to the APBB2 genetic variant, with these results likely to elucidate important cellular and molecular aspects that uniquely lead to this increased risk for glaucoma among the African ancestry population.

Poster 4

Using Stem Cell Derived Retinal Organoids to Assess Impacts from Galactic Cosmic Radiation

Nicole Bodi | Indiana University School of Medicine

PURPOSE

Galactic cosmic radiation (GCR) is theorized to be among the stressors that leads to spaceflight-associated neuro-ocular syndrome (SANS) during long duration spaceflight. To counteract SANS, our objective is to identify mechanisms induced by simulations of radiation using induced pluripotent stem cell (iPSC) derived retinal organoids to assess efficacy of the countermeasure, Coenzyme Q10 (CoQ10). It is known that there are systemic metabolic changes in space leading to oxidative stress. Therefore, our countermeasure, CoQ10, will be evaluated for protective antioxidant properties within the retinal ganglion cells under radiation simulations.

METHODS

We derived keratocytes from donor eyes (N=3) and reprogrammed those into iPSCs, which were differentiated into retinal organoids. We separated organoids into four groups: travel control untreated, GCR untreated, travel control treated with CoQ10, and GCR treated with CoQ10. Following irradiation at Brookhaven National Laboratory, we collected our samples. RNA was extracted for qRT-PCR to determine changes between experimental groups. We analyzed oxidative, apoptotic, and retinal markers; SOD2, CASP3, BAX, RBPMS, and THY1 to assess the impact of CoQ10 post radiation. We also isolated protein lysates and performed western blot analysis where we probed for SOD2 and BAX. Finally, we collected conditioned media and ran ELISA and Luminex multiplex assays for 8OHdG, IL6, and TNF.

RESULTS

We found that there was a significant ($p<0.05$) increase in SOD2 in the GCR group that was decreased significantly ($p<0.05$) in the GCR CoQ10 treated group. There were also significant differences in 8OHdG in the CoQ10-treated groups compared to the untreated ($p<0.05$). Additionally, there were significant differences between IL6 ($p<0.05$) and TNF ($p<0.05$) within the experimental groups.

CONCLUSION

GCR simulation negatively impaired retinal organoids and increased oxidative stress warranting CoQ10 intervention. Our studies highlight that CoQ10 has the potential to provide therapeutic benefits to retinal ganglion cells and counteract SANS following GCR simulations.

Poster 5

Exploring the Effects of NF2 Mutations on Neural Stem Cell Differentiation

Noah Burket | Indiana University School of Medicine

Background: NF2-related schwannomatosis (NF2) is a tumor predisposition syndrome caused by NF2 gene mutations and characterized by the development of multiple central nervous system tumors, including spinal ependymomas (SP-EPN). Patients with SP-EPNs suffer from neurological deficits from compression of the spinal cord by the tumor, and they often present with tumors at an early age. There are currently no medical therapies for SP-EPN, and surgery remains the standard of care for this tumor. Yet, surgical resection of SP-EPNs is associated with high morbidity, especially in younger patients, like those who suffer from NF2. A previous genetic screen identified NF2 as being implicated in stalled neural stem cell differentiation. Connecting this with other studies suggesting that radial glia may be the cell-of-origin of SP-EPNs, we hypothesize that mutations in the NF2 gene may prevent normal radial glia cell (RGC) differentiation, leading to a persistent, progenitor-like population that may serve as progenitors of SP-EPN.

Methods: We engineered NF2 CRISPR knockouts in human neuroepithelial stem (NES) cells. Clones from this population were selected based on Sanger sequencing showing homozygous gene mutations. RT-qPCR and Incucyte imaging were used to assess cell phenotype and differentiation behavior. Transcriptomic studies on human SP-EPN were performed as a complementary study to our in vitro work.

Results: Our results show that NF2-mutant NES cells do not undergo normal differentiation, as they retain neural progenitor gene expression and morphology. Unlike wildtype NES cells, which differentiate into neurons and glia when growth factors are removed, NF2-mutant NES cells form clusters in vitro and can continue to be expanded even after differentiation is attempted. Transcriptomic analysis of patient SP-EPN shows that while there is abundant expression of mature ependymal cell genes within the tumor, there is also varied expression of neural progenitor genes.

Discussion: Our data supports that altered NF2 expression may cause stalled differentiation in neural progenitors in our model, and this may also be reflected in patient SP-EPN tissue. Future directions include global transcriptomic analysis of our in vitro model, as well as mechanistic studies to investigate the role of NF2 in stalled NES cell differentiation and cell cluster formation.

Poster 6

Characterizing Physiological Features of Long-Term iPSC-Derived RGC and Astrocyte Co-Cultures

Alice Yoon Kyung Cho | Indiana University School of Medicine

Human induced pluripotent stem cell (iPSC)-derived cells provide amazing opportunities for in vitro modeling of neurodegenerative diseases. However, generating electrophysiologically mature neural network from iPSCs has been challenging. Human iPSC-derived retinal ganglion cells (RGCs) and astrocytes were cultured on a planar 16-electrode multielectrode array (MEA) plates for over eight weeks. Each MEA plate was recorded for 5 minutes, and physiological parameters such as mean firing rate, inter-spike interval (ISI) coefficient of variation, and burst activity were analyzed to assess differences among culture conditions. For network-level features, functional connectivity, network topology, and network dynamics analysis were conducted.

Poster 7

Patient-Derived Intestinal Organoids Reveal Neurogenic Defects in Hirschsprung's Disease

TIANCI CHU | Cincinnati Children's Hospital

The enteric nervous system (ENS) regulates gastrointestinal function through complex networks of myenteric and submucosal plexuses. Developmental defects in the ENS cause enteric neuropathies such as Hirschsprung's disease (HD), a congenital disorder marked by distal intestinal aganglionosis and life-threatening dysmotility. Although surgical resection restores bowel continuity, many patients experience persistent symptoms, highlighting the need for patient-specific human models to study ENS development and disease mechanisms.

Methods

We generated intestinal organoids from human induced pluripotent stem cells (iPSCs) of HD patients and healthy controls using a Confined Culture System (CCS) that enables large-scale growth and spontaneous ENS development. Organoids were evaluated for ganglia formation, neuronal subtypes, and neuromuscular function following transplantation into immunodeficient rats. Human intestinal tissues resected during HD pull-through surgery were analyzed to compare pathological features with those in organoids.

Results

Control intestinal organoids developed organized enteric ganglia, diverse neuronal subtypes, and functional neuromuscular units. In contrast, HD intestinal organoids showed early and persistent defects in neuronal lineage development, failing to establish functional ENS networks. Despite neurogenic deficits, the mucosal and muscular layers remained morphologically intact. Quantitative analysis in control organoids revealed 79.28 ± 20.47 glial cells/mm and 23.80 ± 15.10 neurons/mm along the myenteric plexus, all confirmed to be of human origin. The resulting glia-to-neuron ratio of 4.14 ± 1.84 closely aligns with values reported for the human colon. HD organoids contained <1 glial cell/mm and no detectable neurons, indicating a profound disruption in ENS development. Functional evaluation using organ bath chamber demonstrated ENS-dependent contractile activity in control organoids. In contrast, HD organoids retained smooth muscle contractility but lacked ENS-mediated responses, recapitulating the aganglionic phenotype characteristic of HD.

Conclusion: Our study demonstrates intrinsic neurodevelopmental defects in HD patient-derived intestinal organoids. It establishes a physiologically relevant, patient-specific model for investigating ENS development, disease mechanisms, and potential therapeutic strategies in enteric neuropathies.

Poster 8

Pharmacologic Reprogramming of Mitochondrial Metabolism Links Human Stem Cell Differentiation to Neuroprotection in Retinal Ganglion Cells

Sayanta Dutta | Indiana University School of Medicine

Metabolic collapse with mitochondrial damage presents the early pathology for retinal ganglion cell (RGC) degeneration in optic neuropathies such as glaucoma, yet no RGC-targeted therapies exist. Here, we identify a clinically tested small molecule that reprograms mitochondrial maturation and metabolic state to both promote differentiation and protect mature human RGCs.

We performed a high-content live-cell mitochondrial screen in human pluripotent stem cell-derived RGCs (hRGCs), using the mitochondrial membrane potential reporter Mito-Tracker Deep Red (MTDR), to identify compounds that restore mitochondrial health. Healthy mitochondria maintain the membrane potential through active electron transport and proton motive force that is sensed by the MTDR probe to get inside the organelle. Hits were subsequently evaluated for neuroprotection by their ability to reduce cellular apoptosis in the differentiated hRGC culture. This led to the discovery of the 5-HT1A G protein-coupled receptor antagonist WAY-100635 (WAY). In wild-type hRGCs, WAY induced a reversible increase in cAMP via 5-HT1A engagement, followed by reversible activation of mitochondrial biogenesis through the master regulator PGC-1a. In hRGCs containing the glaucoma-associated Optineurin E50K mutation, which impair mitophagy, WAY improved mitochondrial health and conferred neuroprotection while shifting metabolism toward aerobic glycolysis, a metabolic reprogramming known to support neuronal survival in humans. Importantly, in mouse models of acute optic nerve injury and elevated intraocular pressure (a glaucoma risk factor), systemic WAY treatment robustly protected RGC axons, preserved cell bodies, and maintained functional circuitry between eye and brain. Strikingly, when applied at the progenitor stage, WAY accelerated mitochondrial maturation (increased cristae complexity) and promoted a metabolic switch toward oxidative phosphorylation (OXPHOS), thereby driving early cell-cycle exit and enhanced differentiation of RGCs. Together, our findings establish a first-in-class pharmacologic paradigm for simultaneously protecting and maturing RGCs via reversible metabolic and mitochondrial reprogramming, advantages that avoid the delivery challenges and constitutive effects of gene therapy. Given the central role of mitochondrial dysfunction across neurodegenerative disease, this approach may extend beyond glaucoma and applicable to Parkinson’s disease and ALS, facilitating translation via repurposing of a drug with established safety.

Poster 9

A Human iPSC-Derived Model to Assess Microglial Dysfunction in Neurofibromatosis Type 1

Teresa Gomez Indiana University School of Medicine

Neurofibromatosis type 1 (NF1) is a common neurogenetic disorder caused by mutations in the NF1 gene, leading to tumor formation and neurodevelopmental abnormalities. Microglia, the resident immune cells of the central nervous system, have emerged as key contributors to glioma development and disease progression in NF1. While animal studies have provided valuable insights into microglia-driven mechanisms, translating these findings into human models remains a major challenge. To overcome this, we used CRISPR/Cas9 gene editing in human induced pluripotent stem cells (iPSCs) to generate microglia-like cells (hMGLs). Using isogenic iPSC lines, we created control, heterozygous, and homozygous NF1 Arg816X mutant hMGLs to investigate the functional consequences of NF1 loss. iPSC-derived hMGLs expressed key microglial markers, including IBA-1 and TREM2, confirming their identity and successful differentiation. Further functional assays revealed that homozygous NF1 Arg816X hMGLs exhibited increased sensitivity to cytokine starvation, suggesting a heightened susceptibility to stress-induced cell death. Additionally, preliminary evidence of altered phagocytic activity was observed in NF1-mutant hMGLs compared to isogenic controls. These findings suggest that NF1 mutations may disrupt fundamental aspects of microglial biology, potentially contributing to disease pathology. This model offers a promising human-based platform for dissecting the cellular mechanisms underlying

Poster 10

Investigation of p75-RET Death Complexes in ALS

Hannah Greenland | Indiana University School of Medicine

Amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease, is a neurodegenerative disorder that causes degeneration of motor neurons, impacting the body's ability to move. This is a rapidly progressing disease, as patients die within three years after diagnosis. Most of our understanding of the etiology of ALS comes from familial ALS, with mutations present in genes including SOD1 and C9ORF72. P75 is a receptor within the tumor necrosis family that triggers apoptosis when bound to pro-forms of the neurotrophins, such as BDNF. RET is a receptor tyrosine kinase that promotes growth and survival when bound to GDNF family ligands (GFLs). Ligands in the GFL family include GDNF, neurturin, artemin, and persephin. Previous work in the lab identified the interaction of p75 and RET, and this complex can initiate either survival or death depending on which ligand causes their association.

Our hypothesis is that specific pro-neurotrophins, such as proBDNF, or another pro-apoptotic ligand, NGF, leads to the formation of the RET/p75 complex to a greater extent in motor neurons from ALS patients as compared to healthy controls. Induced pluripotent stem cells with SOD1 and C9ORF72 mutations, along with healthy control cell lines, were differentiated into motor neurons. After maturation, the cells were split into treatment groups that were treated with NGF, proBDNF, or vehicle control for 24 hours. Colocalization of p75 and RET in motor neurons was visualized via immunofluorescence, and their association was examined using co-immunoprecipitations and followed by quantitative Western blotting. The results of these experiments indicate that in ALS motor neurons, p75 and RET form a complex, potentially activating neurodegenerative pathways. We are currently investigating the result of the complex's activation on motor neuron survival in stem cell models and transgenic mice. Ultimately, this research will expand our knowledge of the role of p75/RET complexes in this disorder and could aid in the development of future potential treatments.

Poster 11

TNF-α stimulation Alters Mitochondrial Morphology in Human Astrocytes

MD NAZMUL HASAN | Purdue University

Reactive astrocytes are a key hallmark of neuroinflammation and neurodegenerative diseases. Reactive astrocytes can be induced in vitro under exposure to pro-inflammatory cytokines such as TNF-α. Astrocytes undergo dramatic morphological changes when transitioning from inactive to reactive states, with more pronounced stellate processes and increased cell motility. However, the cellular mechanisms that drive reactive transition remain poorly quantified in astrocytes. Recent advances in high-content imaging and image-based profiling offer a quantitative framework to unbiasedly capture the complex downstream phenotypes of reactive astrocytes. Here, we used Cell Painting to characterize the morphology of iPSC-derived astrocytes as they transition to a reactive state. We used the Cell Painting assay to capture morphology for several key cellular organelles (i.e., nuclei, ER, RNA, Mitochondria, Actin, Plasma membrane, and Golgi) and used a machine learning pipeline (CellProfiler) to process images and extract interpretable features, constructing a high-dimensional morphological profile. We further processed with variance thresholding, MAD normalization, rank-based inverse normal transformation, and correlation pruning ($r>0.9$) to reduce redundancy and noise. Individual feature profiles were analyzed at both the single-cell level and at mean aggregated well-level profiles to capture treatment effects across biological replicates and cell populations. Overall, we measured >4300 morphological features on 1500 hiPSC-derived astrocytes treated with TNF-α and vehicle controls. From the well-level profiles, our feature selection pipeline yielded 800 distinct features suitable for downstream analyses. At the well-level, our analysis pipeline identified significant change ($p<0.05$) in 61 morphology features enriched for mitochondrial, actin, and ER channels. We took a subset of the single-cell profiles to train a Random Forest classifier, which, upon testing on the remaining subset, achieved ~80% accuracy in distinguishing TNF-α vs control cells. Moreover, we mapped the contribution of feature channels to the model's performance, which corroborates our previous observation that the features relating to the mitochondria are mostly affected due to TNF-α treatment. Finally, our analysis identified several features with measurements indicative of reduced mitochondrial fission, suggesting that TNF-α stimulation alters mitochondrial morphology in human astrocytes. Altogether, our results demonstrate that TNF-α induces mitochondrial phenotypic changes in human astrocytes.

Poster 12

Doxycycline-Inducible Cyclin D2 Overexpression to Increase Proliferation in HPSC-Derived Hemogenic Endothelial Cells

Katherine Hebert | Purdue University

Cyclin D2 (CCND2) facilitates passage through the cell cycle checkpoint from G1 to S phase, driving cells towards a proliferative state. While various differentiation techniques have been developed to produce hemogenic endothelial cells from human pluripotent stem cells, the variability in differentiation efficiency can result in a relatively low yield. The generation of a doxycycline-inducible system increasing CCND2 transcription provides the ability to tightly control the proliferation of cell populations throughout differentiation. By inducing the overexpression of CCND2 through the addition of doxycycline during specific time windows during differentiation, the yield of hemogenic endothelial cells may be significantly increased. Furthermore, addition of doxycycline following purification of hemogenic endothelial cells may more rapidly expand this population for use in follow-on hematopoietic differentiation, as well as in disease modelling and potential clinical applications. A more robust hemogenic endothelium may lead to higher differentiation efficiency of hematopoietic stem cells, in turn increasing production of mature granulocytes, such as neutrophils. Endothelial progenitors produced can also be directed to differentiate into endothelial cells which form blood vessels in the process of angiogenesis, suggesting a possible avenue for ischemia treatment. Understanding the balance between proliferation and directed differentiation can help improve the consistency and reproducibility of stem cell differentiation, with the goal of optimizing yield without compromising cell fate or function. The focus on the hemogenic endothelial stage of development aims to improve this understanding while also exploring these cells' potential in disease modelling and treatment applications.

Poster 13

Establishing an In Vitro Model of Eye-to-Brain Connectivity Using Human Pluripotent Stem Cells to Study Optic Nerve Injury

Shelby Hetzer | Indiana University School of Medicine

Glaucoma and other optic neuropathies affect millions of people each year, leading to progressive visual loss 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 (RGCs). There is currently no known method to completely regenerate these long 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 connectivity. Thus, we aim to create a functional eye-to-brain model on a chip using human pluripotent stem cells (hPSCs). hPSCs were directed to differentiate into RGCs, thalamic neurons, or glia, and the orientation of these cells along the optic pathway was established in a microfluidic-based system. This 3-chamber system allows the study of each cell type and its interactions with RGCs in a compartmentalized nature. For example, oligodendrocytes were grown only along RGC axons, while thalamic cells were grown only at RGC terminals to encourage synaptic contacts as opposed to using a traditional coverslip-based co-culture. We show that when RGCs are grown in the presence of other cell types, RGC outgrowth and complexity are altered. Additionally, we show that we can model optic nerve injury using the microtubule destabilizer, Colchicine, which we aim to use moving forward as a model of optic neuropathy. These results represent the first application of hPSC-derived RGCs in a manner that effectively recapitulates their highly compartmentalized properties, as well as the use of microfluidic platforms to model eye-to-brain connectivity in a dish. Taken together, these results should profoundly impact future studies, providing a much more physiologically relevant in vitro model for the development and degeneration of the optic pathway.

Poster 14

Modeling Cardiac Neural Crest Contribution to Heart Development Using Human Pluripotent Stem Cells

Eri Iwasawa | Cincinnati Children's Hospital Medical Center

Congenital heart defects (CHD) are the most common birth anomalies and frequently involve malformations of the outflow tract (OFT) and semilunar valves. These structures depend on contributions from a transient, multipotent cell population known as cardiac neural crest cells (CaNCCs), which give rise to vascular smooth muscle cells (SMCs), cardiac ganglia, and valve components. Recent advances in human stem cell–derived heart organoids ("cardioids") have enabled modeling of early cardiac morphogenesis, including chamber formation and cardiomyocyte differentiation. However, these systems lack key features of later-stage development, including the OFT and valve structures that are the primary sites of pathology in CHD. This limitation stems, in part, from the absence of CaNCC-derived lineages, which are not included in cardioid models. Despite the importance of CaNCCs, no established protocols exist for generating human versions or their derivatives, limiting our ability to model their developmental contribution or identify disease mechanisms. To address this, we developed a directed differentiation protocol to generate axially patterned CaNCCs from human pluripotent stem cells (hPSCs). By optimizing BMP, WNT, and retinoic acid signaling, we obtained NCCs with a transcriptional profile enriched for cardiac-related ontologies such as heart and OFT morphogenesis, as well as robust expression of a CaNCC-enriched gene TBX2 along with canonical NCC signature genes including TFAP2A, SOX10, and PAX3. To evaluate their migratory capacity, we transplanted these cells in ovo at the mid-otic to somite 3 level, which showed preferential migration toward the heart compared to cranial and vagal NCCs.

In vitro, CaNCC can be differentiated into PHOX2B+ and CHAT+ parasympathetic neurons, resembling post-ganglionic cardiac neurons, and TAGLN+ mature SMCs resembling the inner OFT layer, consistent with lineage contributions observed from mouse. Excitingly, when co-cultured with cardiomyocyte-rich cardioids, CaNCCs enhanced mesenchymal cell recruitment and promoted maturation of SMCs, partially modeling OFT morphogenesis. In summary, this study establishes a scalable platform for generating human CaNCCs. This approach enables future studies modeling CHD using patient specific iPSCs to dissect cell-intrinsic and extrinsic mechanisms driving neural crest-associated disease pathogenesis.

Poster 15

Chronic non-cytotoxic manganese exposure disrupts glutamate homeostasis and pathways involved in the integrated stress response in Alzheimer's disease

Hyunjin Kim | Purdue University

Alzheimer's disease (AD) is a chronic multifactorial neurodegenerative disorder. Most AD cases involve contributions from both genetic and environmental risk factors. Manganese (Mn) is an essential metal widespread in the environment that in excess can cause neurotoxicity. Acute exposure to high Mn can inhibit extracellular glutamate uptake and trigger the integrated stress response (ISR), both of which are molecular pathologies implicated in AD. Additional mechanisms of Mn neurotoxicity in AD etiology remain unclear. We hypothesized that chronic exposure to environmentally relevant Mn levels drives dysregulated ISR that manifests as transcriptional/functional maladaptation. Further, we propose that severity of response to Mn is dictated by individual genetic risk to AD. To address our hypothesis, we utilize cortical neurons and astrocytes from induced pluripotent stem cells differentiated from neurotypical control and AD patients. Cells were cultured for ≈100 days and subsequently exposed to Mn (vehicle, 0.5, or 5.0μM) for 4-6 weeks. Net glutamate uptake was quantified using 14C-glutamate. We observed a significant decrease in glutamate uptake only in AD neuron-astrocyte co-cultures. Gene expression and immunocytochemical analyses indicated absence of astrocyte reactivity, suggesting impaired glutamate uptake is likely a direct Mn effect rather than an epiphenomenon caused by neuroinflammation. scRNA-sequencing and bioinformatic analyses identified alterations in pathways associated with AD and Mn neurotoxicity. These included pathways involved in energy metabolism, glutamate neurotransmission, and ISR-related pathways such as EIF2 signaling. Consistently, gene ontology analysis revealed enrichment of terms associated with mRNA translation, ribosome homeostasis, and cellular energy, suggesting dysregulated ISR. In summary, we provide an initial framework for Mn x AD interaction model wherein chronic Mn exposure leads to differential ISR activation and transcriptional/functional maladaptation as a function of AD genetic risk.

Poster 16

Impact of Human Neuritin 1 on Glaucomatous Human Retinal Ganglion Cells Seeded on Fibrin Scaffolds

Ksenia Lewyckyj | Indiana University School of Medicine

Ksenia N. Lewyckyj1, Shahna S. Hameed1, Tasneem P. Sharma,1,2*

1Department of Ophthalmology, 2 Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indiana University

Purpose: Glaucoma is a group of optic neuropathies characterized by progressive loss of retinal ganglion cells (RGC) and corresponding visual field deficits. Current treatments can only slow the progression of glaucoma, but RGC death is not preventable and irreversible. Thus, neurotrophic factor therapy may be a suitable therapeutic approach. Human Neuritin 1 (NRN1) has demonstrated neurodegenerative and neuroprotective properties, and our group has previously established that NRN1 is downregulated in glaucomatous RGCs. We will investigate the therapeutic potential of NRN1 on human RGCs using a 3D fibrin-based scaffold model.

Methods: Corneal fibroblasts from glaucomatous and non-glaucomatous post-humous donor tissue were reprogrammed into induced pluripotent stem cells (iPSCs) using the CytoTuneTM-iPSC 2.0 Sendai reprogramming kit. These iPSCs were characterized via PCR (C-MYC, KLF4, SOX2, NANOG) and immunofluorescence staining (TRA-1-60, SOX2) and karyotyped. The iPSCs were differentiated into three-dimensional retinal organoids (ROs) from which RGCs were dissociated. PCR (RBPMS, THY1) and immunofluorescence staining (RBPMS, BRN3A, DAPI) were used to characterize RGCs. A fibrin scaffold was created via simultaneous administration of fibrin gel and crosslinking solution. RGCs were then seeded onto the fibrin scaffold to create a 3D microenvironment. Cultures were treated with or without recombinant NRN1. RGC apoptosis and neurite outgrowth were assessed by immunostaining for CASP3 and NEFL.

Results: NRN1 treatment enhanced neurite outgrowth and reduced RGC apoptosis in NRN1 treated RGCs compared to untreated cells.

Conclusions: These findings confirm that NRN1 enhances neurite outgrowth and RGC survival in human glaucomatous cells cultured in a biomimetic fibrin scaffold, further demonstrating its potential as a candidate for glaucoma therapy. Furthermore, they highlight the utility of 3D models for translational neuroregenerative research.

Poster 17

Hematopoietic Stem Cell Characteristics as a Potential Biomarker for Diabetic Retinopathy

Neha Mahajan | Indiana University School of Medicine

Purpose: Diabetic retinopathy (DR) is a major cause of blindness globally and often remains undetected until advanced stages. This highlights the need for new, easily assessable biomarkers. Our study focuses on hematopoietic stem cells (HSCs), which play a crucial role in DR's pathophysiology and can be easily isolated from blood, making them an ideal choice for biomarker research.

Methods: We recruited individuals with 1) no diabetes 2) diabetes, and 3) varying severity of DR, including mild, moderate, and severe non-proliferative DR (NPDR), and proliferative DR (PDR). HSCs (lin-CD34+CD45mid cells) were purified from the blood samples using flow cytometry. Cell surface inflammatory markers (TLR2, TLR4, TLR8), monocyte and macrophage markers (CD11c and CD14), and HSCs markers (lin-CD34+CD45mid) were analyzed. The HSCs were sequenced for miRNA and mRNA, and the data were analyzed using Ingenuity Pathway Analysis (IPA) software. Finally, we will be using machine learning tools to find a biomarker utilizing the big data from physiological parameters, flow cytometric surface expression and HSC's mRNA and miRNA sequencing.

Results: HSC levels remained unchanged across different stages of DR; however, the number of inflammatory macrophages (CD14+ cells) increased in diabetes and PDR. Moreover, lin-CD34+CD45midTLR4+ cells significantly increased in the PDR stage, suggesting an inflammatory phenotypic shift in HSCs as DR progresses from NPDR to a severe form of PDR. miRNA sequencing identified 13 significant miRNAs that target 209 mRNAs. Further gene ontology analysis linked these mRNAs to extracellular matrix and collagen fibril organization, HSC differentiation and migration, as well as inflammation-associated pathways. Interestingly, mRNA sequencing data identified TGFBI, a gene involved in cell adhesion and migration, was significantly elevated in diabetes (169-fold change (FC)) but decreased from NPDR (55 FC) to PDR (11FC), reflecting early genotypic changes in the HSCs. Our big data analysis using SAS Viya identifies TMEM126, FBXO3 and GADD45A as top variables associated with DR.

Conclusion: Our findings highlight a physiological shift towards inflammation in HSCs, which could serve as a potential biomarker for early diagnosis of DR. Additional longitudinal and confirmatory studies on the miRNAs and their targets are necessary to strengthen the current findings.

Poster 18

Glioblastoma Stem Cells Evade NK Cell Killing Through Downregulation of NKG2D Ligands

Jensen Mast IU-Bloomington

Glioblastoma (GBM) is the most aggressive primary brain tumor, characterized by inevitable recurrence and poor patient outcomes. GBM stem cells (GSCs) are key drivers of tumor progression, therapeutic resistance, and immune evasion. To thrive within the tumor microenvironment, GSCs must sustain their stem-like properties while escaping immune surveillance, including cytotoxic natural killer (NK) cell activity. While prior studies have largely focused on stemness-associated pathways, the mechanisms by which GSCs evade NK cell-mediated killing remain largely unexplored. Here, we assessed the susceptibility of GSCs and their non-stem GBM cell (NSGC) counterparts to NK cell cytotoxicity. Patient-derived GSC lines were differentiated into NSGCs via serum treatment. In co-culture assays with NK-92 cells, a human NK cell line, NSGCs were efficiently killed, whereas GSCs remained resistant. To investigate the molecular basis of this differential sensitivity, we profiled the expression of NKG2D ligands, which are activating molecules on tumor cells recognized by the NKG2D receptor on NK cells. GSCs exhibited markedly reduced expression of NKG2D ligands such as MICA, MICB, and ULBP1-3 compared to their differentiated NSGC counterparts. Functional relevance was confirmed by blocking NKG2D signaling with a neutralizing antibody, which partially rescued NSGCs from NK-92-mediated killing. Transcriptomic analysis of three matched GSC/NSGC pairs (MGG4, MGG6, MGG8) from the public dataset (GSE54791) validated the downregulation of NKG2D ligands in GSCs. Additionally, RNA-seq data from 44 GSC models and 9 normal brain cell lines (GSE119834) revealed that NKG2D ligand expression in GSCs was comparable to that in normal brain cells, underscoring a distinct NK cell evasion strategy. Collectively, these findings demonstrate that GSCs are intrinsically more resistant to NK cell surveillance, at least in part due to downregulation of NKG2D ligands. Therapeutic strategies that restore NKG2D ligand expression may sensitize GSCs to NK cell-mediated clearance and enhance the efficacy of NK-based immunotherapies for GBM.

Poster 19

Engineering a Psc-Derived Hepatobiliary-Gallbladder Model by Dictating Left-Right Asymmetric Organogenesis

Yuka Milton | Cincinnati Children's Hospital

Hepatobiliary (HB) development is a tightly regulated process likely dependent on precise left-right (LR) mesenchymal patterning around the e9.5 liver bud to spatially coordinate hepatic and biliary morphogenesis. Recent studies suggest that the LR mesenchyme surrounding the HB niche is both mechanically and transcriptionally heterogeneous. For example, the left mesenchyme, enriched in Pitx2hi hepatic stroma, exhibits softer mechanical properties compared to the right, yet the mechanotransduction pathways translating these cues to coordinate HB development remain unclear. We hypothesize that biomechanical signaling from this hepatic mesenchymal niche triggers a migratory and delaminating phenotype in liver progenitor cells to support expansion from the left liver bud while the biliary system invaginates on the right. To test this, we investigated the molecular and cellular roles of two HB-associated Pitx2hi and Pitx2lo mesenchymal populations identified through single-cell RNA sequencing and shown to orient in a LR pattern around mouse liver buds by in situ hybridization. Gene set enrichment analysis between these populations revealed >3-fold enrichment of mechanoresponsive genes, including Periostin (Postn), within the hepatic mesenchyme and POSTN inhibition by neutralizing antibodies disrupted left liver bud cell migration implicating its role in liver morphogenesis. To assess mechanical contributions of mesenchymal LR patterning in vitro, we developed a microfluidic perfusion device delivering controlled medium on the hemisphere of HB organoids containing naïve mesenchyme. Perfusion induced a significant stiffness differential, softening one domain relative to the contralateral side as measured by atomic force microscopy. Furthermore, perfusion increased identified liver specific mesenchyme markers (e.g. Msx1) and left-specific marker Pitx2 by 1.8-fold compared to static culture reinforcing leftward mesenchymal patterning. Perfusion was also sufficient to trigger Prox1-mediated liver progenitor migration, doubling the production of delaminating progenitors with morphologies resembling e10.0 liver buds, an effect inhibited by POSTN-neutralizing antibody treatment. In contrast, on the opposite side of HB organoids, emerging Pdx1-expressing biliary/pancreatic progenitors displayed invaginating structures. Together, these findings reveal a novel biomechanical role for controlling mesenchymal LR patterning to prioritize liver morphogenesis over biliary development and advance in vitro modeling of early liver development by incorporating axial patterning, offering a powerful platform to explore congenital liver malformations and regenerative therapies.

Poster 20

Persistent neurotoxic effects of developmental methylmercury (MeHg) exposure causes alterations in proteostasis pathway of autophagy

Ribhav Mishra | Purdue University

Neurotoxic effects of methylmercury (MeHg) in the human brain are often linked with its chronic or acute exposure to the human population. Interestingly, we observed that neurotoxic effects of MeHg persist to later timepoints even when the exposure of MeHg is limited to early neurodevelopmental stage of cells. To explore these persistent effects at the protein level, we used neurotypical human induced pluripotent stem (hiPSC) cells and differentiated them to a cortical neuron lineage using dual SMAD inhibition. The differentiated hiPSCs were exposed during developmental phase Days 7-12 (D7-12) to an environmentally relevant MeHg (100nM) concentration. We let these cells differentiate and divide until we observed a high number of immature neurons in the culture by D43 when cells were collected to quantify protein alterations using global proteomics. Analysis of significantly altered protein shows a significant enrichment of proteosasis mechanism of autophagy, and its linked pathways e.g. mitophagy, protein ubiquitination /deubiquitination, and mTOR. Hence, we hypothesize that disturbance in autophagy is the major proteostasis mechanism which contributes to the neurotoxic effects of MeHg. To examine molecular level changes in autophagy following developmental MeHg exposure (D7-12), we performed immunofluorescence (IF) image analysis for critical autophagy marker proteins (p62, LC3 and Ub) on hiPSC cortical cells at D43 (31 days after cessation of exposure). Our findings suggest upregulation of p62 and LC3 expression in the hiPSC-derived cortical cells indicating that autophagy-based degradation is compromised as p62 itself is degraded by autophagy and a simultaneous LC3 increase suggests a decreased autophagosome turnover. We further confirmed changes in autophagosome turnover with IF images by detecting alterations in autophagic flux at D43 with the use of the autophagy inhibitor bafilomycin. In IF images, we observed a significant accumulation of autophagy marker proteins (p62, LC3) in forms of puncta in MeHg-exposed cells suggesting that autophagosomes are formed but not degraded at the same rate. Overall, our analysis indicate that developmental MeHg exposure makes cells prone to autophagic dysfunctions and hence can cause them to become more susceptible to developing autophagy-associated neurodegenerative disorders in later life-phase

Poster 21

Neuritin1 Drives Survival of Retinal Ganglion Cells via MEK/ERK, PI3K, and mTOR Pathways

Kai Mongan | Indiana University School of Medicine

Purpose: Glaucoma, a progressive neurodegenerative disease, has traditionally been treated by regulating a patient's intraocular pressure (IOP), yet in many cases, the pathologic deterioration of retinal ganglion cells (RGCs) continues despite lowering IOP. A known contributing factor to the pathological loss of RGCs is the deprivation of neurotrophic factors, making neuroprotective treatment a potential therapeutic strategy. Neuritin1 (NRN1) is a neuroprotective protein, and our prior studies have demonstrated that secreted NRN1 protects RGCs following axonal injury in acute glaucoma rodent models and ex vivo human pressurized eyes. We aim to evaluate NRN1's effects on gene expression in derived RGCs from glaucomatous and non-glaucomatous human donors.

Methods: Human induced pluripotent stem cells (iPSCs) from non-glaucomatous and glaucomatous donors (N=3) were differentiated into retinal organoids (ROs) and RGCs. Protein and gene expression assays characterized hiPSCs (TRA-1-60, OCT4, SOX2, NANOG, C-MYC), RGCs (RBPMS, CD90), and apoptosis (CC3). The hiPSC-derived RGCs were incubated in Neurobasal media for seven days, then separated into NRN1 and non-NRN1 treatment groups. The NRN1 group was incubated with exogenous NRN1 in Neurobasal media for three days, while the non-NRN1 group was incubated with Neurobasal media alone for three days. Both the NRN1 and non-NRN1 treatment groups were then treated with a MEK/ERK, PI3K, and mTOR inhibitor for four days. The NRN1 and non-NRN1 groups without inhibitor treatment functioned as positive and negative controls, respectively. We then performed RNA extraction, RNA reverse transcription to cDNA, followed by qPCR to evaluate RGC gene expression.

Results: Human iPSCs of non-glaucomatous and glaucomatous donors were successfully characterized and differentiated into ROs and RGCs. The NRN1 and non-NRN1 RGC groups treated with inhibitors of the MEK/ERK, PI3K, and mTOR cellular signaling pathways successfully revealed expression of apoptotic and pro-inflammatory genes.

Conclusions: Our study confirmed that NRN1-treated RGCs, when exposed to pro-survival pathway inhibitors, expressed apoptotic and pro-inflammatory genes, emphasizing that NRN1 drives the survival of RGCs in glaucoma.

Keywords: Neuritin1, Glaucoma, Neuroprotection, Retinal Ganglion Cells

Poster 22

Spatial Multi-Omics Reveals Mutation Class–Specific Neurodevelopmental Perturbations in 8p Syndrome

Basil Obodo | Purdue University

Chromosome 8p is highly susceptible to structural variation, and individuals with 8p syndrome present with a diverse spectrum of clinical phenotypes, including developmental delay, intellectual disability, autism spectrum disorder, and epilepsy. Despite its clinical relevance, the underlying biological mechanisms remain poorly understood, particularly regarding how different types of 8p mutations, such as deletions (Del(8p)) and inverted duplication deletion (Invdupdel(8p)), contribute to divergent outcomes. To address this gap, we leveraged the AVITI24 platform, a spatially resolved multi-omic platform that integrates high-dimensional morphology, targeted transcriptomics, and multiplex protein profiling. This approach enables the simultaneous measurement of RNA, protein, and cellular architecture within the same neural tissue context. We applied AVITI24 to iPSC-derived neuronal progenitor cells from individuals harboring diverse 8p rearrangements as well as matched familial controls. We found that Del(8p) was associated with reduced expression of genes involved in synaptic organization and neuronal differentiation, while Invdupdel(8p) disrupted the spatial coherence of protein–RNA localization patterns in developing neural rosettes. Morphological profiling revealed shared disruptions in cytoskeleton organization across both Del(8p) and Invdupdel(8p) carriers, alongside mutation-specific signatures: degraded Golgi architecture in Del(8p) and altered nuclear morphology in Invdupdel(8p). Together, these results support the hypothesis that different classes of 8p structural variation produce non-overlapping yet convergent disruptions of neurodevelopmental programs, demonstrating the power of multi-omic profiling for uncovering new disease biology.

Poster 23

Utilization of Human Induced Pluripotent Stem Cells to Model Retinal Development in vitro

Kevin Quist | Indiana University School of Medicine

Optic nerve pathologies have a myriad of causes but often result in neurodegeneration of the retinal ganglion cells (RGCs) which project from the eye to the brain's visual centers. Whether triggered by inflammation, genetic mutation, or cellular dysregulation, these conditions can result in progressive RGC loss and blindness. Successful treatment depends on the underlying cause of disease, time of intervention, and extent of neuronal damage. Therefore, understanding the early stages of neurodegeneration is essential to prevent worsening outcomes. Induced pluripotent stem cells (iPSCs) offer a powerful platform to model and study retinal and optic pathway disease. In this study, iPSCs engineered to express mNeonGreen and Thy 1.2 under control of the RGC-enriched BRN3B promoter were directed to differentiate into mature RGCs, passing through neural and retinal progenitor stages. Each stage of retinogenesis was validated by immunostaining. Prior to differentiation, stem cells were plated onto coverslips, fixed, and immunostained for known markers of pluripotency, OCT4 and TRA-1-81. The retinal differentiation process was initiated by directing iPSCs toward an anterior neural fate. Eleven days after neural induction began, cellular aggregates were fixed and immunostained for markers of primitive neural anterior epithelium, PAX6 and SOX2. Retinogenesis continued with the formation of optic vesicles and an emerging optic cup. At 25 and 40 days, organoids were isolated, fixed, and sectioned before staining for CHX10 and ISL1, markers of retinal progenitors and mature RGCs, respectively. mNeonGreen visualization served to cross validate ISL1 expression. Finally, RGCs were purified from retinal organoids and cultured
for four weeks. Cells were fixed and immunostained for MAP2 at two days, two weeks, and four weeks after plating to visualize neurite complexity. This work presents a powerful model for retinal development and effective tool for producing mature, human RGCs that can be utilized for understanding diseases of the optic nerve.

Poster 24

Rapid generation of dual iTCR-CAR iNKT cells from hPSCs for potent cancer immunotherapy

Jingqiao Shen | Purdue University

Invariant natural killer T (iNKT) cells represent a rare subset of T lymphocytes characterized by a semi-invariant T cell receptor (TCR) that recognizes glycolipid antigens presented by the monomorphic CD1d molecule. Compared with conventional T cells, iNKT cells have displayed potent anti-tumor efficacy against different cancers through various mechanisms in preclinical animal and human clinical studies, and more importantly, they do not induce graft-versus-host disease (GvHD). However, the extremely low frequency (0.01-0.1%) of iNKT cells in human peripheral blood has hindered their clinical translation, creating an urgent demand for scalable, renewable sources. Given to their unlimited self-renewal and pluripotency to become any human somatic cells, this project proposes a human pluripotent stem cell (hPSC)-based platform via forced expression of an invariant TCR (Vα24-Jα18-Vβ11) by Cas9-mediated gene knockin, leading to a rapid 30-day generation of homogenous, potent iNKT cells with a uniform glycolipid recognition. We hypothesize that engineering hPSCs with a dual TCR and chimeric antigen receptor (CAR) could further enhance their anti-tumor cytotoxicity. In summary, genetic engineering of hPSCs offers a feasible strategy for the scalable and reproducible production of functional dual TCR and CAR iNKT cells, rendering hPSC-derived CAR iNKT cells a genetically stable and expandable source for cancer immunotherapy.