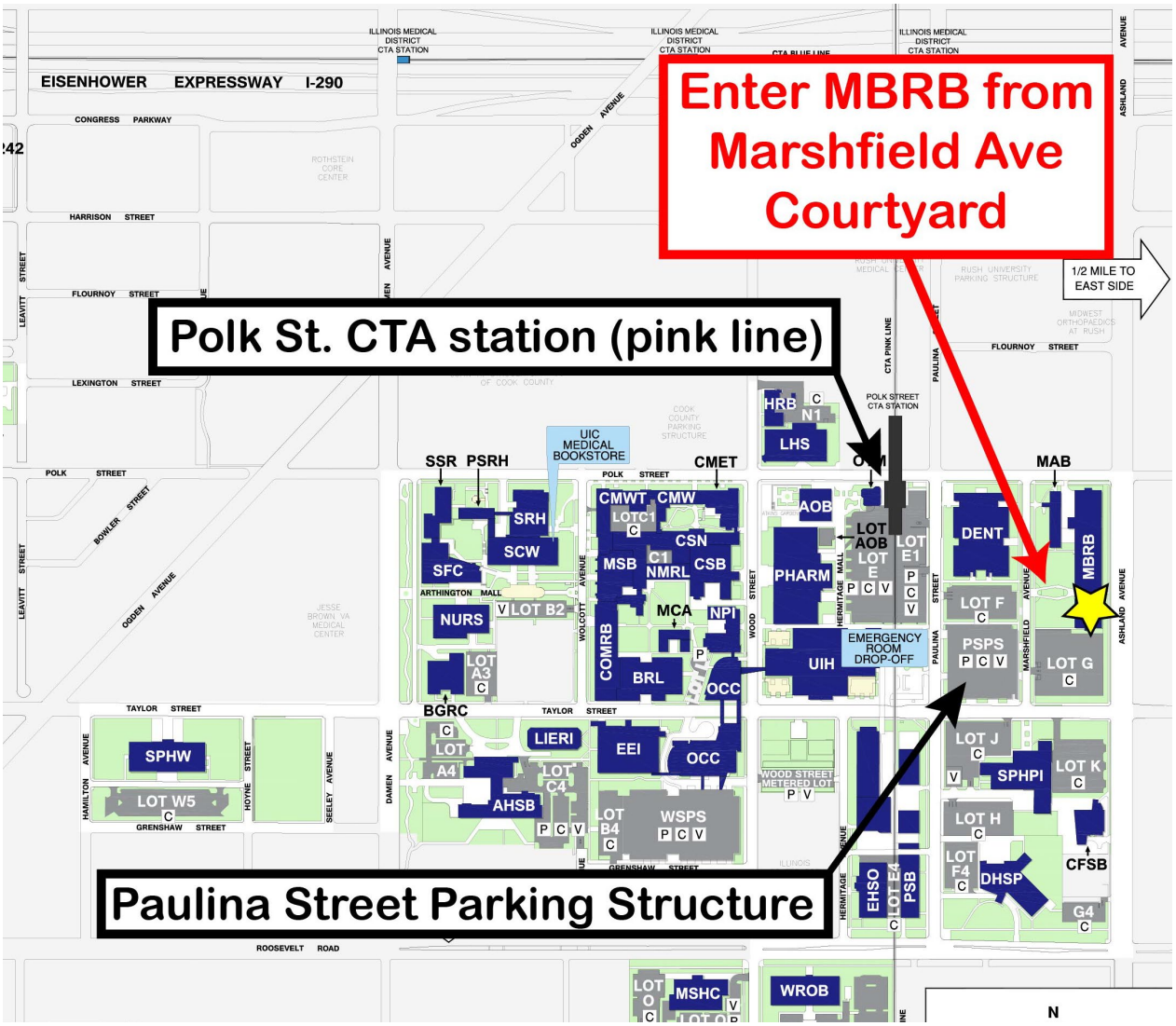
Chicago Neurovascular Meeting

Thursday, February 13th, 2020

THE UNIVERSITY OF ILLINOIS AT CHICAGO

Direction and Connection

Molecular Biology Research Building (MBRB)
University of Illinois at Chicago
900 S. Ashland (enter on Marshfield Avenue)



Wi-Fi

Connect to: UIC-Guest
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8:00 AM **Registration and Poster Setup**

8:45 AM **Introduction and Opening Comments**

9:00 AM **Neurovascular Fundamentals**

9:00 am. Sepideh Amin-Hanjani, MD, UIC. "*Vertebrobasilar Flow Evaluation and Risk of Transient Ischemic Attack and Stroke (VERiTAS) Study*"

9:30 am. Henar Cuervo, PhD, UIC. "*Notch signaling in pericytes*"

10:00 am. Joseph Geraghty, UIC. "*Hemin-induced activation of microglial phagocytosis and therapeutic targeting using the TLR4 antagonist TAK-242*"

10:15 am. Quinn Lee, UIC. "*N-cadherin adhesion induces the assembly of occludin tight junctions to maintain the BBB*"

10:30 AM **Coffee break**

11:00 AM **Neurovascular Inflammation**

11:00 am. Martin Hsu, University of Wisconsin Madison. "*Neuroinflammation dynamically regulates CNS lymphatic vasculature*"

11:25 am. Kelly Langert, PhD, Loyola University. "*Targeted Drug Delivery to the Inflamed Peripheral Nerve Endothelium*"

11:50 am. Sarah Lutz, PhD, UIC. "*Transcellular & paracellular BBB permeability in multiple sclerosis*"

12:15 pm. Ayush Batra, MD. Northwestern University. "*Intravital high resolution imaging of neuroinflammation in acute stroke*"

12:35 PM **Lunch and Poster Competition**

2:00 PM **Neurovascular Degeneration**

2:00 pm. Melissa Lamar, PhD, Rush University. "*Cerebrovascular disease phenotypes and associated cognition profiles: A clinical-neuropathological study*"

2:30 pm. Leon Tai, PhD, UIC. "*The role of APOE genotype in modulating cerebrovascular function*"

2:50 pm. Kejia Cai, PhD, UIC. "*The Development of Advanced Neurovascular MRI for Preclinical Research*"

3:15 pm. Rachel Knopp, UIC. "*Nonselective calpain-cathepsin inhibition preserves BBB-dysfunction in an oxidative-stress based TBI model*"

3:30 PM **Coffee break**

4:00 PM **Current Topics**

4:00 pm. Kari Bonner, Advanced Cell Diagnostics. "*Confirmation and spatial mapping of diverse gene signatures by incorporation of multiplex in situ hybridization technology into single cell RNA sequencing workflows*"

4:15 pm. Qanber Raza, UIC. "*Notch signaling based screen identifies axon guidance receptor Unc5b as a genetic modulator of endothelial cell migration and cell-cell adhesion*"

4:30 pm. Joseph Leasure, UIC. "*Vascularization of human iPSC-derived Cerebral Organoids*"

4:45 pm. Aashutosh Shetti, UIC. "*Depletion of Caveolin-1 in Type-2 Diabetes Model Induces Alzheimer's disease Pathology*"

5:00 PM **Closing Comments**

Neurovascular Fundamentals

Vertebrobasilar Flow Evaluation and Risk of Transient Ischemic Attack and Stroke (VERiTAS) Study

Sepideh Amin-Hanjani, MD FAANS FACS FAHA,
Professor, Residency Program Director, and Co-Director of Neurovascular Surgery, Department of Neurosurgery, University of Illinois at Chicago

Notch signaling in pericytes

Henar Cuervo
Department of Physiology and Biophysics. University of Illinois at Chicago.

Pericytes, mural cells of microvascular capillaries, are regulators of vascular morphogenesis and maintenance. They are closely associated with the endothelium and function as mediators of angiogenesis and blood-brain barrier integrity. Their absence or dysfunction is implicated in several vascular human pathologies such as diabetic retinopathy. Notch signaling regulates vascular morphogenesis and remodeling. In contrast to endothelial cells, in which Notch signaling has been studied extensively, current understanding of the physiological contributions of the Notch pathway in pericytes is limited. Our findings show how absence of Notch-dependent input in perivascular cells results in pericyte dysfunction and changes in blood vessel caliber and functionality.

Hemin-induced activation of microglial phagocytosis and therapeutic targeting using the TLR4 antagonist TAK-242

Joseph Geraghty, Milen Spegar, Jeffrey Loeb, Fernando Testai.
Neurology & Rehabilitation, University of Illinois at Chicago

Subarachnoid hemorrhage (SAH) is a devastating neurological injury resulting from the rupture of cerebral aneurysms. The first 24-72 hours after aneurysm rupture is a critical window during which the degree of early brain injury likely sets the stage for delayed and long-term outcomes. One principal mechanism of early brain injury involves a robust inflammatory response initially generated by microglia. We hypothesize that blood products such as hemin released from degrading erythrocytes trigger this inflammatory response We developed an in vitro system to study the effects of hemin on primary microglia which were exposed to hemin with and without the presence of the Toll-like receptor-4 (TLR4) antagonist TAK-242. We conducted a phagocytosis assay using fluorescent latex beads. Compared to neurons, microglia showed minimal cell death. However, exposure to 40uM hemin resulted in a significant increase in phagocytosis (32.2% increase, p=0.0286). Hemin-treated microglia contained a higher number of beads per cell (6.5 compared to 1.1, p=0.0323). Co-treatment with TAK-242 reduced microglial phagocytic activity in response to hemin, although this remains elevated compared to controls. Hemin and other blood products released into the subarachnoid space following aneurysm rupture therefore can act as damage-associated molecular patterns to trigger inflammatory responses in the brain. Future studies will use the endovascular perforation model of SAH and treatment with TAK-242. This work may offer new mechanistic insight and therapeutic strategies focusing on the role of immune responses after SAH.

N-cadherin adhesion induces the assembly of occludin tight junctions to maintain the blood-brain barrier

Quinn Lee, Kevin Kruse, Shuangping Zhao, Felecia Marottoli, Riya Thomas, Leon Tai, and Yulia Komarova.
Department of Pharmacology, University of Illinois at Chicago

The blood-brain barrier (BBB) is a tight monolayer of brain endothelial cells (BECs) that interact with surrounding pericytes to restrict the exchange of proteins and extracellular fluids in brain tissue. Although BEC-pericyte interactions are critical for maintaining the barrier function of the BBB, the underlying mechanisms are unclear. Both BECs and pericytes express Neural (N)-cadherin, a transmembrane protein that forms heterotypic adhesions between these cells. Utilizing an inducible deletion of N-cadherin gene (Cdh2) under endothelial-specific (end-SCL-Cre-ERT2) or pericyte-specific (Pdgfrβ-CreERT2) Cre-Lox systems, we have investigated the role of N-cadherin juxtacrine signaling in regulating BBB integrity. Our data indicate that loss of N-cadherin results in a size-dependent increase in BBB permeability without changes in pericyte or vessel coverage. This change in permeability is associated with a significantly reduced accumulation of occludin, but not claudin-1 or claudin-5, at tight junctions (TJs). Furthermore, this breakdown of the BBB correlates with a deficit in spatial memory, as determined by Morris water maze. Whereas both control and experimental mice with an inducible deletion of Cdh2 gene exhibited normal learning ability, the experimental mice demonstrated a decline in short-term memory from as early as two weeks after Cre induction. These data suggest that N-cadherin juxtacrine signaling in BECs establishes the BBB permeability set-point and supports cognitive function through the assembly of occludin junctions.

Neurovascular inflammation

CNS lymphangiogenesis regulates fluid homeostasis and leukocyte drainage during neuroinflammation

Martin Hsu, Yun Hwa Choi, Collin Laaker, Matyas Sandor, Zsuzsanna Fabry.
University of Wisconsin

Recent reports have described meningeal lymphatic vessels residing in the dural layer surrounding the dorsal and basal regions of the brain as well as the spinal cord. While all three regions are able to uptake CSF macromolecules, it is unknown how cells and fluid from the CSF-filled subarachnoid space gain access through the blood-CSF arachnoid barrier and into dural lymphatics. In this study, we expand on our previous findings demonstrating the capability of neuroinflammation-induced cribriform plate lymphangiogenic endothelial cells (cpLECs) in draining CSF and leukocytes. These lymphatics reside in an optimal location for CSF drainage due to gaps in the arachnoid epithelial layer separating the dura from the subarachnoid space, correlating both with CSF accumulation and AQP-1 expression near the cribriform plate during neuroinflammation. This is in contrast to other lymphatics residing in the dural layer dorsal and basal to the brain, which are separated from CSF by a complete and uninterrupted arachnoid layer. Additionally, we show lymphangiogenic cribriform plate lymphatic vessels alter their phenotype to increase dendritic cell/T cell binding and may functionally induce tolerance during neuroinflammation. These data implicate cribriform plate lymphatic vessels as dynamic structures that are able to regulate adaptive immunity.

Targeted Drug Delivery to the Inflamed Peripheral Nerve Endothelium

Kelly Langert, Maleen Cabe, Eric Brey.
Department of Molecular Pharmacology and Neuroscience, Loyola University Chicago, Stritch School of Medicine

Despite advances in delivery of therapeutics to the peripheral nerve, targeted delivery for treatment of diseases of the peripheral nervous system remains elusive. We have recently demonstrated that lovastatin-encapsulating poly(lactic-co-glycolic) acid (PLGA) nanoparticles (NPs), administered locally at the sciatic notch, protect against a rat model of inflammatory peripheral neuropathy. In translating this work to the clinic, the sheer length of peripheral nerves and peripheral neuroanatomy necessitate targeted, intravenous delivery. The leukocyte plasma membrane represents a biological material that, when incorporated into a drug delivery system, can enable PLGA NPs to evade degradation, phagocytosis, and opsonization within the circulation and recognize and selectively bind to the inflamed vascular endothelium. We are developing methods to functionalize the surface of PLGA NPs with purified macrophage plasma membranes and characterizing the physical and biological properties of these biomimetic particles. Future studies will investigate adhesion of functionalized NPs to quiescent and inflamed peripheral nerve endothelial cells under physiological flow conditions. Plasma membrane-cloaked, biomimetic PLGA NPs may represent a novel drug delivery system to target the inflamed vascular endothelium.

Transcellular blood-brain barrier permeability

Sarah E. Lutz.
University of Illinois at Chicago

Entry of pathogenic T cells into the central nervous system (CNS) causes disease in the multiple sclerosis (MS) animal model experimental autoimmune encephalomyelitis (EAE). Extravasation across the blood-brain barrier (BBB) occurs via transcellular trafficking within endocytic vesicles such as caveolae, or alternatively via tight junction dissolution. We have previously shown that encephalitogenic Th1 cells utilize caveolae to cross the BBB in vivo in MOG35-55 EAE, whereas Th17 cells primarily engage disrupted tight junctions to cross the BBB (Lutz et al 2017). However, molecular signals targeting infiltrating cells to transcellular or paracellular site of migration remain unclear. We will discuss the role of soluble inflammatory signals in this process. Our findings suggest that chemokine-mediated caveolar transmigration may be a target for modulating BBB permeability.

Intravital high resolution imaging of neuroinflammation in acute stroke

Ayush Batra, Neil Nadkarni, William A. Muller, David P. Sullivan.
Depts of Neurology and Pathology, Northwestern University Feinberg School of Medicine

Ischemic stroke is a leading cause of a death and disability worldwide. Current treatments rely on early restoration of blood flow; however, mitigating the ischemic damage remains a therapeutic target. The innate immune response plays a pivotal role in reperfusion injury, but the mechanisms and time-course of leukocyte recruitment to the cerebrovasculature (CBV) during ischemic stroke remain unclear.

We sought to characterize the real-time leukocyte response at the CBV in a mouse stroke model with reperfusion. Ly6G-tdTomato mice (which have fluorescent red neutrophils) were subjected to 90 minutes of transient middle cerebral artery occlusion (tMCAO) followed by reperfusion. Real-time spinning-disc confocal intravital microscopy of the CBV was performed through intracranial windows. Marked increases in leukocyte rolling, adhesion, and extravasation were observed at 24 and 72 hours following the tMCAO procedure. Interestingly, non-ischemic hemispheres also demonstrated increased leukocyte rolling and adhesion, but not extravasation compared to the tMCAO group. Also, wide-field microscopy on fixed slices showed substantially more neutrophil extravasation in the core of the infarct at 72 hours. These findings are consistent with an early recruitment of neutrophils to the cortical surface at early time points and a delayed infiltration into the core, as reported by others.

These results show that a significant leukocyte response persists after reperfusion and suggest that ischemic stroke triggers a global inflammatory response within the CBV. Further reducing inflammation through immunomodulatory therapy in conjunction with reperfusion therapy may be beneficial in reducing stroke volume but also potentially mitigating inflammation-mediated secondary neuronal damage.

Neurovascular Degeneration

Cerebrovascular disease phenotypes and associated cognition profiles: A clinical-neuropathological study

Melissa Lamar.
Rush University

Cerebrovascular disease (CVD) including vessel disease (atherosclerosis, arteriolosclerosis, cerebral amyloid angioplasty or CAA) and tissue injury (macro-, micro-infarcts) each contribute to dementia including Alzheimer's disease (AD). Less is known about the most prevalent combinations of CVD-related neuropathology, or whether these combinations differentially associate with cognition. We investigated the 32 possible CVD-related combinations involving vessel disease and/or tissue injury using autopsy-confirmed data from 1,528 decedents of Rush Alzheimer's Disease Center cohort studies. We determined the relationships between the most prevalent CVD-related neuropathological phenotypes and global cognition, and domains associated with CVD (working memory, perceptual organization/visuospatial abilities, perceptual speed). Eight CVD-related phenotypes were found in 50 or more decedents, an additional seven in at least 30 decedents, for an analytic sample of 1,153 (age-at-death=8.4y, 66% female). When the eight most prevalent phenotypes were entered into a single mixed effects regression model adjusting for demographics, time before death, AD-related neuropathologies, all other neuropathologies of non-interest, and interactions of these variables with time, results revealed that individuals with CAA or microinfarcts alone. Three mixed CVD-related neuropathological phenotypes contributed to global cognitive decline including atherosclerosis and arteriolosclerosis with and without macroinfarcts and arteriolosclerosis combined with CAA. All three mixed phenotypes contributed to level and change in perceptual speed, however, the patterns of associations to other cognitive domains varied by phenotype. Other CVD-related phenotypes showed domain-specific associations, and not all withstood adjustments for AD-related and/or all other neuropathologies of non-interest. Some CVD-related neuropathological phenotypes are more prevalent than others, and most have domain-specific associates with cognitive decline.

The role of *APOE* genotype in modulating cerebrovascular function

Leon Tai.
University of Illinois at Chicago

There is increasing evidence that cerebrovascular dysfunction correlates with learning and memory alterations in some Alzheimer's disease (AD) patients. However, the specific contribution of cerebrovascular dysfunction to neuronal dysfunction is unresolved, due to the complex interplay of multiple pathogenic elements in AD. One of our goals is to understand the extent that AD risk factors modulate the cerebrovasculature and ultimately alter neuronal function. We have demonstrated that different combinations of key AD risk factors (sex, *APOE* genotype and peripheral inflammation) result in a similar phenotype of cerebrovascular dysfunction, characterized by higher leakiness and lower vessel coverage. In addition, our data suggest that changes in angiogenic growth factor signaling in brain endothelial cells may contribute to AD risk factor associated cerebrovascular dysfunction. For example, we have found that the epidermal growth factor (EGF) reduces brain endothelial cell dysfunction in cell culture models that incorporate AD risk factors. The beneficial effects of EGF extended to mouse models of AD risk factors. *In vivo* EGF treatment improved cerebrovascular and neuronal function as well as behavioral outcomes. Overall, our data suggest a mechanistic link among AD risk factors, angiogenic growth factor signaling and brain endothelial cell dysfunction.

The Development of Advanced Neurovascular MRI for Preclinical Research

Kejia Cai, Fred Damen, Mehran Shaghaghi, Alessandro Scotti, Zhenxiong Wang
Department of Radiology, UIC.

Neurovascular research has gained more and more interest for neuroscientists due to its fundamental role in aging and diseased brain. Within the many cutting-edge techniques, noninvasive MRI represents an advanced, diverse, and fast-growing method, enabling longitudinal monitoring of in vivo neurological systems at all levels from molecular, metabolic, structural, to functional.

Over recent years, besides the conventional structural MRI, we have gradually developed and implemented a few advanced MRI methods related to neurovascular imaging for preclinical applications, including advanced diffusion MRI with comprehensive analysis, endogenous perfusion MRI for cerebral blood flow, diffusion weighted arterial spin labeling MRI for the leakiness of blood-brain barrier, perivascular space mapping, imaging reactive oxygen species due to neuro-inflammation, etc. For each technical development, MR physics has been tailored for solving biological challenges. For instance, full-spectral diffusion MRI was used to extract different neural microenvironments; B0-corrected arterial spin labeling of water protons in the circulation was utilized for more reliable quantification of cerebral flow; diffusion weighted perfusion MRI with fast imaging readout enabled the detection of BBB leakiness; automatic segmentation was implemented for extracting fine perivascular spaces; proton exchange rate imaging on the other hand served as a novel imaging surrogate for reactive oxygen species overproduced under inflammation, and so on.

Supported by the Dept. of Radiology, our group is dedicated to multi-disciplinary MRI research covering MR physics, biomedical applications, and computational analysis. Through collaborations, we aim to develop or customize MRI techniques to meet challenges and needs from biological labs.

NONSELECTIVE CALPAIN-CATHEPSIN INHIBITION PRESERVES BBB-DYSFUNCTION IN AN OXIDATIVE-STRESS BASED TBI MODEL

Rachel Knopp, Ammar Jastaniah, Oleskii Dubrovsky, Sue Lee, Leon Tai, Gregory Thatcher.
University of Illinois at Chicago

The calpain-cathepsin hypothesis (CCH) predicates elevation of calpain-1 (CAPN1) and cathepsin-B (CTSB) as an underlying mechanism in the pathogenesis of Alzheimer's disease (AD) and related dementia, traumatic brain injury (TBI), and ischemic stroke. The hypothesis is supported by studies with small molecule inhibitors, such as NYC-438, that reduce cognitive deficits in AD mouse models. Though they display efficacy, NYC-438, a nonselective CAPN1/CTSB inhibitor and selective CAPN1 inhibitors reported in the literature exhibit poor brain bioavailability. We hypothesized that the CCH could account for dysfunction of the blood-brain barrier (BBB) and, in particular, brain endothelial cell (BEC) dysfunction. To test this theory and further characterize selective vs nonselective targeting of CAPN1 vs CTSB, we developed selective small molecule inhibitors, and characterized their neuroprotective efficacy in in vitro ischemia-reperfusion injury and neuroinflammatory attenuation in an in vivo mTBI mouse model of oxidative-stress (OS). Various inhibition strategies provided the expected dose-dependent neuroprotection in primary neurons. Moreover, they mitigated the post-mTBI neuroinflammatory surge seen in the OS-mice and attenuated the loss in tight junction proteins. We then isolated BECs from WT and OS mice and saw enhanced susceptibility in the OS-BECs after ischemia-reperfusion injury, suggesting a role for oxidative stress and lipid peroxidation in exacerbating CAPN1/CTSB mediated BBB damage. BECs from WT and OS mice provide a platform to assess the role of CCH in cell viability and tight junction proteins, and provides support for targeting CAPN1/CTSB in protecting the BBB, either in early life trauma, such as TBI, or in ADRD itself.

Current Topics

Confirmation and spatial mapping of diverse gene signatures by incorporation of multiplex *in situ* hybridization technology into single cell RNA sequencing workflows

Presented by Kari Halbig Bonner, Ph. D.
Jyoti Phatak, Han Lu, Li Wang, Hailing Zong, Morgane Rouault, Xiao-Jun Ma, Courtney Anderson
Advanced Cell Diagnostics, 7707 Gateway Blvd, Newark, CA 94560

Single-cell RNA sequencing (scRNA-seq) serves as a universal tool for classification and characterization of diverse cell types within heterogenous tissues. Limitations due to the use of dissociated cells results in loss of spatial information of these cells within the tissue context. Incorporating multiplexed spatial approach such as the RNAscope Multiplex fluorescent and RNAscope HiPlex *in situ* hybridization assays to confirm and spatially map these diverse cell types can interrogate gene expression with single cell resolution in the morphological context. In this study, we confirm and spatially map the diverse striatal neurons previously identified by scRNA-seq. Gene signatures of two discrete D1 (Drd1a/Foxp1, Drd1a/Pcdh8) and D2 (Drd2/Htr7, Drd2/Synpr) subtypes of medium spiny neurons , were confirmed, in addition to non-neuronal striatal populations. The utility of RNAscope technology for single cell transcriptomics provides additional biological insights into the organization and functional states of diverse cell types in healthy and disease tissues.

Notch signaling based screen identifies axon guidance receptor Unc5b as a genetic modulator of endothelial cell migration and cell-cell adhesion

Qanber Raza, Bhairavi Swaminathan, Jing Du, Seock-Won Youn, Anne Eichmann, Jan Kitajewski
Department of Physiology and Biophysics, University of Illinois at Chicago.

Notch signaling acts as a central regulator of vascular development in vertebrates and is essential for formation of a functioning blood brain barrier. Activation of Notch receptors by the Notch ligand DLL4 leads to transcriptional changes which influence endothelial cell (EC) processes such as migration, proliferation, and cell-cell adhesion. Nevertheless, Notch dependent mechanisms of angiogenesis and the downstream genetic effectors which elicit Notch activation-based responses in ECs have not been fully characterized. To address this, our lab conducted unbiased transcriptomic profiling utilizing three independent endothelial Notch manipulations: in vitro Notch activation via tethered-ligand DLL4, in vitro rapid Notch activation with EGTA, and in vivo Notch inhibition combined with RiboTag-based EC transcriptomic analysis. All profiles were examined at the earliest stages of transcriptional changes to enrich for direct Notch targets. The genes that were identified as regulated in all screens included established Notch target genes such as DLL4, HEY1 and EFNB2. Novel Notch targets were also identified, including UNC5B, a conserved guidance signaling receptor that has critical functions during angiogenesis but has not previously been recognized as a potential Notch target. We show that, like Notch signaling, Unc5b is enriched in retinal arteries and that Unc5b colocalizes with VE-Cadherin at the cell-cell junctions, suggesting that it has a role in cell-cell adhesion. By utilizing in vivo mouse genetic models combined with in vitro studies in human ECs, we demonstrate that, similar to Notch, Unc5b is required for stabilization of endothelial cell-cell junctions and suppression of EC migration.

Vascularization of human iPSC-derived Cerebral Organoids

Joseph Leasure[1], Li Wang[2], Ankit Jambusaria[3], Regeant Panday[3], David Kukla[3], Peter Toth[1], Sang Ging Ong[1], Orly Lazarov[4], Salman Khetani[3], Jalees Rehman[1,2,3]
[1]Department of Pharmacology, [2]Division of Cardiology, [3]Department of Bioengineering, [4]Department of Anatomy and Cell Biology, The University of Illinois College of Medicine, Chicago

Cerebral organoids derived from pluripotent stem cells are emerging as an important platform to investigate neuronal biology and disease mechanisms. Traditional organoid platforms rely on differentiating induced pluripotent stem cells (iPSCs) into neuroectodermal progenitor lineages with subsequent maturation into neurons and glial cells within a 3D-matrigel environment. However, most current cerebral organoid platforms lack a vasculature, which would allow for studying the blood-brain barrier in health and disease.

We generated vascularized cerebral organoids by combining human endothelial cells (ECs) with differentiating human iPSC-derived neuroectodermal cells. At day 25, cerebral organoids were collected for paraffin embedding, immunohistochemistry and qPCR analysis. Whole mount staining against CD31 and TUBB3 was performed on organoids at days 45 and 72 to visualize vessel formation.

Cerebral organoids integrated with either iPSC-derived brain ECs or the human brain endothelial D3 cell line. The incorporation of brain endothelial cells resulted in the formation of vascular structures in the brain organoid, visualized by immunofluorescence staining for the endothelial marker CD31 and confocal microscopy. Vessels formed in proximity to mature neurons, as indicated by co-staining with the neuronal tubulin marker TUBB3.

Our data suggests that brain ECs can be incorporated into cerebral organoids and form contiguous vascular structures that penetrate deep into the organoid with a diameter approximating that of cerebral capillaries. This novel vascularized cerebral organoid model may be well-suited to study homeostasis and pathogenic mechanisms of the blood-brain barrier, as well as interactions between neural lineage cells and endothelial cells.

Depletion of Caveolin-1 in Type-2 Diabetes Model Induces Alzheimer's disease Pathology

Aashutosh Shetti[1], Jacqueline Bonds[1], Abdullah Bheri[1], Zhenlong Chen[2], Ahmed Disouky[1], Leon Tai[1], Mao Mao[3], Brian Head[5,6], Marcelo Bonini[7], Jacob Haus[8], Richard Minshall[2,4], Orly Lazarov[1].

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Type 2 Diabetes mellitus (T2DM) is a risk factor for the development of Late Onset Alzheimer's disease (LOAD). Vascular dysfunction and impairment in insulin signaling are thought to play a role in the development of dementia and AD in T2DM, but a potential link connecting these pathologies is currently unknown. Here we show that levels of the endothelial-enriched proteins caveolin-1 (Cav-1) and its downstream signal endothelial nitric oxide synthase (eNOS) are reduced in the brains of T2DM patients compared to healthy aging. Cav-1 and eNOS levels inversely correlated with levels of beta-amyloid (Abeta). Depletion of Cav-1 is recapitulated in the brains of db/db diabetic mice and corresponds with recognition memory deficits as well as the upregulation of amyloid precursor protein (APP), BACE-1and hyperphosphorylated tau (p-tau) species. Importantly, we show that restoration of Cav-1 levels in the brains of male db/db mice using adenovirus overexpressing Cav-1 (AAV-Cav-1) rescues learning and memory deficits and reduces pathology, i.e., Abeta, APP, BACE-1 and p-tau levels. Our results suggest restoration of Cav-1 in db/db mice prevents the development of AD-like pathology. Taken together, our study suggests an important role for Cav-1 in regulation of brain homeostasis.

Posters

(Notes: 1-9 are student presenters)

P1.

A CXCL10-CXCR3 axis drives caveolar BBB permeability in neuroimmune disease

Troy Trevino, Elizabeth A. Pietruczyk, Vidya Babu, Andrea Ochoa-Raya, Sarah E. Lutz.
Department of Anatomy and Cell Biology, University of Illinois at Chicago

Entry of pathogenic T cells into the central nervous system (CNS) causes disease in the multiple sclerosis (MS) animal model experimental autoimmune encephalomyelitis (EAE). Extravasation across the blood-brain barrier (BBB) occurs via tight junction dissolution and luminal-to-abluminal trafficking within endocytic vesicles such as caveolae. We have previously shown that encephalitogenic Th1 cells utilize caveolae to cross the BBB in vivo in MOG35-55 EAE (Lutz et al 2017). However, molecular signals targeting infiltrating cells to caveolae remain unclear. Th1 T cells highly express the chemokine receptor CXCR3, and its ligand CXCL10 is upregulated in the CNS in MS and EAE. We have tested the hypothesis that CXCR3 promotes migration of T cells across BBB caveolae. As expected, CXCR3+ T cells were abundant in spinal cords from WT mice with EAE. CXCR3+ T cells were reduced in the spinal cords of Caveolin1-/- mice with EAE. CXCL10 chemotaxis across primary BBB endothelial cells was disrupted in the absence of caveolae. Finally, presentation of CXCL10 on the luminal face of BBB endothelial cells in EAE required endothelial caveolae. This suggests that endothelial cell caveolae 1) deliver chemokine ligands from the abluminal-to-luminal surface, and 2) engage chemokine receptor positive T cells in luminal-to-abluminal trafficking into the CNS. Our findings suggest that chemokine-mediated caveolar transmigration is a target for modulating BBB permeability.

P2.

Optimized tissue clarification of blood brain barrier and lymphatic permeability in neuroinflammatory disease.

Andrea Ochoa-Raya, Mason D. Sutter, Anais K. Mancini, Sarah E. Lutz.
Department of Anatomy and Cell Biology, University of Illinois at Chicago College of Medicine

The blood brain barrier (BBB) is a selectively permeable physical barrier formed by central nervous system (CNS) blood vessel endothelial cells. It becomes increasingly penetrable during neuroinflammatory diseases including multiple sclerosis (MS). In the MS animal model experimental autoimmune encephalomyelitis (EAE), the aberrant trafficking of leukocytes between the blood, CNS, and lymphatics causes neuroinflammatory neurodegeneration. Currently there are several ways of identifying the proteins involved in neuroinflammation, such as immunohistochemistry. Whole organ clearing is the ideal method for imaging entire structures to build a series of 3D images. The current method for doing this is expensive, difficult to implement, and requires a degasser. We sought to optimize a published mPACT organ clearing protocol to visualize BBB proteins in mice with the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis. We optimized antibody combination and incubation times as well as fashioned a home-made apparatus for electrophoretic clearing of opaque proteins and then utilized confocal microscopy to identify protein distribution in neuroinflammatory disease. We were able to construct 3D images of fluorescent endothelial cell proteins in neurovascular segments. Ongoing efforts deploy this technique to assess regional sites of enhanced permeability and cellular trafficking in blood-brain barrier and lymphatic endothelial cells.

P3.

Inhibition of Notch signaling in pericytes leads to AVM-like lesions

Taliha Nadeem, Theressa Ewa, Wil Bogue, Bianca Bigit, Jyotsna Bitra, Henar Cuervo.
Department of Physiology and Biophysics, University of Illinois at Chicago

Pericytes are a subset of mural cells associated with smaller caliber vessels (capillaries). Their close proximity to the endothelium allows for them to regulate vascular homeostasis and act as key components in the blood-brain and blood-retinal barriers. Pericyte dropout, dysfunction and loss are associated with a cohort of pathologies affecting the central nervous system including diabetic retinopathy, Alzheimer's disease and arteriovenous malformations (AVMs).

AVMs are vascular anomalies involving high flow shunts between arteries and veins without an intervening capillary network. It is known that human brain AVMs have significantly reduced pericyte coverage, however the mechanism underlying this reduction and its association with AVM pathogenesis remains elusive.

We investigated the function of Notch signaling in mouse pericytes by ablating canonical Notch signaling in perivascular cells through tamoxifen-inducible deletion of Rbpj. Mice were treated with tamoxifen perinatally. We observed increased pericyte apoptosis and reduced pericyte coverage in the retinal vasculature at postnatal at 5 (P5) followed by capillary enlargement by P14. Over time, capillary enlargement manifested into arteriovenous shunts, a hallmark of AVMs. AVM-like lesions in our mice were also associated with overall poor vascular perfusion and increased vessel regression. Together, our findings demonstrate that Notch signaling is crucial for pericyte survival and capillary endothelium stability and reveal a novel potential mechanism of AVM formation.

P4.

High Resolution Characterization of Oxidative Stress in Endothelial Cells

Anara Serikbaeva, Yueru Li, Andrius Kazlauskas.
Department of Physiology and Biophysics, Department of Ophthalmology and Visual Sciences, UIC, Chicago, IL.

Diabetic complications develop at least in part due to oxidative stress-driven endothelial cell dysfunction. This process starts with activation of enzymes that produce reactive oxygen species, and advances to irreparable damage of the mitochondria. The goal of this project is high resolution characterization of the initial phase of oxidative stress-driven endothelial dysfunction.

We used a set of redox-sensitive GFP sensors that allowed us to determine the effect of high glucose (HG) on the level of hydrogen peroxide and oxidized glutathione (GSH) in distinct subcellular compartments (mitochondria and in the vicinity of the plasma membrane).

Consistent with findings of other investigators, we observed that HG increased oxidative stress within the vicinity of the plasma membrane, and that this was due to an increase in the level of oxidized GSH. In contrast, HG had no effect on the mitochondrial level of either hydrogen peroxide, or oxidized GSH. However, HG did alter the ability of the mitochondria to resolve an acute oxidative insult caused by addition of tert-butyl hydroperoxide (TBH). Surprisingly, TBH triggered a smaller increase in oxidative stress that was resolved more quickly in HG versus normal glucose cells. We conclude that HG causes compartment-specific redox dysfunction. Furthermore, HG improves the capacity of the mitochondria to mitigate oxidative stress. We speculate that this observation reveals the existence of mitochondrial-based processes that protect mitochondria from succumbing to irreparable damage associated with development of diabetic complications.

P5.

Inflammasome-Derived Caspase-1 Is Elevated in the Cerebrospinal Fluid of Subarachnoid Hemorrhage Patients and Correlated to Outcome

Yonatan Hirsch, Joseph Geraghty, Eitan Katz, Jeffrey Loeb, Fernando Testai.
University of Illinois Chicago College of Medicine, American Heart Association

Introduction: The role of neuroinflammation following aneurysmal subarachnoid hemorrhage (SAH) and its relationship to outcome is the subject of many ongoing studies. The proteolytic enzyme, caspase-1, activated by the inflammasome complex, is known to contribute to numerous downstream pro-inflammatory effects. In this study, we investigated caspase-1 activity in the cerebrospinal fluid (CSF) of SAH patients and its association to outcome.

Methods: CSF samples from 18 SAH subjects were collected via an external ventricular drain and obtained within 72 hours of the onset of symptoms. For control subjects, we collected the CSF from 9 patients undergoing lumbar puncture with normal CSF and normal brain MRI. Caspase-1 activity was measured using commercially available luminescence assays. SAH subjects were categorized at hospital discharge into those with good outcomes (Glasgow Outcome Scale, GOS, of 4-5) and poor outcomes (GOS of 1-3).

Results: Caspase-1 levels from SAH patients were higher than that measured from the control group (mean 1.06x10-2 vs 1.90x10-3 counts per second (CPS)/µl*min), p = 0.0002). Caspase-1 activity was significantly higher in the poor outcome group (mean 1.54x10-2 vs 1.60x10-3 CPS/µl*min), p = 0.0012). Additionally, caspase-1 activity had a statistically significant correlation with GOS score (r = -0.60; p = 0.0100).

Conclusions: The inflammasome-dependent protein caspase-1 is elevated in CSF early after SAH and higher in those with poor functional outcome. Inflammasome activity may serve as a novel biomarker to predict outcome shortly after aneurysm rupture.

P6.

Assessing the relationship of vascular dysfunction and inflammation after aneurysmal subarachnoid hemorrhage

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Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating neurological injury commonly resulting from the rupture of cerebral aneurysms. Early brain injury (EBI) occurs within the first 48-72h post hemorrhage and is characterized by an intense neuroinflammatory response and vascular dysfunction. Transcranial Doppler (TCD) ultrasound is routinely used in aSAH to monitor for vascular dysfunction. Pulsatility index (PI), as determined by TCD, correlates with microvascular compliance. In comparison, the Lindegaard ratio (LR), is typically used to estimate the caliber of larger vessels. In this study, we conducted a retrospective study on the relationship between vascular dysfunction (via TCD) and immune responses in the serum and cerebrospinal fluid (CSF). CSF was obtained only from those patients receiving a ventricular drain, while serum samples were obtained from all patients. Samples were collected daily for 5 days following admission. Spearman correlation was used to assess correlation between immune cells, PI, and LR. A total of 244 aSAH patients were included in the analysis. Early neutrophils (day 2) and delayed changes in lymphocytes (day 4) were correlated with vascular dysfunction by day 5 ($p<0.05$). Future studies will compare the systemic immune changes after aSAH and more centralized inflammation within the CSF as predictors of micro- and macrovascular dysfunction. These results may help increase our understanding of the complex interactions between the immune and vascular systems after brain injury and how early immune changes may help predict vascular dysfunction before delayed brain injury fully develops.

P7.

Beyond the Vasculature: Examining the Role of Caveolin-1 in Neural Stem Cell Quiescence

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Adult Hippocampal Neurogenesis (AHN) is the generation of new neurons from neural stem and progenitor cells (NSC, NPC) in the dentate gyrus of the hippocampus. AHN is highly dependent on the neurovascular unit and thought to play a critical role in learning, memory and cognition. Caveolin-1 (Cav-1) is a scaffolding protein and is significantly enriched in endothelial cells. We show that reductions in brain Cav-1 expression is associated with the cognitive deficits seen in Type II diabetes. Global loss of Cav-1 in the mouse (Cav-1 KO) results in reduced numbers of NSCs and NPCs in the hippocampus. However, in vivo rescue of Cav-1 specifically in endothelial cells within the Cav-1 KO mouse did not result in restoration of AHN. We hypothesize that Cav-1 has a novel cell autonomous role in NSCs and is a critical endogenous regulator of NSC quiescence and activation. To test this, we have developed a new mouse model harboring conditional deletion of Cav-1 in NSC and NPCs (NestinCreERT2;Cav-1lox/lox). This model allows for the examination of endogenous Cav-1 in NSC and NPCs. Isolation of NPCs from the hippocampus of these mice revealed that loss of Cav-1 alters NPC proliferation levels. Further, deletion of Cav-1 in NPC results in altered expression of key neurogenic pathways regulating quiescence/activation such as Epidermal Growth Factor (EGF) and Bone Morphogenetic Protein (BMP) signaling. These results suggest that Cav-1 is an important regulator of AHN and as a possible therapeutic strategy in cognitive disorders like Alzheimer's Disease and Type II diabetes.

P8.

Small molecule ABCA1 inducers as potential multifunctional therapeutics for Alzheimer's disease

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There are no disease-modifying therapies for Alzheimer's Disease (AD). Trials with amyloid-targeting agents have consistently failed, indicating a need to explore novel treatment avenues. The cholesterol transporter ABCA1 represents one such avenue, as increased ABCA1 expression has been shown to improve lipid metabolism, insulin signaling, and inflammation, all of which are perturbed in AD brains. However, transcription factors controlling ABCA1 also promote hepatic triglyceride synthesis (via SREBP1c protein), so non-selective activity leads to fatty liver disease. Our objective was to develop novel, selective inducers of ABCA1 that possess therapeutic effects without impacting peripheral lipogenesis. To that end, we conducted a luciferase-based high-throughput screen to identify compounds increasing ABCA1, but not SREBP1c, expression. Following validation, structural analogs of one hit were synthesized to identify new compounds with enhanced potency. Through multiple synthetic iterations, we developed a lead compound with sub-micromolar potency toward ABCA1 induction in vitro but minimal SREBP1c effect. This optimized molecule was validated in phenotypic cell-based assays, in which we observed increased cholesterol transport and reduced inflammatory response to LPS stimulation following treatment. Finally, we tested this compound in high-fat diet mice, demonstrating improved insulin sensitivity in treated mice. Notably, treatment decreased triglycerides in these mice, showing the promise of our strategy to develop small molecules with multifactorial ABCA1-mediated therapeutic activity but minimal adverse effects. Additional work is ongoing to evaluate pharmacokinetic properties, in preparation for long-term studies in AD mouse models to explore efficacy at correcting cognitive deficits and AD pathology.

P9.

Functional Optical Coherence Tomography of Retinal Neurovascular Response and Hyaloid Vessel Regression in Mice

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Quantitative evaluation of retinal neurovascular coupling is essential for a better understanding of visual function and early detection of eye diseases. However, there is no established method to monitor coherent interactions between stimulus-evoked neural activity and hemodynamic responses with high resolution. Here, we report a multi-modal functional optical coherence tomography (OCT) imaging methodology that enables concurrent intrinsic optical signal (IOS) imaging of stimulus-evoked neural activity and hemodynamic response with capillary resolution. OCT angiography (OCTA) guided IOS analysis was used to separate neural-IOS and hemodynamic-IOS changes in the same OCT image sequence. A comparative study using wild-type (WT) and retinal degeneration 10 (rd10) mice revealed IOS distortions due to photoreceptor dysfunction in rd10. We further demonstrate noninvasive concurrent OCT and OCTA monitoring of morphological and physiological regressions of hyaloid vascular system in developing mouse eyes, and rd10 showed early regression of the hyaloid vessels compared to WT.

P10.

S1P/S1P1 signaling axis promotes hyperoxia- induced lung endothelial injury leading to bronchopulmonary dysplasia by suppression of Tie2

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Rationale: We have shown that Sphingosine kinase 1 (SPHK1)/sphingosine 1 phosphate (S1P) signaling contribute to hyperoxia induced BPD like morphology in neonatal mice. Hyperoxia stimulated expression of S1P1 in neonatal lungs/human lung microvascular endothelial cells (HLMVEC). S1pr+/- mice showed protection against hyperoxic lung injury accompanied by improved alveolar formation. Increased expression of S1P receptor 1 was associated with reduced angiogenesis.

Objective: To explore the mechanism by which S1P receptor1 signaling pathway regulates endothelial angiogenesis thereby playing a role in the pathogenesis of BPD.

Methods: Both S1pr+/+and S1pr+/- mice pups were exposed to normoxia or hyperoxia (75% oxygen) for 7 days. Lung injury, lung protein expression and alveolar simplification was evaluated. In vitro experiments were performed using HLMVECs with S1P1 antagonist to interrogate the S1Preceptor 1 signaling pathway.

Results: We noted that S1pr+/- mice pups showed protection against hyperoxia induced BPD in WT neonatal mice accompanied by reduced expression of Lox in lung tissue. This was accompanied by reduced inflammation markers in the bronchoalveolar lavage fluid. HLMVECs exposed to hyperoxia showed increased expression of S1P receptor 1 in cell lysate accompanied by reduced expression of Tie2. S1P1 antagonist attenuated hyperoxia-induced ROS production, enhanced migration of HLMVECs accompanied by enhanced lamellipodia formation and reversal of reduced Tie 2.

Conclusion: Inhibition of S1P receptor 1 has downstream impact under hyperoxia leading to improved lamellipodia formation and migration of HLMVECs thus contributing to the protection against hyperoxia induced neonatal lung injury.

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P11.

Stiffness of Aortic Arch and Carotid Artery Increases in ApoE-knockout Mice with High-fat Diet: Evidence from Echo Cardiography

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Background and aims: Atherosclerosis is a chronic inflammatory disorder that is the underlying cause of most cardiovascular disease (CVD). Arterial stiffness, the expression of reduced arterial elasticity, is an effective predictor of atherosclerosis. Measurement of pulse-wave velocity (PWV) is a gold-standard approach to study the arterial stiffness. In this study, we aimed to measure local arterial stiffness and heart functions in ApoE-knockout (ApoE-/-) mice fed on either normal chow diet (ND) or high-fat diet (HF).

Methods: Arterial stiffness of aortic arch and carotid arteries and heart functions were measured by high-resolution ultrasound (echo); The atherosclerosis phenotypes were assessed by oil red O staining and cholesterol levels. The correlations between PWV, heart functions, and atherosclerosis phenotypes were investigated.

Results: Compared with the ND mice, PWV values in both aortic arch and carotid arteries were significantly increased in HF mice, while the left ventricular diastolic and systolic functions were decreased. In addition, the atherosclerotic plaque stained with Oil Red O and plasma cholesterol levels were significantly increased in HF mice compared with the ND mice.

Conclusions: Stiffness of aortic arch and carotid artery was significantly increased in HF group compared with the ND group, which was consistent with the changes in heart functions and cholesterol levels. All the evidences indicate that echocardiography could represent a new diagnostic strategy for early detection of arterial stiffness.

P12.

Multiplex 3D microscopy for analysis of the tissue microenvironment

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We have developed a novel workflow for 3D tissue imaging that enables comprehensive analysis of the tissue microenvironment. Transparent Tissue Tomography (T3) is a fast and straightforward methodology involving light tissue fixation, thick tissue sectioning, overnight staining with fluorescent antibodies, and tissue clearing with fructose. 3D imaging of thick tissue sections stained for multiple markers can then be performed at cellular resolution. To date, T3 has provided a comprehensive view paired with detailed quantitative analysis of both macromolecular drug distribution and the composition and function of the tumor microenvironment. Here we apply T3 in healthy murine brain tissue in order to analyze the distribution of vascular networks in relation to astrocytes. Our results show that brain tissue can be cleared and imaged in 3D for endothelial cell and astrocytic markers. Future work will focus on implementation of T3 to study development of neurological diseases, including Alzheimer's and Parkinson's, and novel therapies.

P13.

Neurocognitive Profile of a Clinical Sample of Adults with Moyamoya Disease

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Moyamoya disease (MMD) is a rare cerebrovascular condition characterized by occlusion of the internal carotid arteries resulting in collateral vessel formation and poor cerebral blood flow. MMD has been associated with diffuse hypoperfusion and stroke, resulting in cognitive deficits. Though cognition in MMD has not been well-studied, deficits related to subcortical damage have been identified. This study sought to better characterize the neurocognitive profile of adults with MMD. Retrospective data from 18 patients with MMD (Mage =34.4, SD=15.0; 61% female; 69.6% history of clinical stroke) who underwent outpatient neuropsychological evaluation were analyzed. All patients completed the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), and a subset (n=12) had also completed the Trail Making (TMT), Wisconsin Card Sorting (WCST), and Grooved Pegboard (GPT) tests. Impaired performance was defined by z-scores *1.5 standard deviations below the normative means. 47.1% of patients had impaired RBANS total scores, with the highest rates of impairment in the visuospatial (44.4%) and attention (38.9%) domains. Immediate learning (27.8%), language (27.8%), and delayed memory (27.8%) had lower impairment rates. High rates of impairment were found on TMT-B (41.7%) and GPT (41.7% dominant hand, 41.7% non-dominant hand), though TMT-A (25%) and WCST (25% [errors], 16.7% [perseverative responses], and 16.7% [perseverative errors]) were less often impaired. Overall, almost 50% of MMD patients displayed impaired performance on a measure of global cognitive functioning (RBANS). Consistent with previous research, specific deficits in attention/processing speed emerged, although executive functioning was variable. Visuospatial ability and motor dexterity were also commonly impaired.

P14.

The Renin-Angiotensin-Aldosterone System (RAAS) is One of the Effectors used by VEGF/anti-VEGF to Control the Endothelial Cell Barrier

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Leakage of retinal blood vessels, which is an essential element of diabetic retinopathy (DR), is driven by chronic elevation of vascular endothelial growth factor (VEGF). VEGF quickly (within minutes) relaxes the endothelial cell barrier by triggering signaling events that post-translationally modify preexisting components of intercellular junctions. VEGF also changes expression of genes, some of which are known to regulate endothelial cell barrier function. The purpose of this project was to identify effectors by which VEGF and anti-VEGF control the endothelial cell barrier in cells that were chronically exposed to VEGF (hours instead of minutes).

Using in vitro models of DM-induced endothelial dysfunction we discovered that the duration of exposure to VEGF influenced both barrier relaxation and anti-VEGF-mediated closure. Furthermore, the vast majority of VEGF-induced changes in gene expression were not reversed by anti-VEGF. Those VEGF-regulated genes that were also sensitive to anti-VEGF constitute VEGF effectors that are targets of anti-VEGF. By pursuing such candidates, we learned that VEGF used multiple, non-redundant effectors to relax the barrier in cells that were exposed to VEGF for a prolonged time period. Furthermore, one of these effectors was ACE (angiotensin converting enzyme), which is a member of the renin-angiotensin aldosterone system (RAAS). Finally, activating the RAAS reduced the efficacy of anti-VEGF. These discoveries provide a plausible mechanistic explanation for the long-standing appreciation that antagonizing RAAS is beneficial for patients with DR and suggests that antagonizing the RAAS improves responsiveness to anti-VEGF.

P15.

Ischemic Stroke Induces Striking Heterogeneity in the Inflammatory Leukocyte Infiltrate across the Infarction

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Current therapies for ischemic stroke focus on reperfusion but do not address the acute inflammatory response. Previous clinical trials aimed at modulating the inflammatory milieu by disrupting leukocyte infiltration failed to show clinical efficacy. One possible explanation for this unexpected shortcoming is an incomplete understanding of the precise spatio-temporal underpinnings of leukocyte extravasation and infiltration.

Here we describe the evolution of the inflammatory response in a mouse transient middle cerebral artery occlusion stroke model at several time-points post reperfusion. We used widefield and confocal microscopy to examine the exact location of invading myelomonocytic populations, with close examination of their position relative to the brain vasculature and the perivascular space.

Our findings suggest that the vast majority of infiltrating myelomonocytic cells escape the perivascular compartment and enter the parenchyma. Interestingly, neutrophil extravasation was initially restricted to the cortical surface; their appearance deep in the subcortex was substantially delayed. In addition, dramatic heterogeneity in the inflammatory infiltrate was observed across the infarcted tissue, but also in the surrounding penumbra and adjacent cortical surface. In addition, triphenyl tetrazolium chloride staining, a common indicator for infarcted tissue, did not correlate with the amount or location of leukocyte infiltration.

Taken together our findings demonstrate that the infiltration of leukocytes dynamically evolves over several days following reperfusion. Furthermore, leukocytes infiltrate in a heterogeneous pattern that does not correlate well with traditional markers of cellular dysfunction. A better understating of the precise spatio-temporal infiltration of inflammatory cells could help inform the next generation of therapeutic interventions.

P16.

Endothelial Notch signaling dysfunction may contribute to Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive, degenerative disease affecting memory, thinking, and behavior, which is projected to afflict 34 million people worldwide by 2025. Presently, there is no cure or means of stopping or slowing AD progression. While a major pathological hallmark of AD is the accumulation of amyloid beta (Aβ) plaques, changes in cerebral perfusion and cerebrovascular function have been observed prior to onset of plaque development or cognitive impairment, and the link between vascular and cognitive health has gained deeper appreciation in recent years.

Early-onset AD is caused by mutations in amyloid precursor protein (APP), presenilin 1 or 2 (PSEN1 or 2). PENS1 and 2 are the catalytic core of the γ-secretase complex, which is involved in the cleavage of APP into Aβ. In addition to APP cleavage, the γ-secretase complex is also involved in activation of Notch proteins, and alterations in γ-secretase activity have been observed in AD models. In the endothelium, Notch signaling contributes to the regulation of angiogenesis and vascular integrity, and dysregulation of Notch activity has been shown to result in changes in vascular density and barrier function. Thus, we hypothesize that the changes in γ-secretase activity associated with AD development may affect Notch activity, leading to endothelial dysfunction in the AD brain and further contributing to AD development and progression. To test this hypothesis, here we investigate Notch activity in AD brain endothelium, and test the effect of inhibition of endothelial Notch signaling on cognition and pathology in a mouse model of AD.

P17.

Live Animal Imaging of Vascular Remodeling in Ischemic Stroke Using Visible Light Optical Coherence Tomography

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Stroke is a leading cause of death and morbidity in the United States. Neovascularization, the formation of new capillaries from blood vessels, is an emerging target in neurorehabilitation. However, the spatial and physiologic determinants of angiogenesis in vivo are poorly understood. Current studies rely on multiphoton microscopy, which requires extrinsic fluorescent labels and are difficult to reproduce longitudinally.

Visible-light optical coherence tomography (vis-OCT) is a three dimensional, anatomical and functional imaging modality with microscopic resolution. Using visible light illumination, vis-OCT offers higher axial resolution and is more sensitive to differences in oxygenated and deoxygenated hemoglobin absorptions compared to traditional near-infrared OCTs. All reported vis-OCT in vivo brain studies had two major limitations. First, the imaging depth was limited to ~200-400 Âµm due to the relatively high attenuation of visible light in the brain, making it difficult to image the deep cortex. Second, the duration of the reported studies is limited to one week following induction of strokes, although vessel remodeling continues for 60 days post-stroke.

Microprism implantation with a chronic cranial window into the brain has previously been used in other optical microscopy modalities for deep brain imaging. These studies demonstrated that the addition of microprism caused manageable neural damages while dramatically expanding the capability to investigate deep brain and still allowing longitudinal observation of the brain. As such, we sought to investigate the role of an integrated vis-OCT and chronic cranial window with implanted microprism in longitudinally studying cerebrovasculature in vivo.

P18.

Blood Brain Barrier Leakage in Mouse Brains at 9.4T MRI

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The standard way to detect the leakiness of the blood brain barrier (BBB) clinically in the human brain is to acquire T1-weighted images before and at a certain time after an intravenous injection of a gadolinium chelate contrast agent (GD-CA). The delay in post injection imaging gives the Gd-CA time to leak into interstitium and interact with the water molecules, thus, adjusting the T1-weighting proportionally to the concentration of the Gd-CA in the interstitial space. This is an effective means to determine the leakiness when the compromised fenestrations of the BBB are large enough for the passage of the Gd-CA, as in the case of stroke or tumors. Albeit, when the fenestrations are small, as in Alzheimer's Disease (AD), using Gd-CA is an ineffective method to detect BBB leakage. Water can be used as an endogenous contrast agent by marrying two existing imaging techniques, Arterial Spin Labeling (ASL), which determines amount of blood delivered to a voxel of tissue, and Diffusion Weighted (DW), which can nearly totally attenuate flowing blood water while negligibly attenuating water that leaked and is now diffusing in the interstitium. While the potential of this method has previously been demonstrated in the human brain using a clinical scanner, there are significant challenges with mice on a preclinical 9.4T scanner. The challenges and current progress will be presented.

P19.

Transcriptomic Analysis of the Brain Endothelium in an Experimental Model of Alzheimer's Disease

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Brain endothelial cells are key components of the blood brain barrier and recent studies point towards a mechanistic role for endothelial dysfunction in the development and progression of Alzheimer's Disease (AD). To understand the mechanisms by which brain endothelial cells (ECs) may affect AD progression, we used an unbiased transcriptomic analysis approach to analyze gene expression in brain endothelial cells derived from the transgenic mouse line (APP/PS1DE9) that expresses Amyloid Precursor Protein (APP) in the brain, leading to amyloid plaque formation. RNA-seq was used to analyze the transcriptional profiles of ECs isolated from forebrain of young (~4 months) and old (~13 months) transgenic AD (APP)/presenilin (PS1DE9) mice as well as age matched non-transgenic control mice (strain C3H/BL6). Pathway analysis was used to identify gene expression pathways that were selectively upregulated or downregulated in AD mice versus their age-matched controls. The transcriptomic analysis was validated by qRT-PCR and cytospin. Pathways analysis indicated that during early AD, the brain endothelium transcriptome demonstrates features of endothelial remodeling and changes in endothelial junctions as well as decreased metabolic stress tolerance. In advanced AD, the brain endothelium surprisingly demonstrates downregulation of genes typically found in neurons such as those involved in synaptic regulation and neurotransmitter transport. Our findings provide vital information on the signature patterns of gene expression changes in brain endothelial cells of young and aged transgenic AD mice and raise the intriguing question of whether endothelial downregulation of synaptic regulation and neurotransmitter transport genes may impact AD progression.

P20.

Cardiac Neuroanatomy and Chronotropic Modulation of the Adult Giant Danio Heart

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Studies in non-mammalian models species have contributed significantly to our understanding of the biology and the nature of innervation in the heart. The giant danio (D. malabaricus) is a teleost fish species closely related to zebrafish, that is also capable of heart regeneration. We previously described the development and maturation of the giant danio (GD) heart. However, little is known about its innervation. We hypothesized that the pattern of innervation in the GD heart is anatomically and physiologically complex, and that the heart is responsive to physiological modulation similar to that seen in cyprinid fish and mammals. Using various neuronal markers and electron microcopy, we described the presence, distribution, and nature of nerves in the GD heart. Our study shows first that fine intrinsic cardiac nerve fibers are present throughout the heart chambers. Second, nerve soma and ganglia are highly concentrated at nerve plexuses located near the sinoatrial (SA) and atrioventricular (AV) junctions. However, the volume density of axonal processes located over the ventral aorta_ is highest over the corpus of the bulbus arteriosus. Third, using an ex vivo GD heart preparation, we found that the GD heart responded to both adrenergic and cholinergic agonists, in a manner that mirrors mammalian and teleost hearts. Taken together, our studies show that the GD heart displays complex patterns of innervation, and conserved cardiac physiological responses, and strongly suggest that the GD could be used as a viable model for investigating cardiac biology.