BRAIN BARRIERS SIGNALING

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Abstracts

Uncovering the role of connexins and Yap in blood brain barrier hyperpermeability and microvascular injury in cerebral amyloid angiopathy

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Background (165)
Cerebral Amyloid Angiopathy (CAA) is a cerebral small vessels disease characterized by accumulation of amyloid-β (Aβ) around the small caliber vessels (arterioles and capillaries). Structural alterations and loss of integrity in the neurovascular unit/blood-brain barrier (NVU/BBB) in CAA results in "leakier" microvessels, microhemorrhage, increased stroke risk, and cognitive impairment. Although a significant body of evidence has pinpointed several potential mechanisms (i.e., neuroinflammation and oxidative metabolism) to drive BBB injury in CAA, molecular mechanisms remain to be fully elucidated. To determine the profile of microvascular injury in CAA murine model (Tg-SwDI mice), we performed RNA sequencing analysis on the isolated brain microvessels. Transcriptome profiling revealed alteration in genes like gap junction proteins, connexin 43 (Cx43) and Cx45, and Yap1 (Yes-associated protein), a component of the Hippo signaling pathway involved in remodeling of actin cytoskeleton, extracellular matrix, and regulation of inflammation. Our present study aims to understand how changes and interactions between Cx43/Cx45, and Yap modifies barrier integrity and promotes cerebrovascular injury in the CAA condition.

Methods (57)
Using in vitro (Aβ 1-40 and Aβ 1-42 exposure) and in vivo models (Tg-SwDI mice), we performed assessment of cell structural changes (immunocytochemistry, confocal microscopy), functional (optical tweezers method), FRAP assay, in vitro permeability assay) and signaling events (western blotting and proximity ligation assay) associated with alteration of brain endothelial cells (BECs) mechanics and barrier permeability in CAA settings.

Results (90)
Consequences of Aβ-afflicted brain microvessels and Aβ exposure in BECs promotes an overall increased protein expression and a redistribution of Yap expression from the cytoplasm to the nucleus, contributing to structural changes, such cellular stiffening and cell permeability. Similarly, Aβ promotes upregulation of Cx43 and Cx45 (i.e., GJs and HCs formation and activity) as well as loss of tight junction integrity resulting in a hyperpermeable barrier. Inhibition (via selective inhibitors) and/or modifications of each component expression (via siRNA transfection) or whole Yap/Cx43/Cx45 axis alters the cell mechanics and consequently barrier permeability. Conclusion (36) Collectively, our data suggest that vascular Aβ deposition in CAA pathology promotes injury and junctional alterations through a Cx43/Cx45/Yap axis in the brain endothelium contributing to development of CAA associated pathology (i.e., BBB hyperpermeability, and microbleeds).

cARLA: a small molecule cocktail for robust induction of blood-brain barrier properties

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Blood-brain barrier (BBB) models derived from human stem cells are powerful tools to improve our understanding of human cerebrovascular diseases and facilitate drug development for the brain. Yet providing endothelial cells with the appropriate molecular cues to both retain a vascular identity and acquire BBB characteristics remains challenging. Here we present cARLA, an easy-to-use and affordable small molecule cocktail that robustly induces BBB properties in vitro. By activating cyclic AMP and Wnt/β-catenin signaling while inhibiting the transforming growth factor beta (TGF-β) pathway, cARLA synergistically enhances barrier tightness in a range of BBB models. We demonstrate that, upon cARLA treatment, human stem cell-derived endothelial cells have lower rates of transcytosis, higher glycocalyx density and increased efflux pump activity with a shift in gene expressional profile towards the in vivo brain endothelial signature. Our work provides mechanistic insight into how endothelial signaling is orchestrated during BBB maturation and leverages this to advance the prediction of drug delivery to the human brain.

Tanycytic VEGF receptor signaling in the blood-hypothalamus barrier: a new player in the communication between the brain and the periphery

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Abstract Text

The communication between the brain and the periphery is tightly regulated by the blood brain barrier (BBB), in which endothelial cells, sealed by tight junctions, and perivascular cells contribute forming the neurovascular unit. However, specific brain regions known as circumventricular organs (CVOs) lack this type of barrier. CVOs are characterized by the presence of fenestrated vascularization and the translocation of barrier properties to non-endothelial cells.

Among CVOs, the median eminence (ME) is located in the base of the hypothalamus, ventral to the third ventricle and adjacent to the arcuate nucleus (ARH). The ME is a key interface between the neural and endocrine systems involved in the hypothalamic control of energy homeostasis. The presence of a fenestrated endothelium in the ME allows the passive diffusion of blood-borne molecules into the parenchyma and vice-versa. However, the passage of molecules beyond the ME to the ARH or cerebrospinal fluid is tightly regulated by tanycytes. These specialized ependymoglial cells that line the wall of the third ventricle are sealed by tight junctions, forming the blood hypothalamus barrier. Tanycytes send long processes to contact the wall of the fenestrated vessels in the ME, but also of the BBB vessels in the ARH.

In the ME as elsewhere, VEGFR signaling is not specific to blood vessels. Using different approaches, we have observed that a subset of tanycytes expresses VEGFR2, and that in mice, tanycytic VEGFR2 signaling is involved in the maintenance of body homeostasis. A potential role in metabolic diseases such as obesity is also being explored.

Altogether, our results suggest that within tanycytes, vascular-specific pathways are directly involved in the communication between hypothalamus and vasculature, and ultimately between brain and periphery.

TRIM47 is a crucial regulator of brain endothelial cell functions

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Cerebral small vessel disease (cSVD) is a leading cause of strokes and a major contributor to vascular dementia. Evidence indicates that BBB dysfunction may play a significant role in vascular dementia pathogenesis. However, the molecular determinants and responsible mechanisms are still not resolved. Recently, we reported, using a human genome-wide association study, an inverse correlation between the expression of an ubiquitin ligase, TRIM47, and extensive cSVD severity. TRIM47 is highly expressed in human and mouse brain endothelial cells (EC), indicative of its putative role at the BBB level.

We demonstrate that TRIM47 knockdown in human brain microvascular EC (HBMEC) impairs BBB properties with an increased permeability, decreased directed migration, and blockage of endothelial sprouting. TRIM47 loss led to profound alteration in the actin cytoskeleton organisation assembly via the inhibition of the RhoA/ROCK1/LIMK1 pathway.

By performing RNA-sequencing, we identify that loss of TRIM47 represses NRF2 associated gene expression in HBMEC. NRF2 is an important transcriptional regulator of antioxydant and anti-inflammatory enzymes. It binds to its inhibitor KEAP1 in the cytoplasm under normal conditions. Using proximity labeling (BioID) and co-immunoprecipitation assays, we report an interaction of TRIM47 with KEAP1. Repression of TRIM47 increases KEAP1 level. Finally, we showed that KEAP1 inhibitor treatment restores HBMEC functions impaired by TRIM47 loss. In summary, we report that TRIM47 dysregulation affects EC properties by dysregulating the RhoA/ROCK1 signaling via the NRF2/KEAP1 pathway. It may, per se, reduce stress resistance to EC at the BBB level.

Estrogen signaling contributes to Group B Streptococcal disruption and invasion of brain endothelial cells

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Bacterial meningitis is a serious life-threatening infection of the central nervous system (CNS) that occurs when blood-borne bacterial pathogens can disrupt the blood-brain barrier (BBB) and enter the CNS. The BBB is comprised of highly specialized brain endothelial cells (BEC) that serve to protect the CNS from toxins and pathogens while supporting proper brain function. Group B Streptococcus (GBS) is the leading cause of neonatal meningitis and mechanisms of how the BBB fails to protect the CNS during infection remain unclear. We have conducted microRNAseq on BECs either infected with GBS or mock infected and strikingly we found that globally microRNAs are downregulated. Estrogen signaling has been demonstrated to contribute to global microRNA downregulation and we hypothesize that estrogen signaling may play a role in GBS – BEC disruption. Our preliminary findings demonstrate that treatment of BECs with the estrogen receptor (ER) antagonist is sufficient to inhibit GBS invasion of BECs and rescue candidate microRNA expression. Additionally, we find that BECs treated with the ER agonist beta-estradiol is sufficient to reduce microRNA expression, reduce trans-endothelial electrical resistance, and increase rates of bacterial invasion. Our findings suggest that GBS may utilize estrogen signaling to gain access to the CNS. Future work will determine if rescue of microRNAs can restore BBB function during GBS infection. Additionally, we will determine if GBS produces an estrogen-like molecule to induce ER signaling or if GBS induces endogenous ER signaling. We show for the first time, that ER signaling can contribute to GBS invasive disease.

Vascular basement membrane laminins contribute to the functional integrity of the blood vessels

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The neurovascular unit (NVU) is the interconnection between the blood vessels and the surrounding neurons and is constituted by several cell layers, including endothelium, perivascular cells, astrocytes, and neurons, but also acellular layers that are mainly basement membranes (BMs). BMs underlie endothelium, encase pericytes and smooth muscle cells and mark the pial border and, as such, are in contact with several cellular layers of the NVU. Laminins are essential components of vascular BMs, but little is known about their contribution to the NVU. In the brain, laminin α4 and α5 occur in endothelial and mural BMs. To define their contribution to NVU integrity, we analysed blood vessel enriched, neuron depleted samples from laminin α4 knock out (Lama4 -/-) and endothelial-specific laminin α5 knock out (Tek-cre:Lama5 -/-) mice using single cell RNA sequencing. The data was clustered into endothelial, mural and myeloid cell types and unsupervised clustering revealed several sub-types of each category that were altered in the laminin knock out mice. Lama4 -/- endothelium showed enhanced large artery and reduced postcapillary venule sub-clusters. Accordingly, mural cells of Lama4 -/- mice presented augmented contractile gene signatures. Although devoid of laminin expression, resident myeloid cells exhibited an activated phenotype in Lama4 -/- . Analysis of Tek-cre:Lama5 -/- samples exhibited the opposite molecular and cellular phenotype to Lama4 -/- , with enhanced postcapillary venule marker expression and reduced contractile nature of perivascular cells. However myeloid cell populations were not altered in Tek-cre:Lama5 -/- samples compared to WT controls. Our data suggest direct and indirect roles for vascular laminins in the intercommunication between the endothelium, mural cells and myeloid populations associated with the arterial wall, all of which are required for the functional integrity of the NVU.

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An interplay between HIV infection and cerebrovascular toxicity of methamphetamine

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A substance use disorder (SUD) is a well-recognized risk factor for HIV-1 infection; however, little is known about molecular interactions between psychoactive substances and HIV-1 in the brain, and how they impact their long-term effects. The problem is important because SUD, including methamphetamine (METH) use, raises the risk of contracting or transmitting HIV-1 not only for individuals who inject the drug, but also for noninjecting users, as it is associated with an increase in risky sexual behavior. Moreover, METH use, either alone or in combination with other drugs, is associated with failure of viral suppression. People living with HIV-1 (PLWH) who engage in METH use display exacerbations in key pathophysiologic processes that are linked to faster clinical HIV-1 progression. Indeed, the combination of HIV-1 infection and METH dependence causes more profound neurocognitive deficits and structural brain abnormalities than either condition alone. METH use increases genomic activities to become transcriptional events related to neurodegeneration, dopaminergic deficits and decreased cognitive and executive functions. Our research has focused on the central hypothesis that the immunomodulatory impact of METH on BBB pericytes drives their HIV-1 infection and alters their interactions with neural progenitor cells (NPCs), which are located in perivascular niches in a close proximity to the BBB. We identified that both BBB pericytes and NPCs can be infected with HIV and characterized the signaling pathways by which exposure to METH and HIV contributes to aberrant neurogenesis, migration, and proliferation of NPCs. Overall, this research contributes to a better understanding of the influence of psychoactive substances on pericyte HIV-1 reservoirs in the CNS in order to design future therapies for reservoir clearance and a cure for HIV-1, especially for individuals living with HIV-1 who experience a SUD.
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Extracellular vesicle-mediated transfer of functional molecules from the periphery to the brain

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Extracellular vesicles (EVs) are emerging as important carriers for intercellular and interspecies communication and have been implicated in many biological processes. However, due to their small size and the difficulties in manipulating them, data on EV biology have thus far been based mainly on in vitro or indirect in vivo evidence. Therefore, the extent and pathogenic role of EV signaling in vivo, particularly with regard to the transfer of functional molecules, remains poorly understood. To overcome some of the major shortcomings in this field, we introduced the Cre-Lox system to establish a method to trace the functional transfer of RNA or protein by EVs in vivo. We showed that the expression of Cre recombinase in blood cells and bacteria leads to the release of EVs containing Cre mRNA and proteins, respectively. As a result, in mice with a Cre reporter background, the uptake of Cre-containing EVs leads to an irreversible induction of marker gene expression. This allowed us to identify a novel route of communication between the immune system, gut bacteria, and the brain, crossing the intestinal epithelial and blood-brain barriers.

Placenta-derived extracellular vesicles: their unique characteristics of the blood-brain barrier transport

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The placenta, an organ specific for pregnant women, secrets the unique nano-size particles which we call placenta-derived extracellular vesicles (pEVs). The pEVs encapsulate nucleic acid, e.g., miRNA, and functional proteins as message substances and play a role in the placenta-to-maternal organs signal transductions. An interesting report has shown that pregnancy causes a reduction in the brain gray matter region subserving social cognition (Nat Neurosci 20:287-296, 2017). This implies that pEVs could mediate the placenta-to-brain delivery of the message substances such as miRNA beyond the blood-brain barrier (BBB). In support of this notion, we found that the placenta-related miRNAs are present in the pregnant mouse brain as well as in blood-circulating extracellular vesicles. Thus, the purpose of the present study was to clarify the pEVs transport at the BBB. The pEVs were obtained from the human placental trophoblast cells (BeWo cells) by ultracentrifugation. The three-dimensional human brain microvasculature model was our originally constructed on a microfluidic device. We succeeded in visualizing the pEVs which were transported to the brain parenchymal cells across the brain microvasculature on a microfluidic device. We also identified the virus receptor as the potent transport system of pEVs at the BBB by proteomics- and gene knockout-based studies. In this symposium, I will introduce our recent data on the uniqueness of pEVs and the transport characteristics of pEVs at the BBB, which have been clarified by human BBB on-a-chip and proteomics. I will also propose a concept of pEVs-mediated placenta-to-brain signal transduction via the BBB.

Multi-omics characterization of the blood-brain barrier in molecular groups of ependymoma

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Ependymoma (EPN) represents the third most common pediatric CNS tumor. While recurrence rates remain high, systemic therapies have so far failed to lead to clinical benefit. A better understanding of pathophysiological blood-brain barrier (BBB) characteristics represents an important component in developing effective (pre-)clinical trials.

Our study seeks to increase knowledge of molecular EPN group-specific BBB compositions as a proof-of-concept for other brain tumor entities. Furthermore, we explore the correlation between BBB characteristics and their functional impact to adapt an established in silico model that currently predicts drug penetration over the healthy BBB.

T-distributed stochastic neighbor embedding (tSNE)-based clustering analyses using the most relevant tight junction and transporter gene sets revealed distinct molecular EPN group-specific expression patterns. While PDX models (n=20) showed high similarity with patient tumor samples, IUE mouse models (n=2) did not fully recapitulate these BBB characteristics. Single-cell analyses and spatial mapping of protein abundance allowed dissection of BBB gene expression patterns in endothelial cells (e.g. Claudin5). Functional validation on protein level showed that coherence of RNA and protein is BBB gene-dependent.

The differences in BBB markers between molecular EPN groups may partly explain drug resistance of aggressive EPN as especially ZFTA fusion-positive tumors are characterized by high tight junction expression suggestive of low BBB permeability. Our multi-omics approach is intended to develop a score that further complements our established in silico prediction tool for BBB drug penetration.

These findings will be validated in preclinical studies while molecular BBB characterization will be further expanded to other brain tumor entities.

Unraveling the role of biological barriers in the development of chemotherapy-induced peripheral neuropathy

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Although drug distribution into the central nervous system (CNS) has been extensively studied, little is known on how drugs are transported into the peripheral nervous system (PNS). It has been shown that blood-tissue barriers in the CNS and PNS differ in their morphological structures, including tight junctions and transporter expression profiles. However, the functional role of PNS barriers on target-site exposure compared to CNS barriers remains poorly understood, requiring systematic neuropharmacokinetic (neuroPK) evaluations. This study aimed to quantitatively assess and compare the extent of drug transport across the blood-brain barrier (BBB), blood-spinal cord barrier (BSCB), blood-nerve barrier (BNB), blood-dorsal root ganglia barrier (BDB), and parenchymal cellular barriers in respective tissues in rats using a set of 11 small molecular weight drugs. Two key neuroPK parameters, namely unbound tissue-to-plasma concentration ratio ($K_{p,uu}$) and unbound intracellular-to-extracellular concentration ratio ($K_{p,uu,cell}$), were estimated using the Combinatory Mapping Approach. We found that the extent of BDB and BNB transport was significantly greater than that of BBB and BSCB transport, with the difference in $K_{p,uu}$ up to 635-fold observed for vincristine. The extent of drug transport at the BDB was overall the highest. The discrepancy in cellular transport properties was also observed between the investigated PNS and CNS tissues without any universal pattern. This study sheds light on the key features of unbound drug disposition into the dorsal root ganglia and the sciatic nerve vs. brain and spinal cord, providing invaluable insight into the target-site pharmacological/toxicological effects and development of chemotherapy-induced peripheral neuropathy.

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A sticky situation: the influence of microvessel mechanics on cerebral malaria pathogenesis 2. Brain barriers in disease

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Cerebral malaria is the most severe and fatal consequence of malaria infection and occurs when Plasmodium falciparum-infected red blood cells (iRBCs) become sequestered in the brain vasculature, leading to vascular obstruction and disruption of the blood brain barrier (BBB). Interactions between iRBCs and cerebral endothelial cells that line the blood vessels occur under dynamic mechanical conditions due to changing flow conditions within vessels and heterogeneity in tissue stiffness surrounding the vessels. We utilise both 2D brain endothelial monolayers and 3D bio-engineered cerebral blood vessels that allow for the visualisation of iRBC binding, disruption of flow and BBB breakdown in real-time. By customising both the matrix composition, geometry and flow rate in these models we can precisely tune the mechanics of our system to observe their role in endothelial cell function and cerebral malaria pathogenesis. We reveal that when brain endothelial cells and vessels are cultured with iRBC products they demonstrate increased permeability and altered adherens junctions and increased actomyosin contractility, ICAM-1 expression. Furthermore, when cultured on collagen I coated matrices or substrates, brain endothelial cells show reduced disruption by these iRBC toxins. Our results demonstrate that both intracellular mechanics (cytoskeletal organisation) and extracellular mechanics (cell-matrix interactions) play critical roles in regulating brain endothelial cell and BBB function in both health and disease. Further work is currently ongoing to directly measure changes to endothelial monolayers and 3D vessel mechanics, via atomic force and Brillouin microscopy, when cultured on substrates of varying stiffness and after iRBC addition.

Open pathways for cerebrospinal fluid outflow at the cribriform plate

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Routes along the olfactory nerves crossing the cribriform plate to lymphatic vessels in the nasal submucosa are critical cerebrospinal fluid (CSF) outflow pathways. However, it remains unclear how fluid pathways along the nerves connect to the lymphatic vessels and where the arachnoid barrier is breached. Here, we anatomically defined the connections between the subarachnoid space (SAS) of the central nervous system and the lymphatic system.

PEGylated fluorescent microbeads were infused into the CSF space of Prox1-GFP reporter mice to identify the CSF outflow pathways. A labeled anti-CD31 vascular antibody was infused into the cisterna magna to study the connections to the lymphatic vessels in the olfactory region. In some samples, we did immunofluorescence staining on decalcified sections to detect the arachnoid barrier.

PEG microbeads were within lymphatic vessels in the nasal submucosa and the lumen of lymphatic vessels that crossed the cribriform plate alongside the olfactory nerves. The labeled antibody revealed a continuous functional network of lymphatic vessels draining CSF from the SAS through the nasal submucosa. In addition, we observed discontinuity of the arachnoid barrier at the olfactory region.

Micron-sized PEG beads are suitable for studying CSF bulk flow and clearance pathways. We observed direct and open connections from the CSF space to the lymphatic vessels crossing the cribriform plate and in the nasal submucosa. Finally, the discontinuous distribution of the arachnoid barrier in this area could explain how CSF within the SAS accesses the lymphatic system.