The PDA Annual Conference 2025

May 29-30, 2025

Johns Hopkins University (Homewood & East Baltimore Campuses)

ABSTRACTS

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P

Posters

There is a total of 33 poster presentations in the PDA Annual Conference of 2025. The poster session will take place at the Turner Concourse, in the East Baltimore Campus, from 1:00 PM to 2:30 PM on Friday May 30th.

The judge committe will be composed by the following six judges: Dr. Shreya Banerjee, Dr. Jonathan Coulter, Dr. Ryuya Fukunaga, Dr. Joanne Kotelawala, Dr. Iratxe Niño Adan, Dr. Andrea Pereyra, Dr. Meenakshi Pillai and Dr. Sunayana Syed.

P.1

Evaluation of Efficacy of Hypoxia-Inducible Factor-1 alpha mRNA on Dermal Flap Survival in Sprague Dawley Rats

Farzad Mokhtari-Esbuie
(SOM: Surgery)

Work with: Mirza Farhana Iqbal Chowdhury, Amid Yazdani, Azadeh Nourmohammadi, Sepideh Siadati, John M Abraham, and John W Harmon

Abstract *Introduction*: Dermal flaps are a widely used approach for managing large wounds and skin defects. However, ischemia and necrosis of portions of the flap remain significant challenges. Hypoxia-Inducible Factor-1 (HIF-1) is a transcription factor that upregulates the expression of numerous genes including VEGF (vascular endothelial growth factor), PGF (placental growth factor), and ANGPT1 (angiopoietin) under hypoxic conditions, enhancing angiogenesis in ischemic environments such as ermal flaps. We investigated efficacy of HIF-1 mRNA for promoting dermal flap survival.

Methods: Five Sprague Dawley rats were included in this study. Two rectangular caudally based dermal flaps, each measuring 1.5 cm in width and 7.5 cm in length, were created on the dorsum of each rat. The treatment flaps received eight injections of HIF-1 mRNA (5 g) encapsulated in LNP in defined spots, 7 days before surgery, on the day of surgery, and 7 days after surgery. The control flaps also received eight injections of Polypeptide Lipid Nanoparticles (LNP) alone at the same point and locations. Flap necrosis was assessed through imaging on postoperative days, followed by histological evaluation.

Results: Analysis of flap survival demonstrated that the percentage of surviving skin was significantly higher in the HIF-1 mRNA-treated group compared to the LNP group on postoperative days 7 and 14 (90.60% vs 73.08%, P=0.004). Histological analysis revealed reduced inflammation, ischemic damage, and necrosis in the middle and distal portions of flaps treated with HIF-1 mRNA, with improved vascularity compared to the control group.

Conclusions: HIF-1 mRNA encapsulated in LNP significantly improved dermal flap survival.

P.2

Non-Invasive Monitoring of rAAV-Mediated Gene Expression Using Chemical Exchange Saturation Transfer (CEST) MRI

Zinia Mohanta
(SOM: Radiology & Kennedy Krieger Institute)

Work with: Aruna Singh, Hernando Lopez Bertoni, Sophie Sall, Julia Stabinska, Irini Manoli, Hilary Vernon, Charles P. Venditti, Assaf A. Gilad, and Michael T. McMahon

Abstract Non-invasive imaging of gene expression remains a critical challenge in gene therapy. Here we report the first in vivo demonstration of a genetically encoded chemical exchange saturation transfer (CEST) MRI reporter expressed from a recombinant adeno-associated virus (rAAV). The genetically encoded peptide-based reporter, superCESTide, enables detection of transgene expression without the need for exogenous contrast agents. Mice were systemically administered rAAV encoding superCESTide and fluorescence reporter, tdTomato, and evaluated using CEST MRI, fluorescence imaging, and RT-PCR. CEST MRI revealed dose-dependent signal enhancement in the liver, with a strong correlation between CEST contrast and superCESTide expression ($R = 0.75$, $P < 0.05$). Mice receiving the highest dose (1.4×1013 vg/kg) exhibited the strongest MTRasym signal (mean $= 0.0225$), and histogram analyses showed distinct expression patterns. These results establish CEST MRI as a powerful, non-invasive method for monitoring rAAV-mediated transgene expression in vivo, opening new possibilities for imaging-guided gene therapy.

P.3

In Vivo MPI tracking of ICV-injected human mesenchymal stem cells in an EAE mouse model

Kritika Sood
(SOM: Radiology)

Work with: A. Thomas, J.W.M. Bulte, and A. Shakeri-Zadeh

Abstract Human mesenchymal stem cells (hMSCs) are a promising therapy for multiple sclerosis (MS) due to their immunomodulatory properties, clinical safety, and potential to promote remyelination. Among CNS delivery methods, intracerebroventricular (ICV) injection allows direct access to cerebrospinal fluid (CSF), bypassing the blood-brain barrier and enabling widespread CNS distribution while minimizing systemic exposure.

To optimize stem cell therapies, *in vivo* tracking of biodistribution is essential. Magnetic particle imaging (MPI) offers a non-invasive, quantitative, and background-free method for tracking magnetically labeled cells, addressing the limitations of MRI.

In this study, we evaluated the biodistribution of Ferucarbotran-labeled hMSCs following ICV administration in a mouse model of experimental autoimmune encephalomyelitis (EAE), a widely used model of MS. Fourteen days post-EAE induction, mice ($n = 3$) received ICV injections of 1.8×10 hMSCs labeled with Ferucarbotran (25 µg Fe/mL) and poly-L-lysine (3 µg/mL). MPI and CT were performed over 7 days to monitor cell fate.

On Day 1, *in vivo* 2D MPI showed labeled hMSCs confined primarily to the brain, with minimal signal in peripheral organs. MPI-based cytometry demonstrated label stability and retention in the brain over 7 days. By Day 7, 3D MPI/CT imaging revealed hMSCs had migrated to the cerebellum and spinal cord regions, which were not involved on Day 1. *Ex vivo* MPI and histological analysis of brain sections stained with Prussian Blue and anti-HuNA confirmed these observations.

These findings validate ICV as a targeted delivery route for CNS-specific therapy and highlight MPI as a powerful tool for quantitative, longitudinal tracking of cell-based interventions in neuroinflammatory diseases like MS.

P.4

Long-Term Therapeutic Effects of Topical Recombinant Human Nerve Growth Factor in Patients with Refractory Epitheliopathy

Meltem Yashar
(SOM: Ophthalmology)

Work with: Meltem Yashar, Meron Haile, Ugur Tunc, Pranav Kotamraju, Cynthia Wang, Maria Jose Hernandez, and Sezen Karakus

Abstract *Purpose*: To evaluate the long-term therapeutic effects of topical recombinant human nerve growth factor (rhNGF) in stage I neurotrophic keratitis (NK).

Methods: Medical records of patients treated with topical rhNGF and followed for at least 12 months at the Johns Hopkins Wilmer Eye Institute between 2019-2024, were retrospectively reviewed. Patients who received a second treatment course were also assessed. Data included demographics, ocular symptoms, medical history (ocular and systemic), and corneal staining score. Measurements from the worse eye were used to compare pre- and post-treatment values. Full response was defined as a corneal staining score of 0 or 1. Partial response was at least a one-grade improvement but with a final score >1. Relapse was defined as a return to baseline or an increase resulting in a score of ≥2.

Results: A total of 20 patients were identified (90% female; mean [SD] age 70.5 [11.0] years), with a mean (SD) follow-up duration of 26.6 (9.3) months. Based on corneal staining score, 11 (55%) achieved a full response, three (15%) a partial response, and 6 (30%) showed no response upon completion of treatment. Among 14 patients with full or partial responses, 5 (36%) relapsed by month 6, four (28.5%) by month 12, three (21%) by month 24 or later. Only one patient continued to show sustained improvement after 36 months. Six patients received a second course, with a mean interval of 19.4 months after initial treatment (range: 8–30). Following the second course, 3 (50%) achieved a full response that was sustained for at least 12 months. None of the patients progressed to stage 2 or 3 NK during the follow up.

Conclusions: This limited case series demonstrated the therapeutic effect of rhNGF in stage I NK lasts 6–12 months. A second course may provide benefits, with effects lasting up to 12 months.

P.5

mRNA Methylation Dynamics Following Sciatic Nerve Injury: An In Vivo Study

Mehmet Can Sari
(SOM: Neurology)

Work with: Xindan Hu, Ashok Patowary, Riki Kawaguchi, and Ahmet Hoke

Abstract *Background*: Peripheral nerve injuries (PNIs) affect millions annually and often result in chronic disability due to ncomplete regeneration. Schwann cells are central to nerve repair, yet the epitranscriptomic mechanisms regulating their function remain poorly understood. Among >170 RNA modifications, N6- ethyladenosine (m6A) is the most abundant and dynamically regulated. Our previous work showed that Schwann cell-specific deletion of Mettl14, a key m6A methyltransferase, results in progressive demyelination, axonal degeneration, and impaired regeneration, highlighting the essential role of m6A in peripheral nerve maintenance and repair.

Methods: To investigate m6A dynamics post-injury, we employed Oxford Nanopore m6A RNA sequencing (m6A-Seq) in wild- type mice following sciatic nerve transection, focusing on day 1 post-injury-a key time point identified via western blot, dot blot, and m6A colorimetric assays. We also analyzing age-dependent methylation patterns across four developmental stages: P21, 2 months, 4 months, and 12 months. In parallel, we performed bulk RNA-seq in Mettl14 conditional knockout (cKO) mice to assess gene expression changes.

Results: We observed a rapid increase in global m6A methylation after injury, increasing at day 1, peaking at day 3 and sustained through day 7. In Mettl14 cKO nerves, bulk RNA-seq revealed down-regulation of genes critical for myelination, axonal regeneration, and immune signaling. Ongoing integration of m6A-Seq with bulk RNA-seq will define m6A-dependent transcriptional programs driving nerve repair.

Conclusions: Our findings suggest that m6A methylation is a key post-transcriptional regulator of the peripheral nerve injury response. Loss of Mettl14 disrupts early transcriptional programs necessary for Schwann cell proliferation, immune recruitment, and axonal regeneration. These insights provide a foundation for RNA-targeted therapeutic strategies aimed at enhancing peripheral nerve regeneration.

P.6

Evaluating the gene expression profile of human nerve injury

Maaz Khan
(SOM: Neurology)

Work with: Ahmet Hoke, Xindan Hu, Sami Tuffaha, Allan Belzberg, and Riki Kawaguchi

Abstract *Background*: Rodent models are typically used to investigate human peripheral nerve injury. However, delayed coaptation for as little as 8 weeks in rodents significantly impairs recovery, whereas humans can withstand longer durations of denervation. Hence, little is known about how the gene expression profile of rodents following denervation maps temporally onto humans.

Methods: Humans: bulk RNA-seq was performed on nerve and muscle samples from 48 unique patients undergoing peripheral nerve surgery (duration of denervation: control, <1wk, 2-5mo, 6-11mo, 12mo-5yr).

Rats: the sciatic nerve was transected, and distal sciatic nerve samples were analyzed using DNA microarrays and qPCR (duration of denervation: control, 1d, 3d, 7d, 14d, 1mo, 2mo, 3mo, 6mo). Bulk RNA-seq and snRNA-seq are now being performed for further analysis.

Results: The majority of patients were male (76.9%), white (65.2%) and underwent traumatic mechanisms of nerve injury (65.6%), of which most were vehicular accidents (61.9%). The median denervation duration until surgery was 7.0mo (IQR: 5.2mo to 10.6mo). Patients were followed up for an average of 13.4mo.

In human denervation, RUV analysis revealed a triphasic temporal pattern of RNA expression, initially with an increased innate immune response and reduced energy production (2-5mo), followed by a change to an adaptive immune response (6-11mo), and finally somewhat reversion to the original state with increased energy production but reduced neurogenesis (12mo-5yr).

Gene ontology pathway analysis revealed persistent downregulation of biosynthetic pathways involved in cholesterol, lipid, secondary alcohol and acetyl coenzyme A metabolism in both humans and rats. In rats, cellular proliferation and immune signaling were upregulated between 1d-14d of denervation, with ficolin-1 RNA upregulated between 1d-7d, whilst pyroptosis was upregulated at 14d.

Overlap analysis showed that 3mo, 6mo, 12mo-5yr of human denervation corresponded most significantly to 3d-7d, 7d-14d, and 6mo of rat denervation, respectively.

Discussion: Denervation induces a differential RNA expression profile that maps temporally between humans and rodents.

P.7

Methylated DNA Markers for the Detection of Cervical Lesions at High Risk of Malignant Progression

Roshni Saravanan
(SOM: Oncology)

Work with: Mary Jo Fackler, Madison Pleas, Liqun Zhang, Deyin Xing, Suzette Jordaan, Eunice Van Den Berg, Pamela Michelow, Reubina Wadee, Maureen Joffe, Carl Chen, Syed Ali, Leslie Cope, and Saraswati Sukumar

Abstract *Background*: Cervical cancer remains a leading cause of death, particularly in developing countries. WHO screening guidelines recommend HPV detection to identify women at risk of developing cervical cancer. While HPV testing identifies those at risk it does not specifically distinguish individuals with neoplasia. We investigated whether a quantitative molecular test that measures methylated DNA markers could identify high risk lesions in the cervix with accuracy.

Results: Marker discovery was performed in TCGA-CESC Infinium Methylation 450K Array database and verified in three other public datasets. The panel was technically validated using Quantitative Multiplex-Methylation Specific PCR (QM-MSP) in tissue sections (N = 252) and cervical smears (N = 244) from the U. S., South Africa, and Vietnam. The gene panel consisted of FMN2, EDNRB, ZNF671, TBXT, and MOS. Cervical tissue samples showed highly significant differential methylation in SCC with 100% sensitivity, specificity of 91% to 96%, and ROC AUC of 1.000 compared to benign cervical tissue, and CIN2/3 with sensitivity of 55% to 89%, specificity of 93% to 96%, and a ROC AUC ranging from 0.793 to 0.99 compared to CIN1. In cervical smears, the marker panel detected SCC at a sensitivity of 87%, specificity 95%, and ROC AUC 0.925 compared to normal, and HSIL at a sensitivity of 70%, specificity 94% and ROC-AUC 0.884 compared to LSIL/normal. In a pilot study of blinded cervical smears in cytolyte (N=250), QM-MSP distinguished HSILs and LSIL/NIELs with ROC-AUC of 0.886, sensitivity 75.8% and specificity 90%. Modifications to the technique are underway to improve sensitivity of detection of HSILs. HPV-negative HSILs were also frequently hypermethylated.

Conclusions: This 5-marker panel detected SCC and HSIL in cervical smears with a high level of sensitivity and specificity. Molecular tests with the ability to rapidly detect high-risk HSIL will lead to timely treatment for those in need and prevent unnecessary procedures in women with low-risk lesions throughout the world.

P.8

Expressivity of Graph Neural Networks: ReLU vs. Sigmoid GNNs

Josué Tonelli-Cueto
(WSE: Applied Mathematics and Statistics)

Work with: Sammy Khalife

Abstract Graph Neural Networks (GNNs) form a powerful computational framework for machine learning on graphs with many applications. However, many open questions exist regarding how the chosen activation function affects expressivity, trainability and generability. In this short communication, we show that ReLU GNNs are more expressive than Sigmoid GNNs. However, we also show that Sigmoid GNNs are expressive enough so that this gap is not significant in practice, meaning that the comparison between ReLU and Sigmoid GNNs should be done in terms of trainability and generability. This is work accepted at ICLR'25.

P.9

Targeting Aberrant N-Glycosylation in ZIP8 A391T-Associated Crohn's Disease: A Potential Pathway for Personalized Treatment

Vartika Tomar
(SOM: Medicine)

Work with: Joanna Melia

Abstract Manganese is essential for the activity of several key enzymes such as glycosyltransferases, and manganese deficiency can cause glycosylation defects due to disruption of manganese-dependent enzymes, such as glycosyltransferases, manganese -dependent antioxidant enzymes, such as manganese superoxide dismutase (MnSOD) and impaired Golgi apparatus functions, leading to impaired glycosylation of proteins and lipids. Aberrant glycosylation has several clinical implications such as neurodevelopment issues, growth and skeletal abnormalities, immune system impairment and congenital disorders of glycosylation (CDG).

The missense mutation ZIP8 A391T (also known as ZIP8 391T) in the manganese transporter ZIP8 has been implicated in altered manganese homeostasis and is associated with various complex diseases, including Crohn's disease. This mutation affects the transporter's ability to properly regulate manganese levels, leading to impaired manganese-dependent processes, particularly in glycosylation pathways. In patients with Crohn's disease, this genotype is linked to abnormal N-glycosylation and changes in intestinal glycan patterns, characterized by increased truncated N-glycans. The rate limiting substrate of the N-glycosylation cascade is N-acetylglucosamine (GlcNAc); in this study, we hypothesize that abnormal N-glycosylation contributes to the pathogenesis of ZIP8 A391T-associated Crohn's disease. Using a mouse model of ZIP8 391T, we recapitulated the intestinal glycophenotype observed in patients, demonstrating the impact of altered Mn metabolism on disease pathology. Zip8 393T-KI mice were treated with GlcNAc (0.25 mg/ml) in drinking water for 7 days, restoring L-PHA (Phaseolus vulgaris leucoagglutinin) staining in IECs (ileal epithelial compartment). Furthermore, GlcNAc supplementation also corrected N-glycosylation defects, susceptibility to colitis, ameliorated intestinal permeability and bile acid homeostasis. These findings suggest that ZIP8 391-Thr impairs N-glycosylation, contributing to disease pathogenesis, and challenge the notion that CDGs are confined to rare diseases. Importantly, the glycosylation defect can be targeted with monosaccharide supplementation, offering a potential avenue for genotype-driven, personalized treatment.

P.10

Investigating the Function of Antigen-Presenting Oligodendrocyte Lineage Cells in CNS Demyelination

Jingwen Hu
(SOM: Neurology)

Work with: Sachin P. Gadani, Matthew D. Smith, Krista C. Solem, Aayush Pokharel, Ryan Z. Yue, Nikhila R. Reddymalla, Jackson Mace, Riley B. Catenacci, Danny Galleguillos, and Peter A. Calabresi.

Abstract Multiple sclerosis (MS) is an immune-mediated demyelinating condition in the central nervous system (CNS). Oligodendrocyte lineage cells (OLGs) that contribute to myelin formation were traditionally considered to be passively targeted by immune cells during inflammatory immune attacks. Recent studies observed OLGs up-regulate genes related to antigen presentation in MS lesions and animal models such as experimental autoimmune encephalomyelitis (EAE), indicating OLGs may play a role in immune response within the CNS. To explore the function of OLGs during inflammatory demyelination, we performed single-nucleus RNA sequencing (snRNA seq) on lumbar spinal cord tissues from EAE mice. Multiple OLG populations were identified to express high levels of genes involved in major histocompatibility (MHC) class I and class II antigen presentation pathways and inflammation-response genes. Notably, these antigen-presenting OLGs (AP-OLGs) up-regulated genes for co-inhibitory (Cd274) as well as co-stimulatory (Icam1) signaling, suggesting that OLGs might actively modulate immune reactions under inflammation. Using TdTomato reporter lines for MHC class I (B2M) and MHC class II (CD74) molecules recently developed in the lab, we were able to track AP-OLGs and observed direct interactions between AP-OLGs and T cells in spinal cord lesions from mice that were adoptively transferred myelin-reactive polarized Th17 cells from 2D2 TCR transgenic mice following lysolecithin-induced focal demyelination. The functions of class I AP-OLGs in disease progression and recovery were further examined by conditionally knocking out the MHC I gene in oligodendrocyte precursor cells (OPCs) with PDGFR-Cre ER ; H2-Kb flox/flox mice in animal models of MS. Our studies on AP-OLGs may bring a new understanding of the pathogenesis of MS and provide evidence to consider targeting subsets of antigen-presenting immune activating oligodendrocytes under inflammation as a therapeutic avenue.

P.11

Changes in membrane fluidity drive directional migration after epithelial-to-mesenchymal transition in neural crest cells

Allison Mancini
(SOM: Cell Biology)

Work with: Erdnic Sezgin, and Michael L. Piacentino

Abstract During development, a transient group of stem cells known as the neural crest (NC) delaminate and migrate away from the embryonic midline before differentiating into diverse craniofacial structures that include cranial neurons, glia, and connective tissues. We recently found that to achieve this migratory phenotype, NC cells must reprogram their lipid metabolism, which enables them to undergo an epithelial-to-mesenchymal transition (EMT). Expression of nSMase2, an enzyme that hydrolyzes sphingomyelin into ceramide in the plasma membrane, is specifically activated in NC cells at the onset of EMT and is necessary for this transition. However, the biophysical effects of spatiotemporally controlled metabolic programming, and the precise role of nSMase2 activation in this process, remains poorly understood. Using spectral imaging and single-particle tracking, we show that NC cells increase their membrane fluidity during EMT, and that nSMase2 is required for this fluidity increase. Surprisingly, this nSMase2-dependent membrane fluidity is not a determinant of migratory speed, but instead is required for effective directionality during migration. Our results demonstrate how ceramide production coordinates migration by changing the biophysical properties of the plasma membrane to enable directionality. These studies show how gaining a finer understanding of the molecular and biophysical mechanisms that drive EMT and migration will reveal novel avenues to diagnose and treat conditions ranging from congenital disorders to cancer metastasis.

P.12

Patient-Derived IgM Autoantibody Reverses autoimmune diabetes in NOD Mice

Rafid Al-Hallaf
(SOM: Medicine & Pathology)

Abstract Type 1 diabetes (T1D) is an autoimmune disease driven by autoreactive lymphocytes that destroy insulin-producing beta cells, necessitating lifelong insulin therapy. The widely used NOD mouse model has been instrumental in understanding mechanisms of autoimmune diabetes and assessing therapeutic modalities. A major challenge that remains elusive, however, is lack of specificity of immunotherapies due to difficulty in distinguishing autoreactive T cells from non-self-reactive T cells. Hence, despite advances in identifying autoantigens and autoimmune mechanisms, most immunotherapies lack efficacy or cause broad immunosuppression. Here, we show that x-mAb, a germline-encoded IgM autoantibody derived from dual-expresser lymphocytes in T1D patients, prevents disease progression and induces remission in newly diabetic NOD mice. Histological analysis confirmed preservation of islets in prediabetic mice by x-mAb and and reduced insulitis in cured mice. x-mAb selectively targets pancreatic tissue-resident memory (Trm) T cells, including islet-specific CD4+ and CD8+ cells identified by tetramer staining and functionally by congenic adoptive transfer. Treatment downregulates CD69, reducing Trm cell retention, while promoting immune tolerance by enhancing apoptotic cell clearance, inducing IL-10, and expanding regulatory T cells (Tregs). Notably, x-mAb identified analogous islet-specific Trm cells in human T1D, underscoring its translational potential. These findings reveal an unrecognized role for dual-expresser lymphocytes and their IgM products in modulating autoimmunity. By selectively targeting pathogenic islet specific Trm cells while preserving immune function, x-mAb offers a promising, precision-based therapeutic strategy for T1D.

P.13

Hippocampal CA1: Early Site of Tau Tangles Associated with Amyloid Aggregates in a Young Population

Rocio Fernanda Rodriguez Reyes
(SOM: Neuropathology)

Abstract *Background*: CA1 in the hippocampus is a relevant structure in cognitive processes and long-term memory consolidation. It is known in primary age-related tauopathy, phospho-tau tends to aggregate more in CA2 than in CA1 relative to Alzheimer's disease, but it is still unknown if this distribution is the same in young, healthy populations without clinical neurodegenerative disease and whether this is associated with Alzheimer pathology or risk factors.

Methods: Of more than 100 individuals under the age of 65 from the Johns Hopkins Brain Bank, 87 cases had phospho-tau positive pathology in the hippocampus. We developed a semiquantitative method to quantify phospho-tau density in CA1 and CA2, which we correlated with age, sex, race, Amyloid and ApoE genotype.

Results: Overall, in our cohort older patients were found to have higher tau aggregates, with more found in CA1 than in CA2. Amyloid positivity had a stronger association with tau aggregate density. The preliminary ApoE results were that ApoE4 carriers have more deposits in CA1 than in CA2. Overall, no significance was observed at the race distribution even though we have a small tendency to have more tau aggregates in white population. There were no differences by biologic sex.

Conclusions: In our young cohort encompassing a wide age range without diagnosed neurodegenerative disease, we observed that tau pathology is more abundant in CA1 than in CA2. Both amyloid and ApoE4 genotype were associated with greater tau pathology in CA1 compared to CA2. This study supports that the initial tau deposition in CA1 and CA2 during aging is influenced by the presence of amyloid and ApoE4 genotype, recapitulating the patterns of tau pathology observed in older individuals with Alzheimer's disease and primary age-related tauopathy.

P.14

Bioengineering CAR myeloid progenitor therapies from improved human blastomere-like stem cells

Willem Buys
(SOM: Pediatric Oncology)

Work with: Ludovic Zimmerlin, Sanskruti Deshmukh, and Elias T. Zambidis,

Abstract *Background*: Due to their excellent homing beyond physiological barriers, cytokine production, and antigen-presentation, CAR myeloid immune therapies may revert the immunosuppressive microenvironment of many tumors to enable host or co-transfused therapeutic lymphocytes. Human induced pluripotent stem cell (hiPSC)-derived myeloid progenitors are particularly promising due to their practically unlimited availability, facile stable gene editing, freeze-thaw tolerance, extended effector cell release, and auto-tolerance induction.

Methods: Feeder-, xeno-, and transgene-free reprogrammed hiPSC are reverted to a blastomere-like hybrid stem cell state using a chemically defined 'cGMP-ready' modification of our published tankyrase-inhibitor regulated naïve (TIRN) protocol. I have developed a 2D protocol to differentiate TIRN stem cells towards committed granulocyte-macrophage progenitors. Effector cell cytokine production, phagocytosis, respiratory burst, and chemotaxis of derived effector cells were compared to conventional iPSC-progeny.

Results: TIRN stem cells reliably generate approx. 10-fold more viable CD33/34/45 progenitors and downstream CD45/11b effector cells per input cell number and per culture volume, than conventional iPSC. TIRN stem cell-derived myeloid effector cells perform LPS-stimulated TNF production and phagocytosis of E. coli at a similar efficiency compared to conventional hiPSC-progeny. TIRN stem cell derived cells migrate along a chemotactic gradient and undergo respiratory burst at a greater efficiency than even primary adult whole blood-derived monocytes.

Conclusions: TIRN stem cells differentiate at manifold greater efficiency towards lineages of all three germ-layers, including hematopoietic mesoderm. This improvement may reduce bioreactor volumes from approx. 50 liter per therapy unit to a much more economically feasible 5 liters. TIRN stem cell-derived myeloid cells perform crucial immune functions at a similar or greater efficacy compared to conventional iPSC. By functionalizing cells with an adaptor-based uniCAR, myeloid cells can be easily re-targeted against a range of solid tumors, improving therapy control and possibly allowing the use of one iPSC-derived unmatched progenitor line against a range of cancer types.

P.15

Tissue transcriptomics of endomyocardial biopsies reveals widespread molecular perturbations independent of leukocyte-rich loci in human myocarditis

Charles Cohen
(SOM: Cardiology)

Work with: Harriet J. He, Kevin C. Bermea, Sylvie T. Rousseau, Marc K. Halushka, and Luigi Adamo

Abstract *Introduction*: Myocarditis is an inflammatory disease of the cardiac muscle, with a mortality rate of over 400,000 annually. Myocarditis has traditionally been viewed as a focal disease, characterized by localized immune infiltrates and necrosis. The gold-standard for diagnosing the disease is endomyocardial biopsy (EMBx). However, EMBx fails to detect myocarditis in >50% of cases, when focal immune cell infiltrates are missed.Hypothesis: We propose that myocarditis is not a focal disease, but rather a diffuse disease of the entire cardiac muscle.

Aim: To assess whether myocarditis exhibits widespread transcriptional dysregulation independent of immune cell localization using spatial transcriptomics.

Methods: We performed spatial transcriptomics (10X Visium-FFPE) on EMBx from patients with myocarditis of various etiologies (n = 10), HFpEF, (Controls, n = 12) and borderline myocarditis (n = 15). Differential gene expression analysis was computed using multiple algorithms to determine the top common differentially expressed genes (DEGs) among experimental groups. Ingenuity Pathway Analysis (IPA) was subsequently performed to assess the biological significance of these findings.

Results: We identified 16,802 barcoded spots from all EMBx samples, whereby UMAP projections showed myocarditis tissue as transcriptionally distinct to controls. Differential expression analysis revealed known and novel myocarditis-associated genes. Samples from borderline myocarditis exhibited an intermediate transcriptional state. Leukocyte-rich regions were removed, and spots were subsequently enriched for cardiomyocytes. DEGs confirmed widespread transcriptional dysregulation independent of immune cells, congruent with IPA showing the dysregulation of unexpected biological pathways.

Conclusions: This seminal spatial transcriptomics study of human myocarditis demonstrates that myocarditis is a diffuse pathological state independent of immune cell foci, with dysregulation of novel pathways not currently described. These findings provide a renewed perspective into the biology of myocarditis and provide a framework for developing novel diagnostics, which could surpass traditional histology-based criteria.

P.16

Photopatterning hyaluronic acid matrices for cellular mimicry of neuronal circuits

Rohan Choraghe
(SOM: Neuropathology)

Work with: Yush Kapoor, Sharanyan Raghavan, Chris Cottone, Hyeoncheol Park, Junjie Chen, Xingde Li, Yun Chen, and David Nauen

Abstract Functions of neural tissue are made possible by precise anatomic arrangements of cells. Mimicking these patterns is essential for modeling diseased neuronal circuits in vitro. We developed a system for finding regional boundaries in histology photomicrographs and recreating these in cell growth matrices with patterned stiffness. Based on hyaluronic acid, the primary component of the brain extracellular matrix, the matrix is cross-linked using blue light, with riboflavin (vitamin B2) as a catalyst. Exposure to more light causes more crosslinking, increasing matrix stiffness up to 50 kPa. The hardware is assembled from low-cost off-the-shelf components, and the open-source software framework performs photocuring to create regions seen in histology, using a menu of shapes, or following any input image. We demonstrate examples using the PC-12 neuronal cell line. The system provides a means of patterning cell growth to recreate histology and neural circuits in vitro.

P.17

Evaluating patient awareness of upper extremity reconstructive procedures for cervical spinal cord injuries

Fares Lebbos
(SOM: Plastic and Reconstructive Surgery)

Work with: Rachana Suresh, Alec J. Chen, Mohammed Shahid, Mark A Poisler, Jeffrey Khong, Kitae Eric Park, William Padovano, Sami H. Tuffaha, and Ala Elhelali

Abstract *Background*: Upper extremity reconstructive (UER) procedures can improve motor function and manage spasticity and contractures in patients with cervical spinal cord injury (CSCI), often leading to high patient satisfaction. However, the uptake of these surgeries remains low. Limited knowledge about UER may be a barrier to surgery. This study aimed to assess awareness of UER surgeries among individuals with CSCI.

Methods: A cross-sectional survey was administered via a public link on the REDCap platform. Participants were recruited through social media and peer networks (PatientsLikeMe, Reddit, Discord). The survey evaluated awareness of UER procedures, sources of information, timing of learning post-injury, attitudes toward surgery, barriers to care, healthcare provider interactions, and knowledge of the specialists performing these procedures.

Results: Eighty-five participants (mean age: 40.3 ± 16.4 years; 64.7% men) responded. Most had injuries at C3–C5 (54.1%). Nearly all reported upper extremity weakness (96.5%), with 80% experiencing rigidity and 76.5% spasticity. Overall, 44.7% were aware of UER procedures. Tendon transfers (78.9%) and nerve transfers (68.4%) were the most recognized interventions. The most common sources of information were from online platforms (60.5%) and healthcare providers (57.9%). Despite this awareness, only 13.2% of participants had undergone any UER surgery. Among those, nerve transfers were the most common (60%). Reported barriers to undergoing surgery included fear or anxiety about the procedure (53.1%), difficulty accessing specialized surgeons nearby (15.6%), and financial or insurance-related obstacles (15.6%).*Conclusions*: Fewer than half of the participants in our survey were aware of UER procedures available to improve function after CSCI and only 13.2% undergone surgery. These results highlight significant gaps in awareness and access to UER care, underscoring the need for targeted patient education and healthcare system interventions to increase utilization of these potentially life-enhancing procedures.

P.18

Synthesis of amyloid PET images based on structural MRI data using specialized VQGAN

Zongpai Zhang
(SOM: Radiology)

Work with: Jingpu Wu, Puyang Wang, Keyi Chai, Shanshan Jiang, Chiadi Onyike, and Jinyuan Zhou

Abstract *Motivation*: Alzheimer's disease (AD) is marked by amyloid-beta plaques, typically detected through amyloid-PET imaging, which is expensive and limited in availability. Developing a more accessible diagnostic tool is essential for early detection and monitoring.

Goals: This project aims to create a deep learning model that synthesizes amyloid-PET images from widely available MRI scans, offering a cost-effective, non-invasive alternative.

Approach: A specialized VQGAN is trained to map MRI features to amyloid-PET images, utilizing datasets from patients across various stages of AD.

Results: The model demonstrates accurate amyloid-PET synthesis, showing potential for early diagnosis and broad clinical application.

P.19

Developing Genetic Manipulation Tools for The Gut Microbiota Member Turicibacter sanguinis

Arren Liu
(SOM: Biological Chemistry / Molecular & Comparative Pathobiology)

Work with: Yutong Zhu, and Jonathan Lynch

Abstract The mammalian microbiome is home to trillions of microbes that impact aspects of host physiology. One microbiota genus of interest are Turicibacter, which interact with neurotransmitters, SSRIs, lipids, and cholesterols found in their hosts. Although Turicibacter have been implicated in several aspects of host physiology, compatible genetic manipulation tools have yet to be reported, making mechanistic studies difficult to conduct. The work presented here aims to develop genetic tools capable of manipulating gene expression in Turicibacter sanguinis. To start, we assessed the minimal inhibitory concentration of T. sanguinis MOL361 to antibiotics that will be used selection markers for transformants. T. sanguinis MOL361 growth was inhibited at 2 g/mL chloramphenicol, 50 g/mL kanamycin, and 450 g/mL spectinomycin. Next, we developed an electroporation method to deliver plasmid DNA and assessed how various conditions impacted transformation efficiency. The best condition tested using unmethylated DNA, resulted in a transformation efficiency of 2.7 x 10 cfu/ g DNA (about 20-30 colonies per reaction). In accordance with our goal to manipulate gene expression in T. sanguinis, we identified multiple compatible origins of replication (pBP1, pCD6, and pIP404) and are developing a library of plasmids containing promoters with different activities. To assess the compatibility of the tools developed from this study, they will be assessed in other Turicibacter strains found to also influence host-physiology. All in all, this work is the first to report a method capable of transforming DNA into the Turicibacter genus. Future work will look to expand the toolkit to include gene editing methods (i.e. CRISPR-technologies) and improve DNA electroporation efficiency. This work will enable future studies to specifically investigate mechanisms employed by T. sanguinis and opens the potential to engineer them as biotherapeutics for host health.

P.20

Chronic smoking mediates lineage-specific alterations for initiating non-small cell lung cancer

Na Wang
(SOM: Oncolgy)

Work with: Raksha Padaki, Ray-Whay Chiu Yen, Sara-Jayne Thursby, Leslie M. Cope, Malcolm V. Brock, Edward Gabrelson, Hariharan Easwaran, Stephen B. Baylin, Michelle Vaz

Abstract *Background*: Chronic exposure to cigarette smoke is a major driver of lung cancer and contributes to its pathogenesis by inducing genetic and epigenetic abnormalities. The lung's distinct cells of origin give rise to the pathological subtypes of non-small cell lung cancer (NSCLC), suggestive of cell type-specific changes in driving their development. We employed lung organoid models with chronic exposure to cigarette smoke condensate (CSC) to elucidate sequential epigenetic and transcriptomic alterations and their role in co-operating with genetic events to drive development of NSCLC.

Methods: We generated lung organoids composed of stem and differentiated cells representative of the lung airway and alveolar compartments. Organoids were exposed to CSC for 6 months to mimic chronic inflammatory exposure. Epigenomic and transcriptomic alterations were evaluated over the exposure period by DNA methylation arrays, ATAC-seq, and RNA-seq. Changes were evaluated by flow cytometry. Finally, key driver lung mutational events were engineered in the control and CSC-exposed organoids.

Results: Chronic CSC exposure causes distinct morphological changes in organoid structure and composition, increasing proliferative potential. This is accompanied by key shifts in basal stem cell populations along with a reduction in differentiated cell types. We identified distinct temporal changes in CSC exposed organoids suggestive of an immune evasive and pro-tumorigenic phenotype. Notably, we demonstrated their ability to induce shifts in immune cells from a pro- to anti- inflammatory state. Finally, we showed that CSC methylated genes were associated with differentiation, cell fate commitment and transcription factor activity, and that introduction of key NSCLC-specific driver events at the 6-month time point sensitizes only CSC treated organoids to tumor formation in vivo, leading to the development of distinct genetic driver-specific subtypes of NSCLC.

Conclusions: The ability of chronic inflammation to drive key cell type-specific transcriptomic and epigenetic changes leading to the development of distinct subtypes of NSCLC. These findings will aid in identifying molecular correlates for early detection as well as developing targeted therapeutic strategies for interception of early-stage diseases.

P.21

Cilia-dependent hedgehog signaling controls meibomian gland development and unveil potential therapeutic targets for treating meibomian gland dysfunction (MGD)

Huanhuan Xiao
(SOM: Ophthalmology)

Work with: Xiaoying Liao, Céline Portal , Mahtab Nateghi, Igor Butovich, and Carlo Iomini

Abstract *Purpose*: MGD often driven by age-related gland deterioration, is the leading cause of dry eye disease. Effective treatments remain limited. This study aims to define the role of Hh signaling in MG differentiation and maintenance, and to investigate the signaling cascades that regulate MG stem cell function.

Methods: A comparative transcriptomic analysis was performed on tarsal plates (TPs) from 8- and 70-year-old human eyes. Hh pathway activity during early postnatal stages was assessed using the Gli1-LacZ reporter mouse line. To trace Hh-responsive cells in adults, tamoxifen-inducible Cre recombinase was activated in Gli-CreERT2;mTmG reporter mice. Hh signaling in the MG was altered by ablating floxed (Fx) alleles of the Smoothened (Smo) and Ift88 in K14-expressing epithelial cells. MG morphological changes were visualized by ORO staining and GFP expression in K14-Cre; Rosa26mTmG mice by two-photon microscopy at P6 and P8. We used RT-qPCR to assess the relative expression levels of genes potentially linked to cilia-mediated pathways.

Results: A significant decline in Hh signaling in the TPs of aging human eyes was revealed. Similarly, Hh pathway activity decreases as MGs mature, with only a few Hh-responsive cells remaining. Ablation of Smo in adult mice led to progressive MG shrinkage and acinar atrophy. On the other hand, the inactivation of Hh signaling at early developmental stages via Smo deletion disrupted MG formation. Interestingly, ablation of the primary cilium, which functions downstream of Smo, resulted in MG hypertrophy. Moreover, the combined deletion of Smo and Ift88 in K14+ epithelial cells partially rescued the MG defects caused by Smo loss alone. qPCR analysis at P6 revealed compensatory increases in Gli1, Gli2, Gli3, and Ptch1, along with decreased expression of Dhh and Ihh, in Hh pathway of Smo;Ift88 double mutants compared to Smo single mutants at P10. Additionally, we observed upregulation of genes in FGF, BMP, and TGF- pathway.

Conclusions: Our findings suggest that modulation of cilia-mediated signaling pathways offers a promising therapeutic strategy for treating MGD.

P.22

Tumor–neuron crosstalk via Semaphorin 4D/Plexin B1 signaling enhances the migration potential of gastric cancer epithelial cells

Ying Xia
(SOM: Gastroenterology)

Work with: Rong Wu, Xuejiao Han, Adithi Banavar, Hua Zhao, Shayan Gheshlaghi, Yuanlan Cheng, Dechen Lin, and Stephen J Meltzer

Abstract Crosstalk between the nervous system and tumors is known to promote cancer progression, but its role in the early stages of gastric tumorigenesis remains largely undefined. In this study, we reveal a novel mode of tumor–neuron crosstalk in gastric cancer. A TP53/SMAD4 double-knockout gastric organoid model was established using CRISPR/Cas9 and characterized by multi-omics and functional analyses as representative of early gastric cancer. Transcriptomic profiling of organoids and single-cell RNA sequencing of gastric cancer tissues demonstrated early activation of the Semaphorin 4D/Plexin B1 (SEMA4D/PLXNB1) signaling axis. Loss of TP53 and SMAD4 enhanced epithelial cell proliferation and migration, accompanied by upregulation of PLXNB1 expression. Immunofluorescence revealed PLXNB1 expression predominantly in tumor epithelial cells, while SEMA4D localized to neural structures within the tumor microenvironment. In co-culture with human iPSC-derived neurons, gastric organoids induced elevated levels of soluble SEMA4D. Functionally, recombinant SEMA4D promoted cancer cell migration, Functionally, recombinant SEMA4D promoted cancer cell migration, which was effectively blocked by either the anti-SEMA4D monoclonal antibody (Pepinemab) or knockdown of PLXNB1. These findings identify the SEMA4D/PLXNB1 axis as a key mediator of early tumor–neuron crosstalk in gastric cancer and suggest a promising target for early therapeutic intervention.

P.23

Magnetohydrodynamic Forces Enhance Cerebrospinal Fluid Flow in a Physical Model of the Aqueduct

S. Farzad Maroufi
(SOM: Neurosurgery)

Work with: Mohammad Shamsodini Lori, Mohamad Ali Bijarchi, Jiangtao Cheng, and Mark G. Luciano

Abstract *Background*: CSF circulation plays a vital role in maintaining central nervous system homeostasis by facilitating nutrient delivery, waste removal, and intracranial pressure regulation. Impaired CSF flow is associated with neurological disorders such as hydrocephalus, Chiari malformation, and syringomyelia. Existing treatment approaches often involve surgical interventions with significant limitations. Magnetohydrodynamic (MHD) force, which arises from the interaction of electric and magnetic fields, has shown promise in other biomedical applications but has not been studied for CSF flow modulation.

Methods: A three-dimensional (3D) model of the human cerebral ventricular system (CVS) was reconstructed from MRI data and imported into COMSOL Multiphysics for numerical simulation. The model incorporated the lateral ventricles, third ventricle, cerebral aqueduct, and fourth ventricle. The Navier-Stokes equations, modified to include Lorentz force terms, were solved alongside Maxwell's equations to simulate MHD-induced CSF flow. Both invasive (electrodes and magnets placed within the brain) and non-invasive (electrodes and magnets applied externally) configurations were evaluated. Additionally, the impact of adding conductive nanoparticles to CSF to enhance MHD effects was investigated.

Results: Application of MHD force led to a substantial increase in CSF velocity through the cerebral aqueduct, with flow speed enhanced by up to 11.8 times compared to baseline. Pressure within the lateral and third ventricles decreased by up to 18%, depending on the strength of the applied fields. The addition of nanoparticles further improved performance, resulting in up to 48% higher peak velocity and a 15% additional reduction in ventricular pressure. The non-invasive setup achieved comparable flow modulation to the invasive configuration but required higher electric potential and magnetic field strength.

Conclusions: This study demonstrates that MHD force can effectively modulate CSF flow within the brain's ventricular system, offering a novel, potentially non-mechanical strategy for influencing intracranial dynamics. Enhancing CSF conductivity via nanoparticles further amplifies these effects. These findings open avenues for developing MHD-based adjuncts to existing CSF diversion therapies and merit further experimental validation and safety evaluation.

P.24

Preparation of staged *Caenorhabditis elegans* embryos using size filtration

Nikita S. Jhaveri
(KSAS: Biology)

Work with: Maya K. Mastronardo, J.B. Collins, and Erik C. Andersen

Abstract The free-living nematode *Caenorhabditis elegans* has been routinely used to study gene functions, genetic interactions, and conserved signaling pathways. Most experiments require that the animals are synchronized to be at the same specific developmental stage. Bleach synchronization is traditionally used to obtain a population of staged embryos, but the method can have harmful effects on the embryos. The physical separation of differently sized animals is preferred but often difficult to perform because some developmental stages are the same size as others. Microfluidic device filters have been used as alternatives, but they are expensive and require customization to scale up the preparation of staged animals. Here, we present a protocol for the synchronization of embryos using mesh filters. Using filtration, we obtained a higher yield of embryos per plate than using the standard bleach synchronization protocol and at a scale beyond microfluidic devices. Importantly, filtration has no deleterious effects on downstream larval development assays. In conclusion, we have exploited the differences in the sizes of *C. elegans* developmental stages to isolate embryo cultures suitable for use in high-throughput assays.

P.25

Minimal-Measurement Detection of Small Elliptical Anomalies in Electrical Impedance Tomography

Aseel Titi
(WSE: Applied Mathematics and Statistics)

Abstract We study the inverse problem of identifying a small elliptical conductivity anomaly within a unit disc using boundary measurements. The anomaly has a conductivity that slightly deviates from the constant background. Voltage measurements are taken between two point-electrodes on the boundary under a constant current. In the limiting case where the electrode separation tends to zero, the resulting field approximates a dipole. We demonstrate that three dipole measurements uniquely determine the anomaly's location and size, while two additional measurements are required to recover its aspect ratio and orientation. Furthermore, we analyze the stability of this inverse problem and investigate optimal experiment design to ensure robust reconstruction.

P.26

Comparative Analysis of iPSC and Mammary Tissue-Derived Lactogenic Organoids as Models for Human Lactation

Misba Majood
(SOM: Physiology)

Work with: Rajini Rao

Abstract Human lactation is a critical process for infant health, governed by the transport of nutrients across epithelial barriers via specialized membrane transport proteins. Modeling this process has been constrained by the limited availability and ethical concerns of human tissue. To address these challenges, we developed two distinct organoid models: one derived from human induced pluripotent stem cells (iPSCs) and the other from mammary tissue obtained via mammoplasty. Both models were induced into a lactogenic state using a combination of hormones and growth factors, and their functional characteristics were assessed.

Methods: iPSCs were differentiated into mammary-like organoids using a 40-day stepwise protocol and further stimulated with lactogenic factors. Mammary tissue was digested, dispersed and similarly induced into a lactogenic state. Both models were characterized using mammary markers, including milk protein, CK14, CK18, E-cadherin, and estrogen receptor. Functional analysis, such as calcium transport by the secretory pathway pump SPCA2, will be conducted to assess physiological relevance.

Results: Both organoid models successfully mimicked the lactating mammary gland.

Conclusions: This comparative study highlights the strengths and limitations of distinct organoid models in studying human lactation. iPSCs offer an ethically sound, renewable and scalable source with broad applicability, whereas tissue-derived organoids retain donor-specific characteristics and provide immediate functional relevance and physiological complexity. Together, these models represent a complementary approach to advancing our understanding of lactation and developing targeted nutritional interventions for maternal-infant health.

P.28

Suppression of HIV-1 transcription and latency reversal via ectopic expression of the viral antisense transcript AST

Rui Li
(SOM: Molecular and Comparative Pathobiology)

Abstract The mechanisms that regulate HIV-1 latency are not fully elucidated. Our previous studies showed that an HIV-1 antisense transcript (AST) promotes the deposition of histone modifications at the HIV-1 5′ long terminal repeat, causing a closed chromatin state that suppresses viral transcription. Here, we report that ectopic expression of AST in CD4+ T cells from people living with HIV-1 undergoing antiretroviral therapy hinders the reactivation of viral transcription in response to ex vivo stimulation with pharmacologic and T cell receptor agonists, thus preventing the reversal of latency. We defined the structural domains and sequence motifs of AST that contribute to its latency-promoting functions. Last, we carried out an unbiased proteomic screen of AST interactors that revealed an array of host factors both previously known and not known to suppress HIV-1 expression. Our studies identify AST as a first-in-class biological molecule that is capable of enforcing HIV-1 latency and with actionable curative potential.

P.29

Seismoacoustic tracking and characterisation of re-entering space debris

Benjamin Fernando
(KSAS: Earth & Planetary Sciences)

Abstract As Earth orbit becomes more crowded, uncontrolled re-entries of space debris pose increasing risks to both life and the environment. A key part of responding to and mitigating the impacts of re-entry events is being able to rapidly track objects' trajectories once they are burning up in the atmosphere, and identifying likely fall locations of any surviving fragments. At present, such tracking is undertaken primarily with radar assets, however these have limited global coverage and are often access-restricted, hampering debris characterisation. Here, we show that existing seismic networks can be used to identify, track, and characterise re-entering space debris through analysis of the sonic booms they produce. Using the April 2024 uncontrolled re-entry of Shenzhou-15 over California, we show that seismic analysis can determine the module's speed, trajectory, and fragmentation. Our derived trajectory indicates that debris travelled significantly further downrange than was expected from pre-entry predictions, posing a potential hazard from Los Angeles to Las Vegas. More broadly, this work illustrates that that in-atmosphere space debris tracking and characterisation are possible using the worldwide seismic network, enabling near-real-time, open-source predictions for debris fall locations and associated hazard management.

P.30

Human milk choline concentration in relation to infant intake recommendations: findings from the JiVitA-3 study

Sulagna Bandyopdhyay
(SPH: International Health)

Work with: Kerry J Schulze, Daniela Hampel, Lee S-F Wu, Sarah Baker, Katherine Stephenson, Hasmot Ali, Saijuddin Shaikh, Setareh Shahab-Ferdows, Lindsay H Allen, Keith P West Jr, and Parul Christian

Abstract *Introduction*: Choline is an essential nutrient for growth and neurodevelopment in infants and has been associated with maternal dietary intakes. We assessed water-soluble choline compounds in human milk, compared total choline concentrations to Adequate Intake (AI) recommendations for infants, and explored maternal intake of choline-rich foods at 3 months postpartum in rural Bangladeshi women.

Methods: Within the JiVitA-3 trial (comparing antenatal multiple micronutrients without choline to standard iron-folic acid), casual milk samples and dietary data (weekly food servings) were collected at 3 months postpartum. Phosphocholine (PCho), glycerophosphocholine (GPCho), free choline, betaine, and dimethylglycine (DMG) were quantified using UHPLC-MS/MS in 204 samples. Total water soluble choline was calculated as: [free choline + (PCho×0.566) + (GPCho×0.405)]. Milk choline concentration was evaluated against the AI reference concentration (160 mg/L) and the deficiency threshold (90 mg/L).

Results: The concentrations (mean ± SD) of PCho, GPCho, free and total choline were 116.3±38.0, 129.0±35.4, 13.6±8.3, and 131.6±28.9 mg/L, respectively. The concentration (median, IQR) of betaine was 0.59 (0.42) mg/L, and DMG was 0.13 (0.11) mg/L. Only 15% of samples met the AI, while 6.4% were below the deficiency threshold. Weekly consumption of ≥3 servings of choline-rich foods was: fish 60.3%, meat 11.8%, milk 13.2%, and eggs 8.3%.

Conclusions: Choline in human milk of Bangladeshi mothers met the AI for only 15% of infants and the concentrations were similar to those reported elsewhere. Women's consumption of choline-rich foods was limited except for fish, which is habitually consumed in this setting. The results contribute to our knowledge of global variability in human milk composition and warrant further analysis to explore associations of milk choline with maternal diet and infant developmental outcomes.

Funding: BMGF, and USDA, ARS

P.31

P2X4 Purinergic Receptor activation on macrophages inhibits phagocytosis and promotes tumor promoting cytokine secretion

Vahinipriya Manoharan
(SOM: Pathology)

Work with: Kennedy M Rains, Jillian K Landers, Tristan R Ibarra, Angelo M De Marzo, David E Sanin, and Janielle P Maynard

Abstract Prostate cancer (PCa), the most commonly diagnosed cancer and the second leading cause of cancer related deaths among men in the United States, necessitates improved therapeutic strategies. Our previous studies demonstrated that P2X4 Purinergic Receptors (P2X4R) are elevated in PCa tissues and correlate with poor prognosis. Genetic knockdown of P2X4R reduced tumor growth in vivo, and pharmacologic blockade inhibited PCa cell viability, migration, and invasion in vitro. However, the expression pattern and functional role of P2X4R within the prostate cancer tumor microenvironment (TME) remain poorly understood. This study aims to characterize P2X4R expression and explore its functional significance within the TME.

Multiplex immunohistochemistry (mIHC) was performed to evaluate P2X4R expression in the TME of human PCa tissues and mouse allograft tumors. Human THP-1-derived M2 macrophages were treated with CTP (400 μM), a P2X4-specific agonist, and phagocytic activity was assessed in vitro. A human phospho-kinase array was used to identify, and western blotting used to confirm, signaling pathways modulated by P2X4 activation. Cytokine profiling was performed via a human cytokine array. A macrophage-specific P2X4R knockout mouse (LyzM-P2X4- /-) was generated, with successful knockout confirmed by flow cytometry and IHC. Phagocytosis by peritoneal TIM4+ F4/80+ macrophages from LyzM-P2X4- /- mice was compared to wild-type controls.

mIHC analysis showed P2X4R expression on CD163+ M2 macrophages in human PCa tissues and F4/80+ macrophages in mouse tumors. CTP significantly reduced phagocytosis in human M2 macrophages ($p = 0.0115$), while LyzM-P2X4- /- macrophages exhibited enhanced phagocytosis ($p < 0.0001$). P2X4R activation decreased phosphorylation of AKT and p70S6, suggesting suppressed Fc receptor-mediated phagocytosis. Reduced Src family kinase phosphorylation and altered cytokine secretion profiles further support a pro-tumorigenic role.

P2X4R expression on M2 macrophages may promote PCa progression through effects on phagocytosis and cytokine secretion. Targeting P2X4R represents a promising therapeutic avenue.

P.32

Glucose-Stimulated CEST MRI pHe Mapping for Noninvasive Assessment of Tumor Hypoxia and HIF-1 Activity

Aruna Singh
(SOM: Radiology)

Work with: Julia Stabinska, Balaji Krishnamachary, Farzad Sedaghat, Sridhar Nimmagadda, Jeff W.M. Bulte, Zaver M. Bhujwalla, and Michael T. McMahon

Abstract Accurate differentiation between aggressive and less malignant tumors remains a clinical challenge. The hypoxia-inducible factor-1 (HIF-1) plays a central role in tumor aggressiveness by modulating metabolic reprogramming, vascular remodeling via VEGF, and extracellular acidification through upregulation of glycolysis and proton transporters. Tumor acidosis, a hallmark of malignant progression, has emerged as a promising biomarker of proliferation, metastasis, and therapeutic resistance. This acidification is primarily attributed to increased glycolytic flux and lactate accumulation driven by stabilized HIFs.

Chemical exchange saturation transfer (CEST) MRI offers non-invasive, high-resolution mapping of extracellular pH (pHe), and has demonstrated utility in phenotyping tumors based on metabolic and microenvironmental alterations. In this study, we optimized a glucose stimulated CEST MRI protocol to accentuate tumor acidosis for improved pHe-based stratification of tumors with differential HIF-1 expression.

Using an orthotopic xenograft model of triple-negative breast cancer (MDA-MB-231), we compared wild-type (231-WT), HIF-1 knockdown (231-HIF-1 -shRNA), and VEGF overexpressing (231-VEGF) variants. Following iopamidol injection and glucose stimulation (1 M, intraperitoneal, 1 hr prior to imaging), pHe maps revealed significantly greater acidification in 231-WT tumors (6.10 ± 0.12) compared to 231-HIF-1 -shRNA (6.58 ± 0.04) and 231-VEGF (6.30 ± 0.04), as seen in Figs.1a-g. In the absence of glucose, group differences were less pronounced. These imaging findings were supported by immunoblotting and immunohistochemistry, which showed higher lactate dehydrogenase A (LDHA) expression in 231-WT tumors (Fig.1h). NMR-based metabolomics further confirmed trends in lactate levels consistent with imaging and molecular profiles.

Collectively, our results demonstrate that glucose-enhanced CEST MRI pHe mapping sensitively distinguishes tumors with altered HIF-1 expression. This approach holds promise as a non-invasive biomarker strategy for characterizing tumor hypoxia and metabolic phenotype and may guide personalized therapeutic interventions.

P.33

PAI-1's role in defining the "myospan" of skeletal muscle

Indira Paddibhatla
(SOM: Cardiology)

Work with: D. Brian Foster, Anthony A. Kalousdian, and Douglas E. Vaughan

Abstract By mass, skeletal muscle is the largest human organ, and it is a potent mediator of health and longevity. The inevitable and progressive loss of muscle with advanced age negatively impacts movement and mobility, and numerous other physiological parameters. The biological mechanisms that cause age-related changes in skeletal muscle remain poorly understood. PAI-1 is a secretory protein involved in diverse biological processes including inflammation, cell death, and senescence. PAI-1 regulates several pathophysiological processes and contributes to multimorbidity of aging. The impact of elevated PAI-1 on aging skeletal muscle is also largely unknown. Here, we tested the hypothesis that PAI-1 and its homologs are evolutionarily conserved, negative regulators of muscle function and, thereby, directly contribute to its age-related deterioration. To examine the effects of augmented PAI-1 levels on skeletal muscle function, we evaluated activation and relaxation, kinetics and mechanics, of single myofibrils isolated from mice overexpressing (OE) PAI-1, which developed accelerated vascular aging. Relative to age-matched controls, skeletal myofibrils from 7-mo-old PAI-1 OE animals generated 40% less maximum force, which was roughly same as that produced by myofibrils from a 56-wk old control mouse. In Drosophila (model used for aging) we observed a complete loss of flight ability from one-week through seven-weeks of age. Quantitative proteomics of dissected one-, four-, and seven-week-old indirect flight muscle (IFM) fibers revealed several proteins and pathways significantly up-regulated over time, including several PAI-1 homologs, i.e., members of the serine protease inhibitor family (Spn42D). Muscle-specific knockdown of Spn42Da preserved flight-ability over seven-weeks. These results indicate transcriptional repression of Spn42Da mitigates age-associated skeletal muscle degeneration and preserves flight-ability. In conclusion, our findings imply that PAI-1/Spn42Da contributes to age-related pathologies across phyla, and as a key regulator of skeletal muscle function and longevity. PAI-1 inhibition improves the "myospan" to counter age-related deficits in muscle mass, strength, and function.

P.34

The Role of Attention in Multi-Attribute Decision Making

Aaron Sampson
(KSAS: Zanvyl Krieger Mind/Brain Institute)

Work with: You-Ping Yang, Marius Usher, Dino Levy, Ernst Niebur, and Veit Stuphorn

Abstract Real-life decisions typically require the consideration of multiple options, each with multiple attributes. To investigate the neuronal mechanism underlying dynamic preference formation, we recorded pre supplementary motor area (preSMA) activity in two macaques engaged in a multi-attribute decision making task. Options in this task were defined by two attributes each: amount of water reward and probability of receiving that reward. Each attribute was revealed only while fixated and monkeys indicated their choices via arm movement after freely inspecting the options. Neuronal activity was aligned to both the information-gathering and option-selection stages of the task and analyzed using a generalized linear model and de-mixed principal components analysis. We found preSMA neurons encoded action value signals reflecting the sequentially sampled attributes of the options. During each fixation, preSMA activity changes according to the value of each option as indicated by the currently fixated attribute. These value signals were gradually accumulated over multiple fixations, until the preSMA activity strongly indicated the choice. The current focus of attention lead to shifts in firing rates and the gain of value information, but the relative value of both options was continuously represented independent of which option was currently fixated. Thus, our results are not compatible with a strictly serial choice process, i.e. a series of accept/reject decisions with only one option considered at a time. Instead, they suggest a parallel representation of at least two options. The observed dependence of neural activity on where attention is directed suggests a role for attention in gating the representation of options. Rather than a fully parallel process in which all potential options can be represented, attention could serve to “highlight” one option while relevant information is collected an integrated. Such a process might have only the currently attended option and a prior best candidate option represented at a given time point.

S

Slam Talks

There is a total of 17 slam talks in the PDA Annual Conference of 2025. Slam talks will be 3-minutes oral presentations addressed to a general general academic public, being each of the talks a research pitch intended to captivate a broad academic audience. The slam talks will take place at the Tilghman Auditorium, in the East Baltimore Campus, from 2:30 PM to 4:00 PM on Friday May 30th. During the talks, Dr. Josué Tonelli-Cueto will be the master of ceremonies.

S.1

Seeing Is Believing: Real-Time MRI Visualization of Gene Therapy

Zinia Mohanta
(SOM: Radiology & Kennedy Krieger Institute)

Work with: Aruna Singh, Hernando Lopez Bertoni, Sophie Sall, Julia Stabinska, Irini Manoli, Hilary Vernon, Charles P. Venditti, Assaf A. Gilad, and Michael T. McMahon

Abstract Non-invasive imaging of gene expression remains a critical challenge in gene therapy. Here we report the first in vivo demonstration of a genetically encoded chemical exchange saturation transfer (CEST) MRI reporter expressed from a recombinant adeno-associated virus (rAAV). The genetically encoded peptide-based reporter, superCESTide, enables detection of transgene expression without the need for exogenous contrast agents. Mice were systemically administered rAAV encoding superCESTide and fluorescence reporter, tdTomato, and evaluated using CEST MRI, fluorescence imaging, and RT-PCR. CEST MRI revealed dose-dependent signal enhancement in the liver, with a strong correlation between CEST contrast and superCESTide expression ($R = 0.75$, $P < 0.05$). Mice receiving the highest dose (1.4×1013 vg/kg) exhibited the strongest MTRasym signal (mean $= 0.0225$), and histogram analyses showed distinct expression patterns. These results establish CEST MRI as a powerful, non-invasive method for monitoring rAAV-mediated transgene expression in vivo, opening new possibilities for imaging-guided gene therapy.

S.2

Characteristics of Belantamab-Associated Keratopathy in Patients with Refractory/Relapsed Multiple Myeloma

Meltem Yashar
(SOM: Ophthalmology)

Work with: Felipe Barandiaran, Ugur Tunc, Anthony Gonzales, Laura Di Meglio, and Sezen Karakus

Abstract *Purpose*: Belantamab is an antibody-drug conjugate used to treat relapsed/refractory multiple myeloma (RRMM). Patients treated with belantamab pose a high risk to develop keratopathy, most commonly microcyst-like epithelial changes (MECs). Here we report the characteristics of corneal findings observed in this patient population and review a case with a unique presentation.

Methods: We retrospectively reviewed the medical charts of patients with RRMM treated with belantamab and evaluated at the Wilmer Eye Institute between 2020 and 2022. Patient demographics and clinical characteristics, such as visual acuity and corneal findings, were reviewed, and complications related to treatment were discussed.

Results: Twelve patients (3M/9F) were identified with a median age of 68 (range, 55-89) years. Patients received 1 to 6 belantamab infusions, with a median of 3. Out of 12, 5 patients (42%) developed MECs during the treatment course after at least two infusions, four with a decrease in vision. One patient with a previous radial keratotomy (RK) presented with a significant decrease in vision (from 20/20 to 20/150) along with significant myopic shift with topographic changes and a distinctive corneal fluorescein staining pattern around the RK incisions. After stopping belantamab, most of the myopic shift and all the corneal findings improved over two months.

Conclusions: Belantamab can cause severe corneal complications, and its use should be predicated upon close supervision by eye care professionals. Prior corneal procedures like RK or corneal pathologies may be a potential risk factor for significant vision changes and the development of more severe keratopathy.

S.3

The oxidizing power of sulfur and its role in the global energy transition

Patrick Beaudry
(KSAS: Earth & Planetary Sciences)

Work with: Dimitri A. Sverjensky

Abstract Humanity's climate goals require an unprecedented rate of mining of raw materials, such as critical minerals, for the production of clean energy technologies and electric vehicles. Copper, perhaps the most important metal for electricity generation, distribution and storage, has been mined for over 5000 years, yet the amount of copper that must be extracted from the Earth in the next 30 years, just to meet business-as-usual trends, surpasses that which has been mined in the history of humanity. Copper is primarily mined from porphyry deposits. Such deposits form in subduction zone environments, when large volumes of hydrothermal fluids are released by crystallizing magmas that are enriched in copper and other metals. This enrichment is not fully understood, but is the result of magmatic differentiation under oxidizing conditions. Sulfur plays a pivotal role in this process, determining where and how copper is transferred from deeper mantle sources to shallow crustal reservoirs, and how it is removed from a crystallizing magma. My modeling work aims to characterize the complex behavior of sulfur at the high pressures and high temperatures characteristic of subduction zone environments.

S.4

mRNA Methylation Dynamics Following Sciatic Nerve Injury: An In Vivo Study

Mehmet Can Sari
(SOM: Neurology)

Work with: Xindan Hu, Ashok Patowary, Riki Kawaguchi, and Ahmet Hoke

Abstract *Background*: Peripheral nerve injuries (PNIs) affect millions annually and often result in chronic disability due to ncomplete regeneration. Schwann cells are central to nerve repair, yet the epitranscriptomic mechanisms regulating their function remain poorly understood. Among >170 RNA modifications, N6- ethyladenosine (m6A) is the most abundant and dynamically regulated. Our previous work showed that Schwann cell-specific deletion of Mettl14, a key m6A methyltransferase, results in progressive demyelination, axonal degeneration, and impaired regeneration, highlighting the essential role of m6A in peripheral nerve maintenance and repair.

Methods: To investigate m6A dynamics post-injury, we employed Oxford Nanopore m6A RNA sequencing (m6A-Seq) in wild- type mice following sciatic nerve transection, focusing on day 1 post-injury-a key time point identified via western blot, dot blot, and m6A colorimetric assays. We also analyzing age-dependent methylation patterns across four developmental stages: P21, 2 months, 4 months, and 12 months. In parallel, we performed bulk RNA-seq in Mettl14 conditional knockout (cKO) mice to assess gene expression changes.

Results: We observed a rapid increase in global m6A methylation after injury, increasing at day 1, peaking at day 3 and sustained through day 7. In Mettl14 cKO nerves, bulk RNA-seq revealed down-regulation of genes critical for myelination, axonal regeneration, and immune signaling. Ongoing integration of m6A-Seq with bulk RNA-seq will define m6A-dependent transcriptional programs driving nerve repair.

Conclusions: Our findings suggest that m6A methylation is a key post-transcriptional regulator of the peripheral nerve injury response. Loss of Mettl14 disrupts early transcriptional programs necessary for Schwann cell proliferation, immune recruitment, and axonal regeneration. These insights provide a foundation for RNA-targeted therapeutic strategies aimed at enhancing peripheral nerve regeneration.

S.5

Evaluating the gene expression profile of human nerve injury

Maaz Khan
(SOM: Neurology)

Work with: Ahmet Hoke, Xindan Hu, Sami Tuffaha, Allan Belzberg, and Riki Kawaguchi

Abstract *Background*: Rodent models are typically used to investigate human peripheral nerve injury. However, delayed coaptation for as little as 8 weeks in rodents significantly impairs recovery, whereas humans can withstand longer durations of denervation. Hence, little is known about how the gene expression profile of rodents following denervation maps temporally onto humans.

Methods: Humans: bulk RNA-seq was performed on nerve and muscle samples from 48 unique patients undergoing peripheral nerve surgery (duration of denervation: control, <1wk, 2-5mo, 6-11mo, 12mo-5yr).

Rats: the sciatic nerve was transected, and distal sciatic nerve samples were analyzed using DNA microarrays and qPCR (duration of denervation: control, 1d, 3d, 7d, 14d, 1mo, 2mo, 3mo, 6mo). Bulk RNA-seq and snRNA-seq are now being performed for further analysis.

Results: The majority of patients were male (76.9%), white (65.2%) and underwent traumatic mechanisms of nerve injury (65.6%), of which most were vehicular accidents (61.9%). The median denervation duration until surgery was 7.0mo (IQR: 5.2mo to 10.6mo). Patients were followed up for an average of 13.4mo.

In human denervation, RUV analysis revealed a triphasic temporal pattern of RNA expression, initially with an increased innate immune response and reduced energy production (2-5mo), followed by a change to an adaptive immune response (6-11mo), and finally somewhat reversion to the original state with increased energy production but reduced neurogenesis (12mo-5yr).

Gene ontology pathway analysis revealed persistent downregulation of biosynthetic pathways involved in cholesterol, lipid, secondary alcohol and acetyl coenzyme A metabolism in both humans and rats. In rats, cellular proliferation and immune signaling were upregulated between 1d-14d of denervation, with ficolin-1 RNA upregulated between 1d-7d, whilst pyroptosis was upregulated at 14d.

Overlap analysis showed that 3mo, 6mo, 12mo-5yr of human denervation corresponded most significantly to 3d-7d, 7d-14d, and 6mo of rat denervation, respectively.

Discussion: Denervation induces a differential RNA expression profile that maps temporally between humans and rodents.

S.6

Can acetylation improve heart contraction?

Kripa Chitre
(SOM: Cardiology)

Work with: Dawson Lab, Lehman Lab, and Moore Lab

Abstract Cyclic binding of myosin to actin causes a heart muscle to contract. Tropomyosin (Tpm) is a protein that runs along actin filaments and regulates contraction by controlling myosin's access to actin. Two lysine residues on actin (K326 and K328) help Tpm stay in a position that blocks myosin binding. These lysines can be acetylated, which neutralizes their positive charge and may weaken Tpm's grip on actin.

We tested whether mimicking acetylation at these sites—by replacing the lysines with uncharged glutamines (K326Q/K328Q)—would enhance heart contraction. Using fruit flies, we expressed either normal (wild-type, WT) actin or this acetylation-mimetic (AcM) version in the heart and jump muscle. Compared to WT, AcM actin increased the duration of contraction and boosted muscle shortening speed suggesting enhanced cardiac function.We then produced human WT and AcM actin using insect cells and analyzed their structure with cryo-electron microscopy. The overall filament structure and Tpm positioning were unchanged. However, computer modeling showed that the interaction between AcM actin and Tpm was much weaker than in WT, suggesting that myosin could more easily displace Tpm to initiate contraction. Finally, using an in vitro motility assay, we observed that AcM actin-Tpm filaments moved significantly faster over myosin than WT ones, indicating reduced Tpm-mediated inhibition.

Together, these findings suggest that acetylation of actin could be used as a potential tool to modulate heart contraction in hearts that struggle to contract.

S.7

Methylated DNA Markers for the Detection of Cervical Lesions at High Risk of Malignant Progression

Roshni Saravanan
(SOM: Oncology)

Work with: Mary Jo Fackler, Madison Pleas, Liqun Zhang, Suzette Jordaan, Eunice Van Den Berg, Pamela Michelow, Reubina Wadee, Maureen Joffe, Carl Chen, Syed Ali, Leslie Cope, and Saraswati Sukumar

Abstract *Background*: Cervical cancer remains a leading cause of death, particularly in developing countries. WHO screening guidelines recommend HPV detection to identify women at risk of developing cervical cancer. While HPV testing identifies those at risk it does not specifically distinguish individuals with neoplasia. We investigated whether a quantitative molecular test that measures methylated DNA markers could identify high risk lesions in the cervix with accuracy.

Results: Marker discovery was performed in TCGA-CESC Infinium Methylation 450K Array database and verified in three other public datasets. The panel was technically validated using Quantitative Multiplex-Methylation Specific PCR (QM-MSP) in tissue sections (N = 252) and cervical smears (N = 244) from the U. S., South Africa, and Vietnam. The gene panel consisted of FMN2, EDNRB, ZNF671, TBXT, and MOS. Cervical tissue samples showed highly significant differential methylation in SCC with 100% sensitivity, specificity of 91% to 96%, and ROC AUC of 1.000 compared to benign cervical tissue, and CIN2/3 with sensitivity of 55% to 89%, specificity of 93% to 96%, and a ROC AUC ranging from 0.793 to 0.99 compared to CIN1. In cervical smears, the marker panel detected SCC at a sensitivity of 87%, specificity 95%, and ROC AUC 0.925 compared to normal, and HSIL at a sensitivity of 70%, specificity 94% and ROC-AUC 0.884 compared to LSIL/normal. In a pilot study of blinded cervical smears in cytolyte (N=250), QM-MSP distinguished HSILs and LSIL/NIELs with ROC-AUC of 0.886, sensitivity 75.8% and specificity 90%. Modifications to the technique are underway to improve sensitivity of detection of HSILs. HPV-negative HSILs were also frequently hypermethylated.

Conclusions: This 5-marker panel detected SCC and HSIL in cervical smears with a high level of sensitivity and specificity. Molecular tests with the ability to rapidly detect high-risk HSIL will lead to timely treatment for those in need and prevent unnecessary procedures in women with low-risk lesions throughout the world.

S.8

Scalable metagenomic classification in the era of long-read sequencing

Sina Majidian
(WSE: Computer Science)

Abstract Metagenomics is the study of genetic material recovered directly from a specific environment, such as the gut microbiota or soil. High-throughput DNA sequencing technologies revolutionized the field by enabling large-scale metagenomic studies. Initially, these technologies produced short reads of around 200 nucleotides, but recent advances in long-read sequencing allow for the capture of thousands of bases with high accuracy, providing richer information for analysis. By sequencing DNA samples, we can characterize microbial biodiversity and determine which bacterial strains or species are present. This task is known as metagenomic classification. To do so, we compare the DNA sequences of the sample against a reference database of microbial DNA. However, indexing vast microbial genome collections (e.g. in NIH-NCBI containing millions genomes) presents significant computational challenges. One common strategy involves breaking genomes into small fixed-length sequences called k-mers (e.g., 31-mers). Our results show that relying on a single k-mer size is insufficient to discriminate between genomes. We explore the use of variable k-mer sizes to enhance metagenomic classification. By capturing a broader range of sequence patterns, this approach provides a more accurate representation of microbial diversity. As sequencing technologies and genomic databases continue to grow, it is crucial that computational methods evolve accordingly to maintain accuracy and scalability in metagenomic analysis.

S.9

Changes in membrane fluidity drive directional migration after epithelial-to-mesenchymal transition in neural crest cells

Allison Mancini
(SOM: Cell Biology)

Work with: Erdnic Sezgin, and Michael L. Piacentino

Abstract During development, a transient group of stem cells known as the neural crest (NC) delaminate and migrate away from the embryonic midline before differentiating into diverse craniofacial structures that include cranial neurons, glia, and connective tissues. We recently found that to achieve this migratory phenotype, NC cells must reprogram their lipid metabolism, which enables them to undergo an epithelial-to-mesenchymal transition (EMT). Expression of nSMase2, an enzyme that hydrolyzes sphingomyelin into ceramide in the plasma membrane, is specifically activated in NC cells at the onset of EMT and is necessary for this transition. However, the biophysical effects of spatiotemporally controlled metabolic programming, and the precise role of nSMase2 activation in this process, remains poorly understood. Using spectral imaging and single-particle tracking, we show that NC cells increase their membrane fluidity during EMT, and that nSMase2 is required for this fluidity increase. Surprisingly, this nSMase2-dependent membrane fluidity is not a determinant of migratory speed, but instead is required for effective directionality during migration. Our results demonstrate how ceramide production coordinates migration by changing the biophysical properties of the plasma membrane to enable directionality. These studies show how gaining a finer understanding of the molecular and biophysical mechanisms that drive EMT and migration will reveal novel avenues to diagnose and treat conditions ranging from congenital disorders to cancer metastasis.

S.10

Patient-Derived IgM Autoantibody Reverses autoimmune diabetes in NOD Mice

Rafid Al-Hallaf
(SOM: Medicine & Pathology)

Abstract Type 1 diabetes (T1D) is an autoimmune disease driven by autoreactive lymphocytes that destroy insulin-producing beta cells, necessitating lifelong insulin therapy. The widely used NOD mouse model has been instrumental in understanding mechanisms of autoimmune diabetes and assessing therapeutic modalities. A major challenge that remains elusive, however, is lack of specificity of immunotherapies due to difficulty in distinguishing autoreactive T cells from non-self-reactive T cells. Hence, despite advances in identifying autoantigens and autoimmune mechanisms, most immunotherapies lack efficacy or cause broad immunosuppression. Here, we show that x-mAb, a germline-encoded IgM autoantibody derived from dual-expresser lymphocytes in T1D patients, prevents disease progression and induces remission in newly diabetic NOD mice. Histological analysis confirmed preservation of islets in prediabetic mice by x-mAb and and reduced insulitis in cured mice. x-mAb selectively targets pancreatic tissue-resident memory (Trm) T cells, including islet-specific CD4+ and CD8+ cells identified by tetramer staining and functionally by congenic adoptive transfer. Treatment downregulates CD69, reducing Trm cell retention, while promoting immune tolerance by enhancing apoptotic cell clearance, inducing IL-10, and expanding regulatory T cells (Tregs). Notably, x-mAb identified analogous islet-specific Trm cells in human T1D, underscoring its translational potential. These findings reveal an unrecognized role for dual-expresser lymphocytes and their IgM products in modulating autoimmunity. By selectively targeting pathogenic islet specific Trm cells while preserving immune function, x-mAb offers a promising, precision-based therapeutic strategy for T1D.

S.11

Bioengineering CAR myeloid progenitor therapies from improved human blastomere-like stem cells

Willem Buys
(SOM: Pediatric Oncology)

Work with: Ludovic Zimmerlin, Sanskruti Deshmukh, and Elias T. Zambidis,

Abstract *Background*: Due to their excellent homing beyond physiological barriers, cytokine production, and antigen-presentation, CAR myeloid immune therapies may revert the immunosuppressive microenvironment of many tumors to enable host or co-transfused therapeutic lymphocytes. Human induced pluripotent stem cell (hiPSC)-derived myeloid progenitors are particularly promising due to their practically unlimited availability, facile stable gene editing, freeze-thaw tolerance, extended effector cell release, and auto-tolerance induction.

Methods: Feeder-, xeno-, and transgene-free reprogrammed hiPSC are reverted to a blastomere-like hybrid stem cell state using a chemically defined 'cGMP-ready' modification of our published tankyrase-inhibitor regulated naïve (TIRN) protocol. I have developed a 2D protocol to differentiate TIRN stem cells towards committed granulocyte-macrophage progenitors. Effector cell cytokine production, phagocytosis, respiratory burst, and chemotaxis of derived effector cells were compared to conventional iPSC-progeny.

Results: TIRN stem cells reliably generate approx. 10-fold more viable CD33/34/45 progenitors and downstream CD45/11b effector cells per input cell number and per culture volume, than conventional iPSC. TIRN stem cell-derived myeloid effector cells perform LPS-stimulated TNF production and phagocytosis of E. coli at a similar efficiency compared to conventional hiPSC-progeny. TIRN stem cell derived cells migrate along a chemotactic gradient and undergo respiratory burst at a greater efficiency than even primary adult whole blood-derived monocytes.

Conclusions: TIRN stem cells differentiate at manifold greater efficiency towards lineages of all three germ-layers, including hematopoietic mesoderm. This improvement may reduce bioreactor volumes from approx. 50 liter per therapy unit to a much more economically feasible 5 liters. TIRN stem cell-derived myeloid cells perform crucial immune functions at a similar or greater efficacy compared to conventional iPSC. By functionalizing cells with an adaptor-based uniCAR, myeloid cells can be easily re-targeted against a range of solid tumors, improving therapy control and possibly allowing the use of one iPSC-derived unmatched progenitor line against a range of cancer types.

S.12

Evaluating patient awareness of upper extremity reconstructive procedures for cervical spinal cord injuries

Fares Lebbos
(SOM: Plastic and Reconstructive Surgery)

Work with: Rachana Suresh, Alec J. Chen, Mohammed Shahid, Mark A Poisler, Jeffrey Khong, Kitae Eric Park, William Padovano, Sami H. Tuffaha, and Ala Elhelali

Abstract *Background*: Upper extremity reconstructive (UER) procedures can improve motor function and manage spasticity and contractures in patients with cervical spinal cord injury (CSCI), often leading to high patient satisfaction. However, the uptake of these surgeries remains low. Limited knowledge about UER may be a barrier to surgery. This study aimed to assess awareness of UER surgeries among individuals with CSCI.

Methods: A cross-sectional survey was administered via a public link on the REDCap platform. Participants were recruited through social media and peer networks (PatientsLikeMe, Reddit, Discord). The survey evaluated awareness of UER procedures, sources of information, timing of learning post-injury, attitudes toward surgery, barriers to care, healthcare provider interactions, and knowledge of the specialists performing these procedures.

Results: Eighty-five participants (mean age: 40.3 ± 16.4 years; 64.7% men) responded. Most had injuries at C3–C5 (54.1%). Nearly all reported upper extremity weakness (96.5%), with 80% experiencing rigidity and 76.5% spasticity. Overall, 44.7% were aware of UER procedures. Tendon transfers (78.9%) and nerve transfers (68.4%) were the most recognized interventions. The most common sources of information were from online platforms (60.5%) and healthcare providers (57.9%). Despite this awareness, only 13.2% of participants had undergone any UER surgery. Among those, nerve transfers were the most common (60%). Reported barriers to undergoing surgery included fear or anxiety about the procedure (53.1%), difficulty accessing specialized surgeons nearby (15.6%), and financial or insurance-related obstacles (15.6%).*Conclusions*: Fewer than half of the participants in our survey were aware of UER procedures available to improve function after CSCI and only 13.2% undergone surgery. These results highlight significant gaps in awareness and access to UER care, underscoring the need for targeted patient education and healthcare system interventions to increase utilization of these potentially life-enhancing procedures.

S.13

Chronic smoking mediates lineage-specific alterations for initiating non-small cell lung cancer

Na Wang
(SOM: Oncolgy)

Work with: Raksha Padaki, Ray-Whay Chiu Yen, Sara-Jayne Thursby, Leslie M. Cope, Malcolm V. Brock, Edward Gabrelson, Hariharan Easwaran, Stephen B. Baylin, Michelle Vaz

Abstract *Background*: Chronic exposure to cigarette smoke is a major driver of lung cancer and contributes to its pathogenesis by inducing genetic and epigenetic abnormalities. The lung's distinct cells of origin give rise to the pathological subtypes of non-small cell lung cancer (NSCLC), suggestive of cell type-specific changes in driving their development. We employed lung organoid models with chronic exposure to cigarette smoke condensate (CSC) to elucidate sequential epigenetic and transcriptomic alterations and their role in co-operating with genetic events to drive development of NSCLC.

Methods: We generated lung organoids composed of stem and differentiated cells representative of the lung airway and alveolar compartments. Organoids were exposed to CSC for 6 months to mimic chronic inflammatory exposure. Epigenomic and transcriptomic alterations were evaluated over the exposure period by DNA methylation arrays, ATAC-seq, and RNA-seq. Changes were evaluated by flow cytometry. Finally, key driver lung mutational events were engineered in the control and CSC-exposed organoids.

Results: Chronic CSC exposure causes distinct morphological changes in organoid structure and composition, increasing proliferative potential. This is accompanied by key shifts in basal stem cell populations along with a reduction in differentiated cell types. We identified distinct temporal changes in CSC exposed organoids suggestive of an immune evasive and pro-tumorigenic phenotype. Notably, we demonstrated their ability to induce shifts in immune cells from a pro- to anti- inflammatory state. Finally, we showed that CSC methylated genes were associated with differentiation, cell fate commitment and transcription factor activity, and that introduction of key NSCLC-specific driver events at the 6-month time point sensitizes only CSC treated organoids to tumor formation in vivo, leading to the development of distinct genetic driver-specific subtypes of NSCLC.

Conclusions: The ability of chronic inflammation to drive key cell type-specific transcriptomic and epigenetic changes leading to the development of distinct subtypes of NSCLC. These findings will aid in identifying molecular correlates for early detection as well as developing targeted therapeutic strategies for interception of early-stage diseases.

S.14

Milk Matters: Growing Mini-Breasts to Decode Lactation

Misba Majood
(SOM: Physiology)

Abstract Lactation is a biological marvel-yet, for many mothers, it's a daily struggle. From low milk supply to drug transfer through milk, the science behind lactation remains surprisingly understudied. My research tackles this knowledge gap by developing "mini-breasts in a dish"-organoids that mimic the lactating human mammary gland. By combining three unique sources-mammoplasty tissue, breast milk-derived cells, and induced pluripotent stem cells-I aim to create a comprehensive model to decode transporter function and lactation disorders. This tri-source approach doesn't just push boundaries-it redefines how we study human milk biology, drug safety in breastfeeding, and the future of personalized maternal care.

S.15

Damage Characterization of Granite under Hypervelocity Impact

Xingyuan "Zazzy" Zhao
(WSE: Hopkins Extreme Materials Institute)

Work with: Zhifei Deng, Brett Kuwik, Justin Monero, Ryan Hurley, and Todd C. Hufnagel

Abstract Granite is the most common crustal rock and is often subject to extreme dynamic loads, such as earthquakes, meteorite impacts, and underground explosions. A quantitative understanding of the connection between the microstructure of granite and its behavior under extreme pressure and loading rate would assist with the assessment of the effects of events such as planetary impact. This work reports the results of high-velocity impact experiments of metal spheres on Westerly granite at velocities of 1 km/s to 3 km/s. We assess the size and shape of the impact craters as well as the distribution and velocity of ejecta. To characterize damage beneath the crater, we section the material and map the size, shape, and distribution of defects using high-resolution X-ray computed tomography. The 3D damage distribution in relation to the hypervelocity impact will be discussed. These results contribute to a better understanding of damage under impact craters on planetary surfaces and the structural response of granite in real-world applications.

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S.16

A Cost-Efficient Porous Adsorbent for Selective CO2 Separation from Industrial Flue Gas

Dinesh Mullangi
(Institute for NanoBioTechnology)

Abstract Modern societies are largely depending on fossil fuels for various energy requirements. As of 2020, more than 80 percent of the global energy supply was powered by fossil fuel combustion technologies.1 The steady progress in chemical processing industries and rapid growth in the world population causes immense demand for various energy sources.2 Meeting the global energy demand without jeopardizing climate changes has become an immense challenge for researchers, despite tremendous research efforts.3 Owing to their high porosity, surface functionality, and versatile modifications, porous materials such as Metal-Organic Frameworks (MOFs), and Covalent Organic Frameworks (COFs) have stimulated huge research interest in the field of energy and environmental applications.4 Notably, MOF-74-Ni, UiO-66, SIFSIX-2-Cu-I, mmem-Mg2(dobpdc), MIL-53(M), HKUST, the ZIF-series, imine-based COFs, TpPa-1, TaTp-COF, COF-LZU1, ACOF-1, and other porous materials have shown impressive performance for gas capture, storage, catalysis, energy storage, and several other potential applications.4 However, the excessive materials cost, unfavourable synthesis methods, limited large-scale production, and their poor performance under practical conditions are still preventing them from achieving real-life usage. To improve the energy efficiency of industries while mitigating their impact on global warming requires a new class of advanced materials development. In my presentation, I proposed a bottom-up approach to developing inexpensive porous materials to address ongoing energy and environmental challenges.5, 6 Also, I will discuss the most promising methods to capture and store atmospheric CO2 to protect the environment and ecosystems.

S.17

Monkey business: The evolution of cell fate specification mechanisms in primate retinal organoids

Jo Hagen
(KSAS: Biology)

Abstract Photoreceptor (PR) subtype patterning in the primate retina generates high-acuity spatial, trichromatic color, and dim light vision. PRs express light-sensitive opsin proteins, which confer distinct functions. Short-wavelength sensitive cones (S cones) express S-opsin, long/medium-wavelength sensitive cones (L/M cones) express L/M-opsin, and dim light sensitive rods express Rhodopsin (Rho). In humans, opsin expression initiates in a temporal order: S opsin, then L/M opsin, and lastly Rho. In marmoset monkeys, opsin expression is inverted: Rho, then L/M opsin, followed by S opsin, suggesting that primate PR subtypes are born in a different order and/or mature at different rates.

In humans, thyroid hormone (TH) signaling controls the temporality of cone subtype specification. Early, low TH signaling specifies S cones. Later, high TH signaling yields L/M cones and rods. The inverted order of photoreceptor development between species could be driven by evolutionary changes in the timing or effects of TH signaling.

To test this, we developed a new differentiation method capable of generating human and non-human primate retinal organoids that retain all neuronal layers and generate PRs with species-specific developmental timing. In order to determine whether PR subtypes are born in a different order and/or mature at different rates between primates, we have begun generating a birth order timeline using EdU labeling of PR subtypes in each species.

Whereas high TH suppresses blue fate and promotes red/green cones and rods in human organoids, TH promotes blue fate and suppresses red/green fates and rods in marmoset organoids. In both species, we detect an increase in cones co-expressing S and LM opsin under high TH conditions. These data suggest TH is sufficient to drive cone subtype fate conversion in both species, but the direction of the effect has evolved.

These studies will advance our mechanistic understanding of how fate specification evolves across primates.