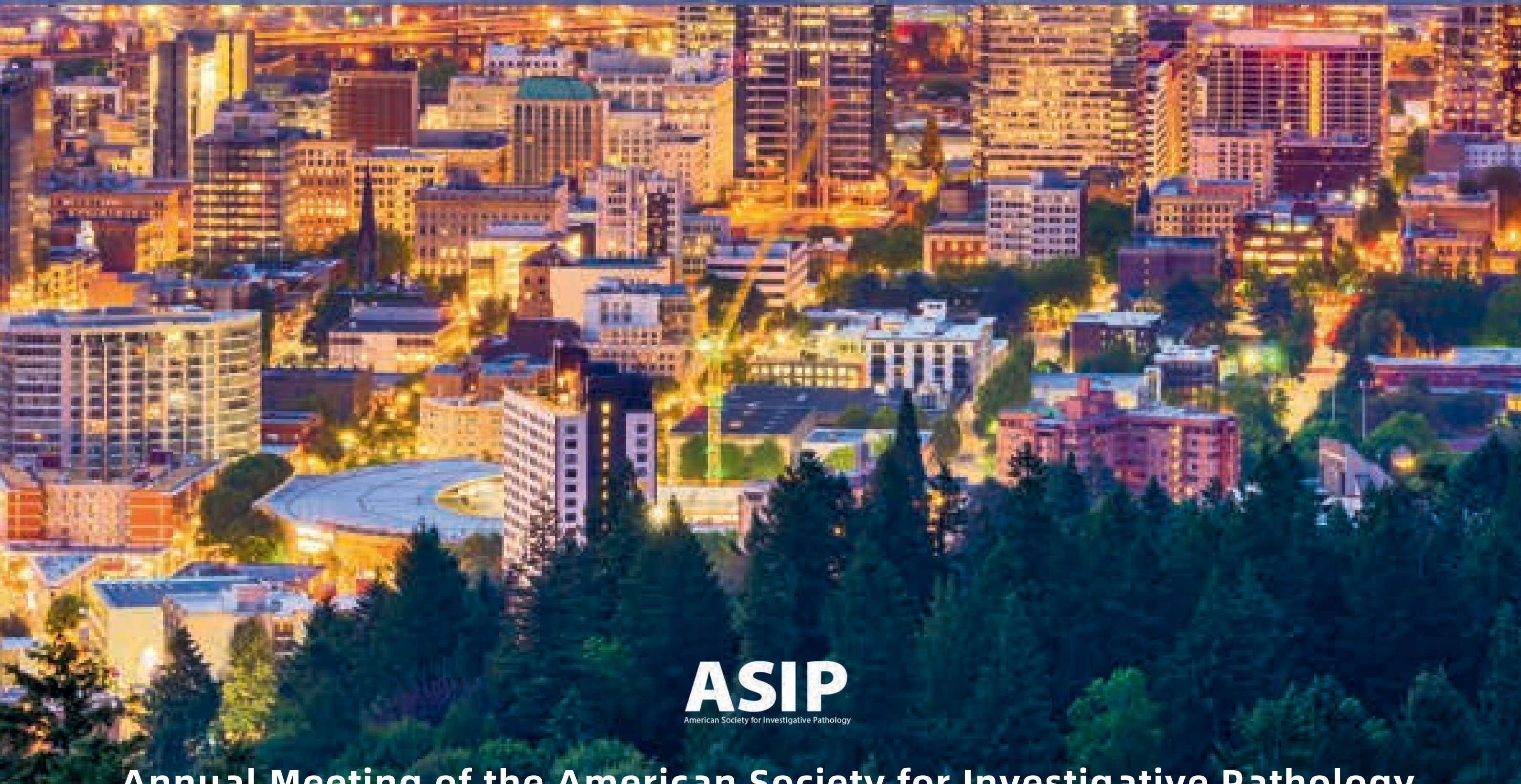
PATHOBIOLOGY 2025

Mechanisms of Disease

Meeting Program

April 26–29 | Portland, OR



ASIP

American Society for Investigative Pathology

Annual Meeting of the American Society for Investigative Pathology

An innovative, inclusive and collaborative team
of academic pathologists in the
heart of the beautiful Pacific Northwest

Department of Pathology and Laboratory Medicine

- The only academic health center in Oregon, we are a rapidly growing healthcare system investing in the highest quality cancer and complex care.
- Our labs process more than 45,000 anatomic pathology cases and approximately 3.5 million billable laboratory tests annually.
- Subspecialized practice offering a complex case mix and growing body of consultation services.
- Vibrant education environment with AP/CP and AP/NP residency program, eight fellowships and a unique medical student fellowship program.
- Research programs range from cutting edge molecular pathology analysis, hematologic malignancies, placental biology and disease, to cardiovascular disease and aging, as well as neurodegenerative disease.
- Strong network of partnerships with OHSU Knight Cancer Institute, Biomedical Engineering, Knight Cardiovascular Center, and Layton Aging & Alzheimer's Disease Center.
- Emerging digital pathology and Artificial Intelligence development and applications.

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2025 Program Committee

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University of Utah

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Northwestern University

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Northwestern University

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Washington University School of Medicine

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Schepens Eye Research Institute of
Massachusetts Eye and Ear

Xiao-Ming Yin, MD, PhD
Tulane University School of Medicine

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2025 Awardees

Gold-Headed Cane Award
Fred P. Sanfilippo, MD, PhD
Emory University School of Medicine

Rous-Whipple Award
Anindya Dutta, MBBS, PhD
University of Alabama, Birmingham

Outstanding Investigator Award
Pilar Alcaide, PhD
Tufts University School of Medicine

Cotran Early Career Investigator Award
Onur Kanisicak, PhD
Ohio State University Wexner Medical Center

Women in Pathology Early Career Award for Advancement of Women in Experimental Pathology
Magali Saint-Geniez, PhD
Novartis Biomedical Research

Women in Pathology Award for Advancement of Women in Experimental Pathology
Martha B. Furie, PhD
Stony Brook University

Young Scientist Leadership Award
Nakisha S. Rutledge, PhD
Temprian Oncology, Inc.

Robbins Distinguished Educator Award
Dani S. Zander, MD
University of Cincinnati College of Medicine

GALL Trainee Scholar Award for Excellence in Cardiovascular Research
Zachary Robbe, BS
Tufts University

Amber Hazzard, BS
Medical University of South Carolina

Gotlieb Undergraduate Student in Pathobiology Scholar Award
Adelaide Horvath
University of Houston

Emily Broberg
Brigham Young University

Monga Family Trainee Scholar Award for Excellence in Cardiovascular Research
Carlos Cosme, Jr., BA
University of Pittsburgh

Monga Family Trainee Scholar Award for Excellence in Liver Pathobiology Research
Yubo Wang, M. Med
The University of Texas at Austin

Vik Meadows, PhD
University of Pittsburgh

Patrick Mireles, PharmD
University of Texas at Austin

Monga Family Trainee Scholar Award for Excellence in Neoplasia Research
Harsh Dongre, PhD
Boston Childrens Hospital

Selene Shore, PhD
Medical University of South Carolina

Charulekha Packirisamy, BS, MS
Medical University of South Carolina

Marion and Lawrence (Larry) Muller Memorial Fund - ASIP Trainee Scholar Award for Excellence in Inflammation Research
Karen Dubois Camacho, PhD
University Medical Center Groningen

Carlos Matellan Martin, PhD
University College Dublin

Jael Miranda-Guzman, PhD
University of Michigan

Marion and Lawrence (Larry) Muller Memorial Fund - ASIP Trainee Scholar Award for Excellence in Neurodegenerative Disease Research
Kathryn Rhodes, BS
University of Texas at Austin

Histochemical Society Sponsored Trainee Travel Award
Makenna Grozis
Elon University

Erin Chard, MS
Medical University of South Carolina

Carly Ramos
Boston Children's Hospital

Tyler Yasaka, BS
University of Pittsburgh

Rachel Edens, BS
Medical University of South Carolina

Adrian Jones, BS
Queens University

Fred Sanfilippo-ASIP Visiting Lectureship Award Recipients

Evan Delgado, PhD
University of Pittsburgh

Nidhi Jalan-Sakrikar, PhD
Mayo Clinic

Ramon Bossardi Ramos, PhD
Albany Medical College

Menglu Yang PhD, MD
Schepens Eye Research Institute/Massachusetts Eye & Ear/Harvard Medical School

George K. Michalopoulos Junior Faculty Scholar Award

Goran Micevic, MD, PhD
Yale School of Medicine

Nidhi Jalan-Sakrikar, PhD
Mayo Clinic

Susana Lechuga, PhD
Cleveland Clinic Foundation

Monga Family Junior Faculty Scholar Award

Ramon Bossardi Ramos, PhD
Albany Medical College

Daisy Shu, PhD
University of New South Wales (UNSW) Sydney

Dani and Erik Zander Junior Faculty Scholar Award

Amy Engevik, PhD
Medical University of South Carolina

Ian Cartwright, PhD
University of Colorado Anschutz Medical Campus

ASIP Experimental Pathologist-in-Training Award (EPIT)

Laura Molina, MD, PhD
University of Pittsburgh

ASIP Experimental Pathologist-in-Training Merit Award (EPIT)

Nathaniel Lartey, PhD, MPhil
University of Michigan

Zhe Zhu, MD, PhD
Columbia University

ASIP Experimental Pathologist-in-Graduate Training Award (EPIGT)

Louisa Tichy MS
University of North Carolina at Greensboro

ASIP Experimental Pathologist-in-Graduate Training Merit Award (EPIGT)

Anna Maria Tingler, BSS
Medical University of South Carolina

Maria Zambrano, BS
Tufts University

A.D. Sobel Trainee Scholar Award

Brandon Lehrich, BS
University of Pittsburgh

Cole Hladik, MSc
University of Oklahoma Health Sciences Center

Laura Manzanares, PhD
Northwestern University

Zoe Libramento
University of North Carolina at Greensboro

Fareeha Siddique
Washington University

ASIP Summer Research Opportunity Program in Pathology (SROPP) Scholar Award

Gillian Moraga
University of Michigan

Amy Li
Boston College

ASIP Trainee Scholar Award

Brandon Lehrich, BS
University of Pittsburgh

Cole Hladik, MSc
University of Oklahoma Health Sciences Center

Laura Manzanares, PhD
Northwestern University

Zoe Libramento
University of North Carolina at Greensboro

Fareeha Siddique
Washington University

2025 Meritorious Award Lectures

Session 8: Cotran Early Career Investigator Award Lecture

Saturday, April 26, from 1:30–2:30 PM
Pavilion Ballroom West

The Fibroblast Odyssey: Journey from Bench to Bedside

Onur Kanisicak, PhD
The Ohio State University

Session 12: Rous-Whipple Award Lecture

Saturday, April 26, from 5:30–6:30 PM
Pavilion Ballroom West

An Exploration of Noncoding RNAs and DNA Variants in Cancer

Anindya Dutta, MBBS, PhD
University of Alabama at Birmingham

Session 18: Outstanding Investigator Award Lecture & 2024 FASEB Excellence in Science Award Lecture

Sunday, April 27, from 1:30–2:30 PM
Pavilion Ballroom West

T-Cells at the Heart of Cardiac Remodeling

Pilar Alcaide, PhD
Tufts University School of Medicine

Session 21: Gold-Headed Cane Award Lecture

Sunday, April 27, from 6:00–7:00 PM
Pavilion Ballroom West

The Importance of Mentorship in Career Development

Fred Sanfilippo, MD, PhD
Emory University

Session 35: Young Scientist Leadership Award Lecture

Tuesday, April 29, from 1:00–2:00 PM
Pavilion Ballroom West

Leveraging the Frontline: Targeting Innate Immunity for Vaccines and Therapies

Nakisha Rutledge, PhD
Temprian Oncology, Inc.

General Information

Welcome to Pathobiology 2025! All sessions will be held on the Plaza Level of The Hilton Downtown Portland Hotel. The Career Development, Poster Blitzes, Education Session, and the Women in Pathology Networking Event will be held on the Skyline Level (23rd Floor) of the Hilton Hotel.

Below you will find information that will be helpful while on-site during the meeting. If you have any questions, make your way to the registration desk located in the Pavilion Foyer or the ASIP Networking Lounge (Broadway I/II).

Registration Hours

The meeting registration desk is located in the Pavilion Foyer on the Plaza Level of The Hilton Downtown Portland Hotel. Name badges can be picked up at the registration desk. The desk will be staffed during the following hours:

- Saturday, April 26 7:00 AM–5:00 PM
- Sunday, April 27 7:00 AM–5:00 PM
- Monday, April 28 7:00 AM–5:00 PM
- Tuesday, April 29 7:00 AM–12:00 PM

Registration

Registration fees include the main conference scientific sessions, access to the meeting online meeting program, poster sessions, and the meals included below. Registration fees exclude hotel costs.

Catering

Included in registration fees are the following catered events:

- Breakfast on Saturday, Sunday, Monday, and Tuesday
- Lunch on Saturday, Sunday, Monday, and Tuesday
- Evening Reception on Saturday, Sunday, and Monday

Internet Access

Internet access is provided in the guest rooms for those staying onsite at The Hilton Downtown Portland Hotel and the Duniway within the meeting block. Complimentary Wi-Fi access is also provided by the ASIP in the meeting spaces.

Network: Hilton Meeting Room
Password: asip25 (case sensitive)

Business Meeting

All ASIP members are encouraged and invited to attend the ASIP Business Meeting and Meritorious Awards Presentations on Monday, April 28 from 5:00–6:30 PM in Pavilion Ballroom West. If you are not currently a member, membership applications are available in the ASIP Networking Lounge (Broadway I/II) and at the ASIP table located in the Pavilion Foyer. Non-members are welcome at the business meeting to learn more about the ASIP community.

Women in Pathology Networking Event

Meeting attendees are invited to celebrate the ASIP community with this Women in Pathology sponsored event taking place on Saturday, April 26 from 6:30–8:30 PM (Skyline I/II, 23rd Floor). Take a break from the science and bring your outgoing, collegial, and team-building self—join us for an evening of treats, drinks, and games! Women in Pathology invites you to shake things up a bit and connect with

your colleagues in a night of conversation, networking, and fun competition playing games outside your everyday routine.

Poster Blitzes
Each poster blitz features short (3-minute) presentations based upon selected posters and presented by a young investigator. The objective is to highlight a subset of posters to be presented in a standard poster session to generate interest. Each short presentation will focus on the objectives of the study, the major results, and the conclusions. Details of the studies will be available during the regular poster session.

Poster Sessions
Poster boards will be set-up in the Atrium Ballroom on the Plaza Level. All posters will be on display throughout the entire meeting and all attendees are encouraged to view the posters.

- Poster Session I (**ODD** Numbered Posters)
  Sunday, April 27
  4:30–6:00 PM
- Poster Session II (**EVEN** Numbered Posters)
  Monday, April 28
  3:30–5:00 PM

The organizers are not responsible for any materials posted. Push pins will be provided. Set-up and breakdown for poster boards is as follows:

Poster Set-up
Saturday, April 26 at 2:00 PM

Poster Dismantle
Monday, April 28 at 5:00 PM

Please note: If your poster is not removed by the designated day and time, it will be thrown away when the poster boards are dismantled.

Society-Wide President's Reception and Networking Event – Portland Spirit Dinner Cruise
Monday, April 28 from 7:00-10:00 PM

- 6:45 PM Walk Over to Salmon Springs Dock from Hilton Hotel
- 7:00 PM Check-In & Boarding Begins
- 7:25 PM Safety Announcement
- 7:30 PM Cruise Begins
- 7:45 PM Buffet Opens
- 10:00 PM Boat Returns to Dock, Guests Disembark

Exhibits
Please take time to visit the exhibit displays in the Pavilion Foyer area during the breaks and poster sessions. See the exhibitor listing for detailed information regarding our sponsoring companies.

Exhibits Schedule:

- Saturday, April 26 10:00 AM–1:30 PM; 4:30–7:30 PM
- Sunday, April 27 12:30–6:00 PM
- Monday, April 28 12:30–6:00 PM
- Tuesday, April 29 9:30 AM–1:30 PM

Exhibitor Spotlight Sessions

New for 2025! During the morning coffee break on Saturday and Sunday, join us for our Exhibitor Spotlight Sessions from two of our 2025 Exhibitors & Sponsors.

> ***Level up Your Research with Comprehensive and Complete Single-Cell Multiomics Solutions, Designed to Streamline Processes and Amplify Insights***
> BD Biosciences
> Saturday, April 26 from 10:10–10:25 AM
>
> ***Advancing Medicine Through Pathology: Shaping the Future at OHSU***
> Oregon Health & Science University
> Sunday, April 27 from 10:10–10:25 AM

Please check the Scientific Program for more details about each of the Exhibitor Spotlight Sessions.

Accompanying Persons

Guests are welcome to enjoy the city during the conference hours. There are many sites and attractions within walking distance. For the most complete information about the local area and things to do while in Baltimore, check out the Travel Oregon website (https://traveloregon.com/)

Special Needs

Registrants with special needs are invited to contact Lisa McFadden or hotel concierge for assistance.

Liability

Neither the host venue nor the organizers can be held responsible for any personal injury, loss, damage to private property or additional expense incurred as a result of delays or changes in air, rail, sea, road or other services. All participants are encouraged to make their own arrangements for health and travel insurance.

Photography and Recording Policy

Pathobiology 2025 is committed to honoring the rights of copyright owners and to respectful sharing of scientific research and data. Attendees are expected to adhere to this policy.

Health and Safety Guidelines

Travel and the gathering of people in a public place incurs the risk of communicable diseases, including influenza and COVID-19. All meeting attendees should take personal responsibility to keep yourselves, other attendees, exhibitors, vendors, and staff safe, prior to and during the meeting.

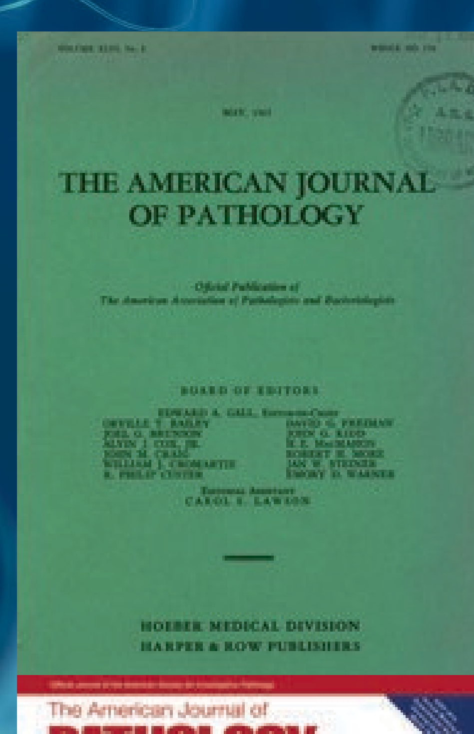
Mask wearing is optional throughout the meeting and masks will be available at the Registration Desk. You are encouraged to practice health and safety habits that make you most comfortable.

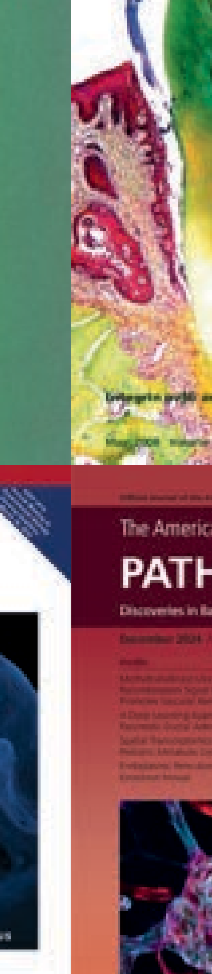
Regardless of vaccination status, if you have symptoms of COVID-19, have tested positive, or are sick prior to traveling to the meeting, please do not attend the meeting. Contact Lisa McFadden (Lmcfadden@asip.org) to discuss your participation as a presenter/chair/poster presenter so that other arrangements can be made.

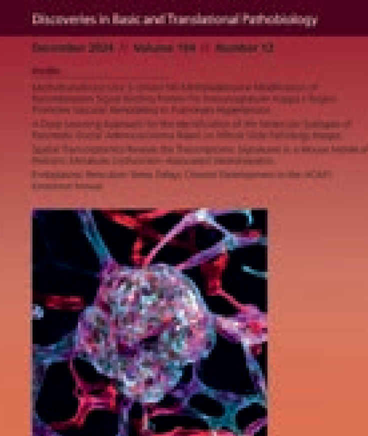
Thank you in advance for your understanding and cooperation!

The American Journal of Pathology

- The most frequently cited pathology journal
- Over 45,000 citations per year
- Over 1.9 million downloads per year
- Discounted charges for ASIP regular members
- Flat-rate publication fee
- Average time to first decision: 35 days







Editor-in-Chief
Martha B. Furie, PhD

AJP.AMJPATHOL.ORG

2025 Exhibitors, Sponsors & Academic Partners

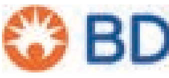
Thank you to our exhibitors, sponsors, and academic partners for making this meeting possible!

American Association for the Study of Liver Diseases (AASLD)
aasld.org

***American Journal of Pathology* (AJP)**
ajp.amjpathol.org
Booth 1

American Society for Investigative Pathology (ASIP)
asip.org
Booth 1

BD Biosciences
bdbiosciences.com
Booth 2

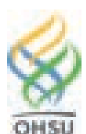


Digital Pathology Association (DPA)
digitalpathologyassociation.org
Booth 5

Earlier.org
earlier.org

Elsevier
elsevier.com

Oregon Health and Science University
ohsu.edu/school-of-medicine/pathology
Booth 3



Pacific Northwest University of Health Sciences
pnwu.edu

Pittsburgh Liver Research Center
livercenter.pitt.edu

Pfizer
www.pfizer.com

The Histochemical Society (HCS)
histochemicalsociety.org
Booth 4

University of Alabama at Birmingham Department of Genetics
uab.edu/medicine/genetics

University of New Mexico School of Medicine
hsc.unm.edu/medicine

UNC Greensboro Department of Kinesiology
kin.uncg.edu

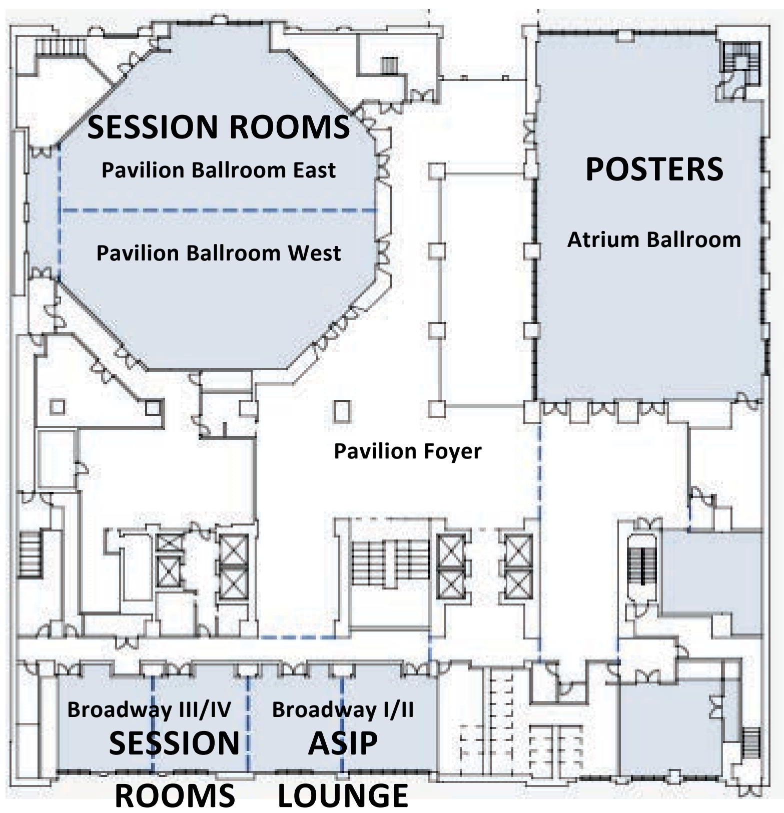
UNC School of Medicine Pathology and Lab Medicine
med.unc.edu

University of Pittsburgh Department of Pathology
path.pitt.edu

University of Pittsburgh Organ Pathobiology and Therapeutics Institute
upddi.pitt.edu

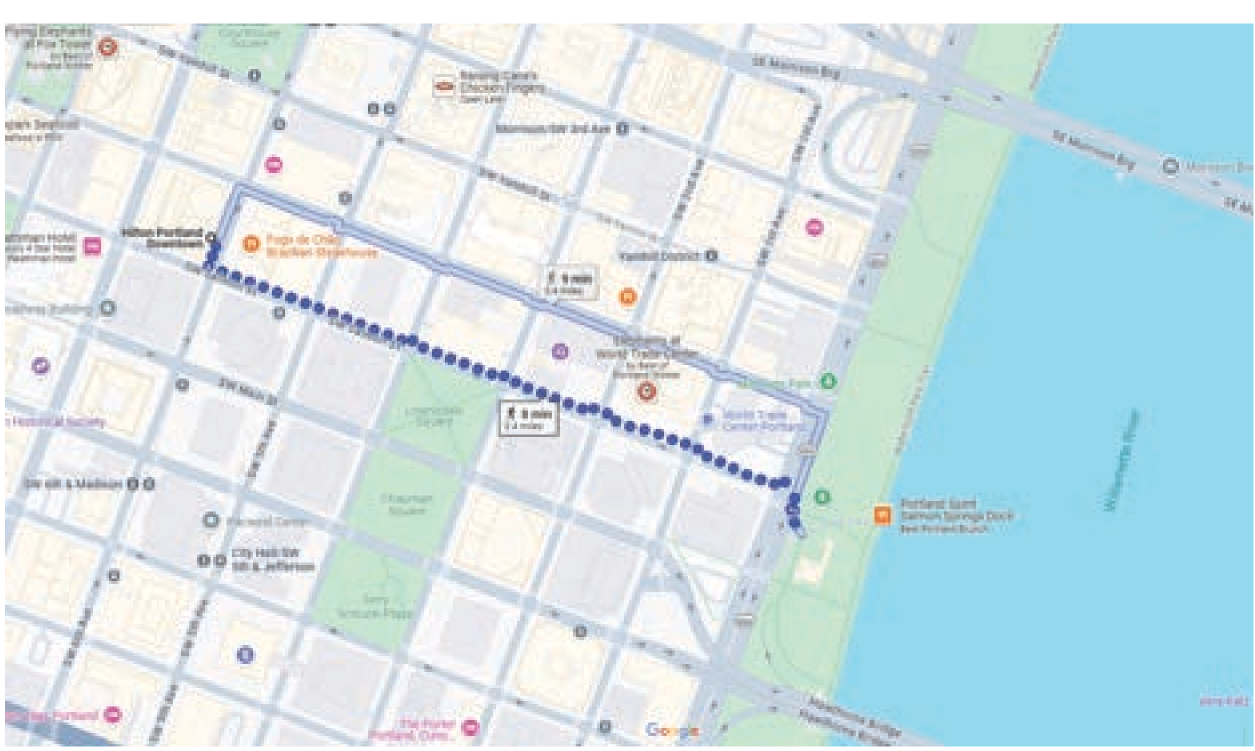
Pathobiology 2025 Meeting Map
The Hilton Downtown Portland Hotel

Plaza Level



Skyline Level (23rd Floor)

Downtown Portland Hilton to Salmons Springs Dock



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Behind Our Science Co-hosts

Roberto Mota Alvidrez, MD
Assistant Professor, Dept. of Pharmaceutical Sciences
University of New Mexico

Daisy Shu, BOptom, PhD
Scientia Senior Lecturer
University of New South Wales

Vik Meadows, PhD
Postdoctoral Research Fellow
University of Pittsburgh

Scientific Meeting Program

FRIDAY, APRIL 25, 2025

ASIP Council Meeting (*By Invitation Only*)
7:00 AM-5:00 PM
Room: Skyline III (23rd Floor)

PathoMingle DIY Tour of Portland
1:00-6:00 PM
Meeting Place: Central Library Stop
SW Yamhill x 9th Avenue, SW

PathoMingle Trainee Dinner Reception: Hosted by the University of Pittsburgh, Department of Pathology
6:00-10:00 PM
Mayrose Tavern, Duniway Hotel
545 SW Taylor Street
Portland OR 97204

ASIP Council Dinner (*By Invitation Only*)
6:30-9:00 PM
Jack's Grill (Hardy Room)
611 SW 10th Avenue
Portland, OR 97205

SATURDAY, APRIL 26, 2025

Continental Breakfast
7:00-8:00 AM
Pavilion Foyer

Session 001: Symposium – *Regenerative Medicine Innovations for Tissue Repair and Cancer Therapy*
8:00-10:00 AM
Session Room: Pavilion Ballroom West
Sponsored by the ASIP Regenerative Medicine and Stem Cells Scientific Interest Group

Chair: Andrew Duncan, PhD ▪ University of Pittsburgh
Co-Chair: Joud Mulla, BS ▪ University of Pittsburgh

Session Overview: This session will explore the latest developments in regenerative medicine, focusing on advancements in stem cells and tissue regeneration. The presentations will cover multiple organ systems and showcase new tissue repair and cancer therapy approaches. Speakers will share recent discoveries, providing attendees with a comprehensive overview of state-of-the-art technologies and the fundamental biology of stem cells and regeneration. Participants will gain valuable insights into the latest scientific advancements. We invite you to join us for an engaging and informative discussion about the future of regenerative medicine.

- **Chair - Welcome and Introductions**
- 8:00-8:30 AM
  Unveiling the Mysteries of Vascular Development and Regeneration with Advanced Human Organoid Models
  Mingxia Gu, MD, PhD ▪ University of Cincinnati
- 8:30-9:00 AM
  Tumor-Immune Hybrid Cells: Harbingers of Metastatic Disease in Gastrointestinal Cancers
  Melissa Wong, PhD ▪ Oregon Health and Science University

- 9:00-9:30 AM
  Allogeneic Hepatocyte Transplantation Without Immune Suppression
  Markus Grompe, MD ▪ Oregon Health and Science University

- 9:30-9:45 AM
  ABSTRACT CANCER001 - Novel Oncogenic Roles of Semaphorin 5B (Sema5B) in Renal Cancer Pathogenesis
  Anirban Kundu, PhD ▪ University of Arizona, Tucson

- 9:45-10:00 AM
  ABSTRACT CANCER002 - Immune Checkpoint Inhibitor Enhances Response to RNAi-Mediated β-Catenin Inhibition in CTNNB1-Mutated Hepatocellular Carcinoma
  Brandon Lehrich ▪ University of Pittsburgh School of Medicine

Session 002: Symposium – *Investigative Pathology in Drug R&D*
8:00-10:00 AM
Session Room: Pavilion Ballroom East
Sponsored by the ASIP Pathology in Biotech and Industry Scientific Interest Group

Co-Chair: Cary Austin, MD, PhD ▪ Genentech, Inc.
Co-Chair: Sripad Ram, PhD ▪ Pfizer, Inc.

Session Overview: Pathology as the tissue-based science of cause and effect of disease is a key discipline in evidence-based drug research and development, and has become an area of focus and expanded capabilities in the translational research industry. This session will explore recent scientific and technological advances involving the application of investigative pathology in drug research and development. Presentations from pathology thought leaders across a broad set of industry settings will provide attendees with an in-depth perspective on how pathology as a discipline is making an impact in tissue-based research and the discovery and development of new drugs to address unmet medical needs, and will highlight some of the pathology activities underway in the Biopharma industry.

- **Chair - Welcome and Introductions**

- 8:00-8:30 AM
  Histopathologic Readouts of Complex In Vitro Models for Research and Drug Discovery
  Eliah Shamir, MD, PhD ▪ Genentech, Inc.

- 8:30 AM-9:00 AM
  Comparative Pathology of Pulmonary Fibrosis in People and Mice: Advantages and Limitations of Mouse Models of IPF
  Hannah Bender, BVSc, PhD ▪ Genentech, Inc.

- 9:00-9:30 AM
  Spatial Omics Study Reveals Colorectal Cancer State Transitions and Immune Interactions for Antigen Target Discovery
  Si Wu, PhD ▪ AbbVie

- 9:30-10:00 AM
  Spatially Resolved Multi-Omics to Unveil Small Intestinal Crohn's Disease Pathobiology
  Sunish Mohanan, DMV, PhD, DAVCP ▪ Gilead Sciences

10:00-10:30 AM
Coffee Break
Pavilion Foyer

Exhibitor Spotlight Session

10:10-10:25 AM

Session Room: Pavilion Ballroom West

Session Overview: Come and immerse yourself in the world of high-dimensional biology and learn about BD Biosciences' continuous ecosystem of single-cell multiomic solutions! From preserving and labeling cells to capturing them, prepping DNA libraries to analysis with bioinformatic tools, we've got everything you need. Our products are designed with high-throughput and automated capabilities that you can rely on through your single-cell workflow. They're not just tools, they're your research partners, streamlining your work and driving greater insights.

We will also be introducing our newly expanded reagent and assay portfolio that includes the BD® OMICS-Guard Sample Preservation Buffer, Intracellular CITE-seq Assay and BD® OMICS-One Panels using BD® AbSeq Antibody-Oligos, BD Rhapsody™ ATAC-seq Assays and improved immune repertoire profiling with the BD Rhapsody™ TCR/BCR Next Multiomic Assays. These are designed and validated to work seamlessly with our existing products, which means you can focus on what truly matters—answering biological questions. Say goodbye to technical hurdles and hello to a smoother, more efficient research journey with BD Biosciences.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

- ***Level Up Your Research with Comprehensive and Complete Single-Cell Multiomics Solutions, Designed to Streamline Processes and Amplify Insights***
  Nicholas DePatie ▪ BD Biosciences

Session 003: Symposium – *Explore Pathology's Potential with AI-Powered Insights*

8:00-10:00 AM

Session Room: Broadway III/IV

Co-Sponsored by the ASIP Digital and Computational Pathology Scientific Interest Group and the Digital Pathology Association (DPA)

Chair: Chhavi Chauhan, PhD ▪ American Society for Investigative Pathology

Session Overview: Explore pathology's potential with AI-powered insights. Delve into the latest research applications of digital pathology, featuring expert presentations on AI-powered biomarker discovery and the transformative use of GenAI/LMMs. Explore the Digital Pathology Association's (DPA) pivotal role in bridging technology and pathology, driving innovation in modern medicine.

- 8:00-8:20 AM
  Welcome and Digital Pathology Association Highlights
  Chhavi Chauhan, PhD ▪ American Society for Investigative Pathology
- 8:20-9:00 AM
  AI Biomarkers: Opportunities and Impact in Pathology and Precision Medicine
  Melissa Alexander, MD, PhD ▪ AstraZeneca
- 9:00-9:40 AM
  Harmonizing Generative AI into Computational Pathology
  Quincy Gu, PhD ▪ University of Pittsburgh
- 9:40-10:00 AM
  Question/Answers and Closing Remarks

Session 004: Symposium – *Gut-Liver Axis in Liver Pathobiology*

10:30-12:30 AM
Session Room: Pavilion Ballroom West
Sponsored by the ASIP Liver Pathobiology Scientific Interest Group

Chair: Vik Meadows, PhD ▪ University of Pittsburgh
Co-Chair: Kari Nejak-Bowen, PhD ▪ University of Pittsburgh
Co-Chair: Satdarshan Paul Monga, MD ▪ University of Pittsburgh

Session Overview: Metabolic dysfunction associated steatotic liver disease (MASLD) and alcohol-associated liver disease (ALD) are among the leading cause of liver-related morbidity worldwide. A highly pertinent role of the gut-liver axis in pathogenesis of MASLD and ALD is being increasingly realized. Key investigators in the field will discuss the mechanisms that alter the gut-liver communication and lead to injury to the liver including gut inflammation, gut leakiness, dysbiosis, and bile acid perturbations. Additionally, there will be discussion on modulation of the gut-liver axis for possible therapeutics of MASLD and ALD.

- **Chair - Welcome and Introductions**
- 10:30-11:00 AM
  Gut-Liver-Immune Axis in MASH
  Reben Raeman, PhD ▪ University of Pittsburgh
- 11:00-11:30 AM
  Exploring Gut Microbiota in Alcohol-Induced Liver Pathology
  Cristina Llorente, PhD ▪ University of California, San Diego
- 11:30-12:00 PM
  Meredith Hullar, PhD ▪ Fred Hutchinson Cancer Center
- 12:00-12:15 PM
  ABSTRACT LIVER001 - CD47 Signaling Contributes to Liver Injury During Cholestatic Liver Injury
  Matthew McMillin, PhD ▪ Baylor College of Medicine
- 12:15-12:30 PM
  ABSTRACT LIVER002 - STARD10 as a Key Modulator of ERBB2-Driven Lipogenesis in Alcohol-Associated Liver Disease
  Manisha Dagar ▪ Cedars-Sinai Medical Center

Session 005: Symposium – *Blood Vessel Club* – *Inflammation in Vascular and Retinal Pathologies: Of Cells and Cytokines*

10:30-12:30 PM
Session Room: Pavilion Ballroom East
Co-Sponsored by the Ocular Pathobiology Scientific Interest Group and the Cardiac and Vascular Pathobiology Scientific Interest Group

Chair: Daisy Shu, PhD ▪ University of South Wales
Co-Chair: Michael Schnoor, PhD ▪ Cinvestav-IPN (Mexico City)

Session Overview: This session will explore the complex interplay between inflammation, vascular biology, and ocular health, highlighting how ocular and systemic vascular pathologies are intricately linked. We will explore the cellular and cytokine mechanisms driving inflammation in conditions such as diabetes, heart disease, and hypertension, emphasizing their impact on ocular health. By examining these connections, we aim to uncover novel insights into the role of inflammation in vascular diseases, providing a comprehensive understanding of how systemic vascular health directly influences ocular pathologies.

- **Chair - Welcome and Introductions**

- 10:30-11:00 AM
  Dual Targeting of Inflammation and Angiogenesis in Neovascular Eye Disease
  Tim Corson, PhD ▪ University of Toronto

- 11:00-11:30 AM
  OCT Angiography and Oximetry
  Yali Jia, PhD ▪ Oregon Health and Science University

- 11:30-12:00 PM
  Mechanobiology of Retinal Inflammation in Early Diabetic Retinopathy
  Kaustabh Ghosh, PhD ▪ University of California

- 12:00-12:15 PM
  ABSTRACT VASCUL001 - The SH2 Domains of RASA1 are Indispensable for Vascular Development
  Nathaniel Lartey, PhD ▪ University of Michigan School of Medicine

- 12:15-12:30 PM
  ABSTRACT VASCUL002 - An Ex-Vivo Human Choroidal Explant Model to Investigate Angiogenesis and Fibrosis in Age-Related Macular Degeneration
  Anton Lennikov, MD, PhD ▪ Schepens Eye Research Institute, Harvard Medical School

Session 006: Lunch and Career Development and Education Session – *Catch You on The Flip Side: Engaging Learners in the Flipped Classroom*
12:30-1:30 PM
Session Room: Skyline I/II (23rd Floor)
Sponsored by the ASIP Education Committee

Co-Chair: Andrew Duncan, PhD ▪ University of Pittsburgh
Co-Chair: Richard Mitchell, MD, PhD ▪ Brigham and Women’s Hospital, Harvard Medical School
Co-Chair: Julie Randolph-Habecker, PhD ▪ Pacific Northwest University

Session Overview: The flipped classroom model has transformed traditional teaching methods, emphasizing active learning and student engagement. This session focuses on the application of case-based learning within a flipped classroom setting to enrich pathology education. The session includes a live demonstration of a case-based activity, allowing attendees to experience the process firsthand. By the end of the session, participants will be equipped with practical tools to begin implementing case-based flipped classroom techniques in their pathology courses, fostering a more interactive and student-centered learning environment.

Session 007: *Lunch and Science Policy Session*
12:30-1:30 PM
Session Room: Broadway III/IV

Session Description: Adapting NIH structure and priorities to take on the challenges of the future is a broadly shared concern inviting a wide range of perspectives and proposals in the science policy and legislative arenas. This session will provide an overview of recently proposed ideas for NIH reform and will seek ASIP community input and feedback.

- 12:30-12:45 PM
  Overview of Proposed Framework for NIH Reform
  Rajanikanth Vadigepalli, PhD ▪ Thomas Jefferson University

- 12:45-1:30 PM
  Roundtable Discussions

  Topic 1: Combining Multiple Institutes into a New NIH Structure
  Topic 2: Indirect Cost Negotiations and Tracking
  Topic 3: Congress’s Role in Directing Funding

Session 008: Cotran Early Career Investigator Award Lecture

1:30-2:30 PM

Session Room: Pavilion Ballroom West

- **Welcome and Introduction**
  Michael Tranter, PhD ▪ Ohio State University

- ***The Fibroblast Odyssey: Journey from Bench to Bedside***
  Onur Kanisicak, PhD ▪ The Ohio State University

Session 009: Symposium – *Chromatin Remodeling and Epigenetics in Cancer*

2:30-4:30 PM

Session Room: Pavilion Ballroom West

Sponsored by the Gene Expression Scientific Interest Group and the Neoplasia, Tumor Microenvironment, and Metastasis Scientific Interest Group

Chair: David Williams, MD, PhD ▪ University of North Carolina
Co-Chair: Qin Yan, PhD ▪ Yale University

Session Overview: Epigenetic changes are a hallmark of carcinogenesis and include alterations in histone modifications, DNA methylation, and chromatin compaction. In addition, many cancer types have recurrent mutations in epigenetic modifiers which drive the malignant phenotype. In some cases, the specific mutations and alterations have become effectively diagnostic for a particular cancer type (e.g., SMARCA4 loss). Notably, the downstream epigenetic alterations may be reversible and, therefore, potential targets for therapy. In this session, we will explore the role of epigenetics in the development of cancer, with a focus on chromatin remodeling complexes. Talks will include discussions of the SWI/SNF complex in cancer and drug resistance and the NuRD complex in leukemia, as well as selected talks from submitted abstracts.

- **Chair - Welcome and Introductions**

- 2:30-3:00 PM
  The Chromatin Remodeling Complex PBAF Functions as a Tumor Suppressor in SCLC
  Arnaud Augert, PhD ▪ Yale School of Medicine

- 3:00-3:30 PM
  SWI/SNF Chromatin Remodeling Complexes in Development and Cancer
  Diana Hargreaves, PhD ▪ Salk Institute for Biological Studies

- 3:30-4:00 PM
  The Role of the Nucleosome Remodeling and Deacetylase (NuRD) Complex in Leukemia
  David Williams, MD, PhD ▪ University of North Carolina

- 4:15-4:15 PM
  ABSTRACT CANCER003 - Single Cell RNA and TCR Sequencing Reveals Novel Regulatory Mechanisms of CD8+ Memory T Cell Formation and Identify a Marker of Response to Checkpoint Inhibitor Therapy in Melanoma
  Goran Micevic, MD, PhD ▪ Yale University School of Medicine

- 4:15-4:30 PM
  Regulation of Cancer Metastasis by an ISWI Chromatin Remodeling Complex
  Qin Yan, PhD ▪ Yale University School of Medicine

Session 010: Symposium – *Guest Society Session – Functional Spatial Multi-Omics Sponsored by The Histochemical Society*
2:30-4:30 PM
Session Room: Pavilion Ballroom East
Sponsored by the Histochemical Society

Chair: Douglas Rosene, PhD, FAAA ▪ Boston University
Co-Chair: Tracy Fischer, PhD ▪ Tulane University

Session Overview: This session will explore cutting-edge approaches integrating spatial omics technologies with multi-omics data to drive advances in understanding complex biological systems. This session highlights the emerging role of spatially resolved omics in bridging the gap between high-dimensional molecular data and functional insights into disease processes. The session will also feature selected talks from abstracts, showcasing innovative research in spatially resolved multi-omics approaches and their potential to revolutionize our understanding of cellular functions, tissue organization, and disease mechanisms. Join us for an exciting exploration of these transformative technologies and their impact on the future of biomedical research and therapeutic development.

- **Chair - Welcome and Introductions**
- 2:00-2:30 PM
  Integrative Multi-Omics Approach to Identify Rationale Therapeutic Targets in Pulmonary Fibrosis: Bridging Gaps Between Animal Model and Human Disease
  Resat Cinar, PhD, MBA ▪ National Institutes of Health
- 2:30-3:00 PM
  Digital Pathology Meets Spatial Omics: Emerging Problems in Data Integration, Solutions, and New Opportunities
  Pinaki Sarder, PhD ▪ University of Florida
- 3:00-3:30 PM
  Integrating Viral Detection with Spatial Imaging
  Sabrina Ramelli, PhD ▪ National Institutes of Health/National Cancer Institute
- 3:30-3:45 PM
  ABSTRACT LIVER003 - Single-Cell Spatial Molecular Imaging of Early and Advanced Fibrosis in Fontan Liver Disease (FALD)
  Brandon Lehrich ▪ University of Pittsburgh School of Medicine
- 3:45-4:00 PM
  ABSTRACT LIVER004 - The Dynamic Role of β-Catenin Activation in Early Liver Regeneration
  Tyler Yasaka ▪ University of Pittsburgh School of Medicine

Session 011: *Minisymposium: Inflammatory and Non-Inflammatory Cellular Alterations in Organ Pathology*
2:30-4:30 PM
Session Room: Broadway III/IV

Chair: Evan Delgado, PhD ▪ University of Pittsburgh
Co-Chair: Anna Tingler, BS ▪ Medical University of South Carolina

- **Chair - Welcome and Introductions**
- 2:30-2:45 PM
  ABSTRACT INFLAM003 - Clostridioides Difficile Reduces Chemosensory Tuft Cells in the Colonic Epithelium
  Adelaide Horvath ▪ University of Houston

- 2:45-3:00 PM
  ABSTRACT INFLAM004 - Investigating the Shrinking Colonic Mucus Layer: Antibiotic Impact on Goblet Cells and the Protective Role of Microbial Metabolites
  Anna Tingler, BS ▪ Medical University of South Carolina
- 3:00-3:15 PM
  ABSTRACT INFLAM005 - Inflammatory Bowel Disease-Susceptibility Genes Display Altered Histone Modification Patterns in TWEAK-Primed Intestinal Inflammatory Fibroblasts
  Cristina Bauset, BPharm, MEd, PhD ▪ University College of Dublin
- 3:15-3:30 PM
  ABSTRACT INFLAM006 - Potential Use of V202, a Novel Small Molecular Bromodomain and End-Terminal Inhibitor in Mitigating Secondhand Smoke (SHS)-Induced Pulmonary Inflammation
  Katelyn Sturgis ▪ Brigham Young University
- 3:30-3:45 PM
  ABSTRACT INFLAM007 - Impact of Secondhand Smoke and E-Cigarette Aerosols on Maternal Lung Inflammation During Pregnancy
  Benjamin Davidson ▪ Brigham Young University
- 3:45-4:00 PM
  ABSTRACT INFLAM008 - CoQ10 and Alpha-Ketoglutarate Effect on Mitochondrial Function and Morphology in a DSS-Colitis Mice Model
  Karen Dubois-Camacho, PhD ▪ University Medical Center Groningen
- 4:00-4:15 PM
  ABSTRACT INFLAM009 - More Than Just Liver: Foxa3-Cre-Mediated YAP Deletion Targets Genitourinary Tissues Causing Polycystic Kidney Disease and Male Infertility
  Laura Molina, MD, PhD ▪ University of Pittsburgh
- 4:15-4:20 PM
  ABSTRACT INFLAM010 - Selective Ablation of FXR in Neurons Is Neuroprotective in A Mouse Model of Acute Liver Failure
  Kathryn Rhodes, BS ▪ University of Texas, Austin

Afternoon Break *(wine and beer will be served)*
4:30-5:30 PM
Pavilion Foyer

Session 012: *Rous-Whipple Award Lecture*
5:30-6:30 PM
Session Room: Pavilion Ballroom West

- **Welcome and Introduction**
  Pilar Alcaide, MD ▪ Tufts University
- ***An Exploration of Noncoding RNAs and DNA Variants in Cancer***
  Anindya Dutta, MBBS, PhD ▪ University of Alabama at Birmingham

Women in Pathology Networking Event
6:30-8:30 PM
Session Room: Skyline I/II (23rd Floor)

Session Overview: Women in Pathology Social: Connect, Communicate & Collaborate
ALL attendees are invited to celebrate the ASIP community with this Women in Pathology sponsored event! Take a break from the science and bring your outgoing, collegial, and team-building self—join us for an evening of treats, drinks, and games! Women in Pathology invites you to shake things up a bit and connect with your colleagues in a night of conversation, networking, and fun competition playing games outside your everyday routine.

SUNDAY, APRIL 27, 2025

Continental Breakfast
7:00-8:00 AM
Pavilion Foyer

Session 013 – Healing Inflammation with Specialized Pro-Resolving Mediators
8:00-10:00 AM
Session Room: Pavilion Ballroom West
Sponsored by the Inflammation and Immunopathology Scientific Interest Group

Chair: Ramon Ramos, PhD ▪ Albany Medical College
Co-Chair: Maria Zambrano ▪ Tufts University School of Medicine

Session Overview: This session will explore specialized pro-resolving mediators (SPMs), focusing on resolvins and their critical roles in the resolution of tissue inflammation and the restoration of homeostasis after injury. The session will cover three pivotal areas: (i) the impact of resolvins on atherosclerosis, (ii) their broader role in inflammation resolution, and (iii) the emerging field of immunonutrition and its connection to SPMs.

- **Chair - Welcome and Introductions**
- 8:00-8:30 AM
  Resolvins in Atherosclerosis
  Gabrielle Fredman, PhD ▪ Albany Medical College
- 8:30-9:00 AM
  Inflammation and Resolvins
  Stephania Libreros, PhD ▪ Yale University School of Medicine
- 9:00-9:30 AM
  Immunonutrition – Specialized Pro-Resolving Mediators
  Robert Martindale, MD, PhD ▪ Oregon Health Science University
- 9:30-9:45 AM
  ABSTRACT MUC001 - Microbial Metabolites Regulate Restitution of Intestinal Epithelial Cells
  Madison Flory ▪ University of Kentucky
- 9:45-10:00 AM
  ABSTRACT MUC002 - Nanoparticle Encapsulation of Protectin D1 and DX: Advancing Colonic Repair Therapy with Pro-Resolving Mediators
  Jael Miranda-Guzman, PhD ▪ University of Michigan School of Medicine

Session 014 – Therapeutic Targeting of the Metastatic Niche in Cancer: Perspectives from Premalignant to Malignant Disease
8:00-10:00 AM
Session Room: Pavilion Ballroom East
Sponsored by the Neoplasia, Tumor Microenvironment, and Metastasis Scientific Interest Group

Chair: Sanjukta Chakraborty, PhD ▪ Texas A&M University
Co-Chair: Evan Delgado, PhD ▪ University of Pittsburgh

Session Overview: Metastatic cancers are a major clinical problem as risk of recurrence often significantly persists despite resection of the primary tumor. One of the primary steps in cancer metastasis is the formation of the metastatic niche that helps in colonization of tumor cells at a distant site. A complex interplay between immune cells and factors in the tumor microenvironment as well as stromal cells in the secondary metastatic sites produce favorable environment for tumor survival and growth. Frequently, these mechanisms are activated in premalignant lesions initiating a cascade

of events that promote tumor metastasis and contribute to therapeutic resistance leading to metastatic relapse. Current research is geared towards the development of new therapeutic interventions that disrupt this metastatic niche and understanding of key driver elements that promote the development and progression of metastasis. This session will explore current basic and translational research that focus on different stages of the metastatic cascade and emerging therapies that seek to redefine the treatment modalities. The presentations will highlight the molecular landscape of the metastatic niche in multiple cancers and discuss how increased mechanistic understanding of the progression of premalignant to malignant disease can unravel key therapeutic targets with transformative clinical value and implications.

- **Chair - Welcome and Introductions**
- 8:00-8:30 AM
  Autophagic Response in Cancer Progression
  Jayanta Debnath, MD ▪ University of California, San Francisco
- 8:30-9:00 AM
  Therapeutic Targeting of the Metastatic Niche: Lessons from Lymphatic Dissemination of Solid Tumors
  Sanjukta Chakraborty, PhD ▪ Texas A&M University School of Medicine
- 9:00-9:30 AM
  Giving Cancer the Flu – Targeting the RIG-I Pathway
  Sudarshan Anand, PhD ▪ Oregon Health and Science University
- 9:30-9:45 AM
  ABSTRACT CANCER005 - Carcinoma-Associated Fibroblasts Induce an Invasive Phenotype and a Preferentially Lymphangiogenic Secretome In E6E7 Transfected Cancer Cells
  Harsh Dongre, PhD ▪ Boston Children's Hospital, Harvard Medical School
- 9:45-10:00 AM
  ABSTRACT CANCER006 - Infiltrating Plasma Cells Promote Glioblastoma Stem Cells Growth Through FCGR2A
  Zhe Zhu, MD, PhD ▪ Columbia University Irving Medical Center

10:00-10:30 AM
Coffee Break
Pavilion Foyer

Exhibitor Spotlight Session
10:10-10:25 AM
Session Room: Pavilion Ballroom West

Session Description: This talk will showcase how the Department of Pathology and Laboratory Medicine at OHSU is at the forefront of medical innovation—transforming patient care and shaping the future of healthcare. Our work spans the full continuum of care, from foundational diagnostics to cutting-edge technologies and research that influence clinical decision-making every day.

The presentation will highlight the department's pivotal role in managing cardiovascular disease, advancing population health, and leading rapid-response efforts during pandemics, while also strengthening preparedness for future emerging infectious disease outbreaks. Equally significant is our leadership in early cancer detection, precision diagnostics, and the integration of targeted therapies. These advances are driven by the incorporation of NGS molecular testing into routine pathology practice, the application of AI in flow cytometry and digital pathology, and innovative, system-wide approaches that enhance laboratory operation efficiency and expand access to care. In addition to clinical and technological innovation, this talk will illustrate how OHSU Pathology is driving excellence in education and research, training the next generation of pathologists, scientists, and laboratory professionals who are redefining the standards of modern medicine. Discover how OHSU Pathology is not just supporting medicine, but leading its transformation.

- ***Advancing Medicine Through Pathology: Shaping the Future at OHSU Pathology***
  Guan Fan, MD, PhD ▪ Oregon Health & Science University

Session 015 – Regulation of Epithelial and Endothelial Junctions: Novel Molecules and Mechanisms

10:30 AM-12:30 PM
Session Room: Pavilion Ballroom West
Sponsored by the Mucosal Pathobiology Scientific Interest Group

Chair: Andrei Ivanov, PhD ▪ Lerner Research Institute, Cleveland Clinic
Co-Chair: Jennifer Brazil, PhD ▪ University of Michigan

Session Overview: Epithelial and endothelial junctions are critical cellular structures that regulate assembly and remodeling of tissue barriers. Junctional integrity and dynamics are controlled by elaborate intracellular mechanisms and various environmental factors. This session discusses recent advances in understanding the structure and regulation of epithelial and endothelial junctions in different tissues. Specific emphasis is placed on the mechanisms and pathophysiologic implications of junctional disruption in human diseases.

- **Chair - Welcome and Introductions**
- 10:30-11:00 AM
  Microbiota-Derived Metabolites and Epithelial Barrier Function
  Sean Colgan, PhD ▪ University of Colorado Anschutz Medical Campus
- 11:00-11:30 AM
  Desmosome Dysfunction in Disease
  Jens Waschke, MD ▪ Ludwig-Maximilians-Universität Munich
- 11:30 AM-12:00 PM
  The Role of the Epigenomics Regulatory Pathways in the Tight Junction Complex Dynamic and Blood-Brain Barrier Function in Health and Disease
  Anuska Andjelkovic-Zochowski, MD, PhD ▪ University of Michigan
- 12:00-12:15 PM
  ABSTRACT LIVER005 - Complete Loss of β- and γ-Catenin in Bile Ducts Leads to Cholestasis and Colonic Inflammation
  Vik Meadows, PhD ▪ University of Pittsburgh School of Medicine
- 12:15-12:30 PM
  ABSTRACT MUC003 - Unconventional Myosin18A is a Novel Regulator of the Intestinal Epithelial Barrier and Mucosal Inflammation
  Susana Lechuga, PhD ▪ Cleveland Clinic Foundation

Session 016 – Intersection of the Vascular and Neural Systems

10:30 AM-12:30 PM
Session Room: Pavilion Ballroom East
Co-Sponsored by the Neuropathology Scientific Interest Group and the Ocular Pathobiology Scientific Interest Group

Co-Chair: Michele Alves, PhD ▪ Florida International University
Co-Chair: Anton Lennikov, MD, PhD ▪ Schepens Eye Research Institute, Harvard Medical School

Session Overview: This session will feature the overlapping advancements in neuroimmune and neurovascular mechanisms involved in neuropathologies. The presentations will showcase the role of inflammation, metabolism, and vascular modifications in rewiring synaptic plasticity and neural processes in neurodegenerative diseases. Insights into the latest findings on brain communication with immune and vascular systems, as essential players in brain physiology and pathology, will be discussed in this session. The Neuropathology Scientific Interest Group and the Ocular Pathobiology Scientific Interest Group invite you to join us for an exciting discussion with the speakers as they share their last scientific advancements.

- **Chair - Welcome and Introductions**

- 10:30-11:00 AM
  Powered by PINK1: Mitochondria and Synaptic Plasticity
  Charleen Chu, MD, PhD ▪ University of Pittsburgh

- 11:00-11:30 AM
  The Pathophysiology of Alzheimer's Disease in the Retina
  Maya Koronyo-Hamaoui, PhD ▪ Cedars-Sinai Medical Center

- 11:30 AM-12:00 PM
  Blood Drivers of Neuroinflammation: From Mechanisms to Therapies
  Katerina Akassoglou, PhD ▪ University of California, San Francisco

- 12:00-12:15 PM
  ABSTRACT NEURO001 - Exploring Vascular Contributions to the Pathobiology of Cognitive Impairment: Focus on Small Vessel Disease of White Matter and Presence of Micro(nano)plastics
  Elaine Bearer, MD, PhD ▪ University of New Mexico School of Medicine

- 12:15-12:30 PM
  ABSTRACT NEURO002 - Microcurrent Electrical Stimulation Activates Small Ca2+-Induced K+ Channel (KCNN) to Promote Axons Growth in Trigeminal Ganglia Neurons and Restore Corneal Nerve Density in Diabetic Keratitis
  Menglu Yang, MD, PhD ▪ Schepens Eye Research Institute, Harvard Medical School

Session 017 – Lunch and Poster Blitzes

12:30-1:30 PM
Session Room: Skyline I/II (23rd Floor)

Moderator: Laura Molina, MD, PhD ▪ University of Pittsburgh

Session Description: Each poster blitz features short (3-minute) presentations based upon selected posters and presented by a young investigator. The objective is to highlight a subset of posters to be presented in a standard poster session to generate interest. Each short presentation will focus on the objectives of the study, the major results, and the conclusions. Details of the studies will be available during the regular poster session

Moderator: Laura Molina, MD, PhD ▪ University of Pittsburgh

- 12:30-12:33 PM
  POSTER 15-CARDIOVAS014 - Immune Checkpoint Inhibitor-Induced Cardiotoxicity is Associated with Dysfunctional Metabolism, Muscle Wasting and Autophagy: An Exploratory Analysis of Pathological Signaling Pathways
  Louisa Tichy ▪ University of North Carolina at Greensboro

- 12:33-12:36 PM
  POSTER 25-LIVER009 - Caspase-11 and GasderminD Deletion Exacerbates Coagulopathy in APAP-Induced Acute Liver Failure
  Joud Mulla, BS ▪ University of Pittsburgh

- 12:36-12:39 PM
  POSTER 27-LIVER011 - Central Administration of Recombinant Insulin-Like Growth Factor 1 Dampens the Neuroinflammatory Response and Attenuates the Cognitive Deficits Observed in a Rodent Model of Hepatic Encephalopathy
  Yubo Wang ▪ The University of Texas at Austin

- 12:39-12:42 PM
  POSTER 33-METABOLOMICS003 - Leveraging Lysosomal Calcium Flux as a Strategy to Induce Autophagy-Lysosomal Biogenesis in Macrophages
  Carlos Cosme, Jr. ▪ University of Pittsburgh

- 12:42-12:45 PM
  POSTER 35-METABOLOMICS005 - Effects of Semaglutide on Trophoblast Cell Function: A Comparative Study
  Kristen Noyes ▪ Brigham Young University
- 12:45-12:48 PM
  POSTER 37-METABOLOMICS006 - Semaglutide Impairs Mitochondrial Bioenergetics and Elevates Oxidative Stress in C2C12 Myoblasts
  Genevieve Parker ▪ Brigham Young University
- 12:48-12:51 PM
  POSTER 41-MUCOSAL005 - Clostridioides Difficile Stimulates IL-22 which Increases Adherent Mucins
  Erin Chard ▪ Medical University of South Carolina
- 12:51-12:54 PM
  POSTER 43-MUCOSAL007 - Mucus-Producing Gastric Metaplasia in Mice Driven by High-Fat Diet Consumption
  Makenna Grozis ▪ Elon University
- 12:54-12:57 PM
  POSTER 45-MUCOSAL009 - High Fat Diet Drives Gastric Metaplasia Through Mast Cell-Driven Inflammation
  Charulekha Packirisamy, PhD ▪ Medical University of South Carolina
- 12:57-1:00 PM
  POSTER 51-TOXPATH002 - Apoptotic Responses in Maternal Lung Following Prenatal Exposure to Secondhand Smoke or E-Cigarette Vapor
  Elizabeth Thurmond ▪ Brigham Young University

Session 018 – Outstanding Investigator Award Lecture and FASEB Excellence in Science Award Lecture

1:30-2:30 PM
Session Room: Pavilion Ballroom West

- **Welcome and Introduction**
  Robinna Lorenz, MD, PhD ▪ Genentech, Inc.
- ***T-Cells at the Heart of Cardiac Remodeling***
  Pilar Alcaide, PhD ▪ Tufts University

Session 019 – President's Symposium – WNT'er in Pathobiology

2:30-4:30 PM
Session Room: Pavilion Ballroom West

Chair: Satdarshan Paul Monga, MD ▪ University of Pittsburgh

Session Overview: The Wnt signaling is vital to the function of many organs and eventually to survival. The past few decades have seen an advancement in our understanding of this complex pathway in development, homeostasis and repair. Aberrant functioning of this pathway has been associated with multitude of diseases ranging from development defects, degenerative diseases and cancer. Improved understanding of the pathway has yielded novel information on the regulation of this pathway in disease pathobiology. This has resulted in new and innovative opportunities in translation ranging from molecular diagnostics, patient stratification and even therapeutics. The current session will discuss advances in understanding of the Wnt signaling pathway in various aspects of organ pathophysiology and targeting various the pathway for innovative therapeutics.

- **Chair - Welcome and Introductions**
- 2:30-3:00 PM
  Wnt Signals Control Cell Proliferation Across Multiple Tissues
  Roeland Nusse, PhD ▪ Howard Hughes Medical Institute

- 3:00-3:30 PM
  Manipulating Wnt Signaling with Antibody Agonists to Restore Tissue Homeostasis
  Stephane Angers, PhD ▪ University of Toronto

- 3:30-4:00 PM
  Intestinal Stem Cell Orchestration of Tumor Microenvironments in Colorectal Cancer
  Marian Waterman, PhD ▪ University of California, Irvine

- 4:00-4:30 PM
  Wnt'er in Liver: Understanding Biology for Therapeutics
  Satdarshan Paul Monga, MD ▪ University of Pittsburgh

Session 020 – Poster Session I (ODD Numbered Posters)

4:30-6:00 PM
Session Room: Atrium Ballroom
Wine and beer will be served

Poster Categories

▪ Cancer Pathobiology ▪ Cardiovascular Pathobiology ▪ Cell Injury and Repair ▪ Gene Regulation in Disease
▪ Infectious Disease ▪ Liver Pathobiology ▪ Lung Pathobiology ▪ Metabolomics ▪ Mucosal Pathobiology
▪ Neuropathology ▪ Ocular Pathobiology ▪ Toxicologic Pathology

Session 021 – Gold-Headed Cane Award Lecture

6:00-7:00 PM
Session Room: Pavilion Ballroom West

- **Welcome and Introduction**
  George Michalopoulos, MD, PhD ▪ University of Pittsburgh

- ***The Importance of Mentorship in Career Development***
  Fred Sanfilippo, MD, PhD ▪ Emory University, Atlanta, GA

Scientific Interest Group Networking Event and Trainee Scholar Awards Presentation

7:00-9:00 PM
Session Room: Atrium Ballroom

MONDAY, APRIL 28, 2025

Continental Breakfast

7:00-8:00 AM
Pavilion Foyer

Session 022 – Diverse Models to Study Microbial-Host Interactions

8:00-10:00 AM
Session Room: Pavilion Ballroom West
Sponsored by the Infectious Disease Scientific Interest Group

Chair: Mindy Engevik, PhD ▪ Medical University of South Carolina
Co-Chair: Anna Tingler ▪ Medical University of South Carolina

Session Overview: In this session, we will explore the intricate relationships between bacteria and host, focusing on how these interactions influence health and disease. Our discussion will be divided into three main topics, each highlighting cutting-edge research and novel insights in the field. These topics include: (1) Bacterial Influence on the Development and Function of the Gastrointestinal Epithelium in Zebrafish, (2) complex interactions between bacterial species in human stool bioreactors, and (3) the role of bacteria in modulating inflammation within the gut epithelium, utilizing

mouse models. Key points include: This session will provide valuable insights into the multifaceted interactions between bacteria and their hosts. By understanding these interactions, we can better appreciate the balance of microbial communities and their critical roles in maintaining health and preventing disease. We look forward to an engaging and informative discussion.

- **Chair - Welcome and Introductions**
- 8:00-8:30 AM
  Pathogenic Inhibition of Epithelial Inflammasome Defenses
  Isabella Rauch, PhD ▪ Oregon Health and Science
- 8:30-9:00 AM
  Investigating Interindividual Differences in Clostridioides Difficile Colonization Resistance and Disease
  Jennifer Auchtung, PhD ▪ University of Nebraska
- 9:00-9:30 AM
  Homeostatic Antiviral Responses of the Intestinal Epithelium
  Timothy Nice, PhD ▪ Oregon Health and Science University
- 9:30-9:45 AM
  ABSTRACT INFDIS001 - Bacterial Polyphosphates Enhance Intestinal Inflammation through Disruption of IL-27/STAT Signaling
  Markus Bosmann, MD ▪ Boston University Chobanian & Avedisian School of Medicine
- 9:45-10:00 AM
  ABSTRACT INFDIS002 - A Two-Hit Sepsis Model Reveals JunB-Driven Endothelial Inflammatory Memory That Exacerbates Secondary Pneumonia
  Ramon Ramos, PhD ▪ Albany Medical College

Session 023 – The Beginning Affects the End: Developmental Origins of Metabolic Disease

8:00-10:00 AM
Session Room: Pavilion Ballroom East

Chair: Michael Thompson, MD, PhD ▪ Washington University, St. Louis
Co-Chair: Traci Parry, PhD ▪ University of North Carolina, Greensboro

Session Overview: Obesity and its associated metabolic complications continue to rise at an alarming rate. An emerging area of research centers on the idea that exposures in utero and shortly after birth can have a significant impact on the development of chronic diseases in offspring, known as the developmental origins hypothesis of adult disease (DOHAD). This session will include talks from leaders in the field studying how developmental programming applies to offspring metabolic disease and provide insight on how early life events contribute to pathophysiology.

- **Chair - Welcome and Introductions**
- 8:00-8:30 AM
  Early Life Factors Predicting Metastatic and Metabolic Disease in Adulthood
  Kent Thornburg, PhD ▪ Oregon Health and Sciences University
- 8:30-9:00 AM
  Lasting Impacts of Maternal Obesity on Skeletal Muscle Growth and Metabolism in Adolescent Macaques
  Carrie McCurdy, PhD ▪ University of Oregon
- 9:00-9:30 AM
  Immunometabolic Reprogramming in the Offspring of Obese Mothers
  Alina Maloyan, PhD ▪ Oregon Health and Science University
- 9:30-9:45 AM
  ABSTRACT Diabetes001 - Impact of Gestational Diabetes Treatment Modalities on Placental Metabolic Adaptations: Differential Roles of PKM2, AMPK, and mTOR Pathways
  Emily Broberg ▪ Brigham Young University

10:00-10:30 AM
Coffee Break
Pavilion Foyer

Session 024 – Tissue Fibrosis: Common and Unique Mechanisms Across Different Organs

10:30 AM-12:30 PM
Session Room: Pavilion Ballroom West

Chair: Andrei Ivanov, PhD ▪ Cleveland Clinic

Session Overview: Fibrosis is a common consequence of chronic tissue inflammation and injury. It leads to poorly reversible alterations in tissue structure and dysfunctions of the affected organs. This session features presentations from leading experts who study fibrosis in different organs. The session highlights diverse mechanisms underlying fibrogenesis under different pathologic conditions and discusses recent advances in identifying novel molecular targets for therapeutic prevention or reversal of fibrosis.

- **Chair - Welcome and Introductions**
- 10:30-11:00 AM
  The Alveolar Stem Cell Niche in Idiopathic Pulmonary Fibrosis: Vulnerable Seed in Hostile Soil
  Dianhua Jiang, MD, PhD ▪ Cedars Sinai Medical Center
- 11:00-11:30 AM
  Multiomic Analysis of Human MASH Identifies Novel Targets for Liver Fibrosis
  David Brenner, MD ▪ Sanford Burnham Prebys Medical Discovery Institute
- 11:30 AM-12:00 PM
  Role of Collagen Binding Receptors in Acute Kidney Injury
  Ambra Pozzi, PhD ▪ Vanderbilt University School of Medicine
- 12:00-12:30 PM
  Mesenchymal Cells as Key Players in Crohn's Disease Associated Fibrosis
  Iryna Pinchuk, PhD ▪ Penn State Health, Milton S. Hershey Medical Center

Session 025 – Pathobiology for Basic Scientists Course: Cancer Vascularization

10:30 AM-12:30 PM
Session Room: Pavilion Ballroom East
Sponsored by the ASIP Education Committee

Chair: Richard Mitchell, MD, PhD ▪ Brigham and Women's Hospital, Harvard Medical School
Co-Chair: Diane Bielenberg, PhD ▪ Boston Children's Hospital, Harvard Medical School

Session Overview: Without new blood vessel growth to sustain the increased metabolic demand of a mass of proliferating cells, malignancies may be constrained to a relatively microscopic focus of cells, or alternatively can become ischemic and die. In addition, neovascularization – including lymphatics – is the pathway by which tumors can escape systemically, but importantly is also the avenue by which immune cells and various treatment modalities can access the original cancer. Thus, understanding the mechanisms by which tumors can drive local vasculogenesis, and the nature of the resulting – often aberrant – vessels can lead to important therapeutic targets. This session is intended to provide foundational knowledge and an overview of the cellular and molecular underpinnings of neovascularization in malignancy, while also pointing toward some translational aspects.

- **Chair - Welcome and Introductions**
- 10:30-11:00 AM
  Anti-Angiogenesis and Immunotherapy for Hepatocellular Carcinoma: Why, When, and How?
  Dan Duda, PhD ▪ Massachusetts General Hospital, Harvard Medical School

- 11:00-11:30 AM
  Targeting Angiogenesis via Resolution of Inflammation
  Dipak Panigrahy, PhD ▪ Beth Israel Deaconess Medical Center
- 11:30 AM-12:00 PM
  Neuropilin Receptors as Critical Mediators of Tumor Angiogenesis, Lymphangiogenesis, and Tumor Immunity
  Diane Bielenberg, PhD ▪ Boston Children's Hospital, Harvard Medical School
- 12:00-12:30 PM
  The Lymphatic System in Cancer Progression
  Tim Padera, PhD ▪ Massachusetts General Hospital, Harvard Medical School

Session 026 – Lunch and Poster Blitzes

12:30-1:30 PM
Session Room: Skyline I/II (23rd Floor)

Moderator: Louisa Tichy ▪ University of North Carolina at Greensboro

Session Description: Each poster blitz features short (3-minute) presentations based upon selected posters and presented by a young investigator. The objective is to highlight a subset of posters to be presented in a standard poster session to generate interest. Each short presentation will focus on the objectives of the study, the major results, and the conclusions. Details of the studies will be available during the regular poster session

- 12:30-12:33 PM
  POSTER 2-CANCER014 - The Impact of Tumor Burden on Skeletal Muscle Function in Tumor-Bearing Mice and the Therapeutic Role of Physical Activity
  Zoe Libramento ▪ University of North Carolina at Greensboro
- 12:33-12:36 PM
  POSTER 6-CANCER018 - Investigating the Neural Progression of Pancreatic Ductal Adenocarcinoma
  Carly Ramos ▪ Boston Children's Hospital
- 12:36-12:39 PM
  POSTER 12-CARDIOVAS011 - Investigating Associations between the Gut and the Heart in PTSD-Like Mice
  Amber Hazzard ▪ Medical University of South Carolina
- 12:39-12:42 PM
  POSTER 22-GENEREG002 - Investigation of Drosophila Spn42Dd Gene to Accurately Model Human SerpinE1 Diet and Stress-Related Phenotypes
  Michelle Thayer ▪ Iowa State University
- 12:42-12:45 PM
  POSTER 24-LIVER008 - Ataxia-Telangiectasia Mutated Mediates Transforming Growth Factor Beta Response in Acetaminophen-Induced Liver Injury in Mice
  Patrick Mireles, PharmD ▪ University of Texas at Austin
- 12:45-12:48 PM
  POSTER 26-LIVER010 - Maternal Obesogenic Diet Exposure Increases Severity of Offspring Cholestatic Liver Disease
  Fareeha Siddique ▪ Washington University, St. Louis
- 2:48-12:51 PM
  POSTER 40-MUCOSAL004 - Proteomic Analysis of Human Colon Organoids: Impact of Multi-Mineral Intervention Alone and with LPS-Cytokines and Mesalamine
  Gillian Moraga ▪ University of Michigan Medical School

- 12:51-12:54 PM
  POSTER 42-MUCOSAL006 - Akkermansia Muciniphila Alters Small Intestine Specialized Cell Populations
  Rachel Edens ▪ Medical University of South Carolina
- 12:54-12:57 PM
  POSTER 44-MUCOSAL008 - Urothelial-Specific Deletion of Semaphorin 3F Leads to Detrusor Underactivity
  Gabriel-Luis Ocampo ▪ Boston Children's Hospital
- 12:57-1:00 PM
  POSTER 50-OCULAR001 - Transcriptomic Profiling of Cytokine-Induced Pathways in Human Retinal Endothelial Cells: Unraveling Angiofibrotic and Inflammatory Signaling in Neovascular AMD
  Daisy Shu, PhD ▪ University of New South Wales

Session 027 – Cardiovascular Disease and Aging: Emerging Science and Clinical Perspectives

1:30-3:30 PM
Session Room: Pavilion Ballroom West
Sponsored by the Cardiac and Vascular Pathobiology Scientific Interest Group

Chair: Yabing Chen, PhD ▪ Oregon Health and Science University
Co-Chair: Alan Daugherty, PhD, DSc ▪ University of Kentucky College of Medicine

Session Overview: This session will bring together leading basic science researchers and physician-scientists, providing new insights and perspectives on (i) genetic, (ii) metabolic regulation of cardiovascular disease, and (iii) new concepts of pan-vascular pathology in cardiovascular disease and aging. Vascular complications and the management of atherosclerosis and aging continue to be a highly active area of research in cardiovascular biology and disease. We anticipate that this forum will generate significant discussion. Attendees will have the opportunity to gain exposure to cutting-edge research in cardiovascular pathology and clinical management. Additionally, by inviting a diverse group of cardiovascular investigators at different career stages and disciplines, this session aims to attract attendees with a research focus on cardiovascular biology and disease to the ASIP annual meeting, with the goal of expanding our membership.

- **Chair - Welcome and Introductions**
- 1:30-2:00 PM
  Vascular Stiffness and Aging: A Pan-vascular Mechanism
  Yabing Chen, PhD ▪ Oregon Health and Science University
- 2:00-2:30 PM
  Mechanobiology in Cardiometabolic Diseases
  Tzung Hsiai, MD, PhD ▪ University of California, Los Angeles
- 2:30-3:00 PM
  Novel Mechanisms Regulating Injury-Induced Neointimal Formation and Atherosclerosis
  Shiyou Chen, DVM, PhD ▪ University of Missouri School of Medicine
- 3:00-3:30 PM
  Aneurysm and Dissection: Mechanisms and Beyond
  Alan Daugherty, PhD, DSc ▪ University of Kentucky College of Medicine

Session 028 – Minisymposium – Immune Cell Activation in Tissue Inflammation and Injury

1:30-3:30 PM
Session Room: Pavilion Ballroom East

Chair: Ian Cartwright, PhD ▪ University of Colorado Anschutz Medical Campus
Co-Chair: Joud Mulla, BS ▪ University of Pittsburgh

- **Chair - Welcome and Introductions**

- 1:30-1:45 PM
  ABSTRACT IMMUN001 - Phosphorylated UBC9 Regulates RAB GTPase Mediated Cytokine Secretion Signaling in Alcohol-Associated Liver Disease
  Swati Chandla, PhD ▪ Cedars-Sinai Medical Center
- 1:45-2:00 PM
  ABSTRACT IMMUN002 - The Protein Tyrosine Phosphatase CD45 Regulates PMN Transepithelial Migration and Inflammatory Function in Mucosal Tissues
  Jennifer Brazil, PhD ▪ University of Michigan
- 2:00-2:15 PM
  ABSTRACT IMMUN003 - Epithelial Immune Memory Imprinted by Transmigrating Neutrophils
  Ian Cartwright, PhD ▪ University of Colorado Anschutz Medical Campus
- 2:15-2:30 PM
  ABSTRACT IMMUN004 - Noncanonical Inflammasome Caspase-11 Promotes Neutrophil Infiltration and Inflammatory Response in Severe Tissue Injury
  Joud Mulla ▪ University of Pittsburgh
- 2:30-2:45 PM
  ABSTRACT IMMUN005 - Neutrophils Migrate Long Distances and Persist for Days Following Reperfusion in a Mouse Model of Ischemic Stroke
  David Sullivan, PhD ▪ Northwestern University
- 2:45-3:00 PM
  ABSTRACT IMMUN006 - An Extracellular Histone-Platelet-Macrophage Axis Drives Pathophysiology in Pulmonary Fibrosis
  Markus Bosmann, MD ▪ Boston University Chobanian & Avedisian School of Medicine
- 3:00-3:15 PM
  ABSTRACT IMMUN007 - Role of Neutrophils in Pathogenesis of Primary Sclerosing Cholangitis
  Nidhi Jalan Sakrikar, PhD ▪ Mayo Clinic

Session 029 – Poster Session II (EVEN Numbered Posters)

3:30-5:00 PM
Session Room: Atrium Ballroom
Beer and wine will be served

Poster Categories

▪ Cancer Pathobiology ▪ Cardiovascular Pathobiology ▪ Experimental Therapeutics ▪ Gene Regulation in Disease ▪ Infectious Disease ▪ Liver Pathobiology ▪ Lung Pathobiology ▪ Metabolomics ▪ Mucosal Pathobiology ▪ Neuropathology ▪ Toxicologic Pathology

ASIP Business Meeting and Meritorious Awards Presentations

5:00-6:30 PM
Session Room: Pavilion Ballroom West

Chair: Satdarshan Paul Monga, MD ▪ University of Pittsburgh

Society-Wide President's Reception and Networking Event – Portland Spirit Dinner Cruise

6:30-10:00 PM

Boarding Time: 7:00 PM

Boarding Location: Salmon Springs Dock (10-minute walk from Hilton Portland), 1010 SW Naito Parkway, Portland, OR

Sailing Time: 7:30 PM (SHARP)
Return Time: 10:00 PM Spirit of Portland - Salmon Springs Dock

TUESDAY, APRIL 29, 2025

Continental Breakfast
7:00-8:00 AM
Pavilion Foyer

Session 030 – Immunity and Innate Environments in Inflammation
8:00-10:00 AM
Session Room: Pavilion Ballroom West
Sponsored by the Mucosal Pathobiology Scientific Interest Group and the Inflammation and Immunopathology Scientific Interest Group

Chair: Ronen Sumagin, PhD ▪ Northwestern University
Co-Chair: Jennifer Brazil, PhD ▪ University of Michigan

Session Overview: This session will provide insights into various aspects of innate and adoptive immunity in inflammation. This will include adopted behavior, heterogeneity, cellular crosstalk and functional implications of innate and adoptive immune cells to disease progression.

- **Chair - Welcome and Introductions**
- 8:00-8:30 AM
  A Tissue Encoded Metabolic Switch Programs Intestinal Tolerance
  Roni Nowarski, PhD ▪ Brigham and Women's Hospital, Harvard Medical School
- 8:30-9:00 AM
  Altered Estrogen Signaling Drives T-Cell Dysfunction in the Inflamed Mucosa
  Wendy Goodman, PhD ▪ Case Western Reserve University
- 9:00-9:30 AM
  Neutrophil-Endothelial Crosstalk Via Cathepsin G and Cortactin Facilitates Neutrophil Extravasation
  Michael Schnoor, PhD ▪ National Polytechnic Institute, Mexico City
- 9:30-9:45 AM
  ABSTRACT INFLAM001 - The TNF Superfamily Factor TWEAK Promotes Inflammatory Stroma Expansion in Ulcerative Colitis and Modulates Fibroblast-Monocyte Crosstalk via STAT3
  Carlos Matellan, PhD ▪ University College Dublin
- 9:45-10:00 AM
  ABSTRACT INFLAM002 - Exploring Neutrophil Contributions to Anti-TNFa Therapy Resistance in IBD
  Laura Manzanares, PhD ▪ Northwestern University

Session 031 – Understanding Liver Cancer Pathogenesis
8:00-10:00 AM
Session Room: Pavilion Ballroom East
Sponsored by the Liver Pathobiology Scientific Interest Group

Chair: Kari Nejak-Bowen, PhD ▪ University of Pittsburgh
Co-Chair: Vik Meadows, PhD ▪ University of Pittsburgh
Co-Chair: Satdarshan Paul Monga, MD ▪ University of Pittsburgh

Session Overview: Globally, liver cancer is the 6th most common cancer and 3rd most common cause of death related to cancer. While the treatment of unresectable hepatocellular cancers (HCCs) has seen a revolution with the introduction of immune checkpoint inhibitors, the response rates are close to 30%. These rates are even more dismal for cholangiocarcinomas. Hence, these tumor types remain under intense research to better understand the cellular and

molecular drivers with an eventual goal of implementing personalized therapies. The current session will hear from experts who have used zebrafish models to unravel the molecular underpinnings of HCC for identifying and targeting key oncogenic pathways. The role of matrix stiffness in pathogenesis of HCC will be discussed to potentially develop better biomarkers and develop therapies to prevent development of tumors in high-risk cases. Eventually, there will be talk on signaling mechanisms that direct tumor microenvironment which could also have therapeutic implications.

- **Chair - Welcome and Introductions**
- 8:00-8:30 AM
  Matrix Regulation of Hepatocellular Carcinoma
  Natalie Torok, MD ▪ Stanford University School of Medicine
- 8:30-9:00 AM
  Zebrafish Models of Liver Cancer
  Kimberly Evason, MD, PhD ▪ University of Utah
- 9:00-9:30 AM
  YAP-Dependent Regulation of Tumor Microenvironment During Cholangiocarcinoma Progression
  Xin Chen, PhD ▪ University of Hawaii Cancer Center
- 9:30-9:45 AM
  ABSTRACT LIVER006 - Deletion of Glutamine Synthetase from Hepatocellular Carcinoma-Associated Macrophages Reduces Glutamine Bioavailability and Disease Severity
  Evan Delgado, PhD ▪ University of Pittsburgh School of Medicine
- 9:45-10:00 AM
  ABSTRACT LIVER007 - A Digital Pathology Approach to Predict Spatial Subtype Signatures of Hepatocellular Carcinoma from Histologic Images
  Tyler Yasaka ▪ University of Pittsburgh

10:00-10:30 AM
Coffee Break
Pavilion Foyer

Session 032 – Advancing Cancer Research Through Artificial Intelligence

10:30 AM-12:30 PM
Session Room: Pavilion Ballroom West

Chair: Wendy Mars, PhD ▪ University of Pittsburgh
Co-Chair: Yabing Chen, PhD ▪ Oregon Health and Science University

Session Overview: AI has the ability to change our worlds in a positive manner when harnessed with forethought and care. This session will focus on cutting edge research that is using AI and machine learning techniques to advance our knowledge of both diagnostics and mechanisms that drive disease, to better our approach to targeted treatment.

- **Chair - Welcome and Introductions**
- 10:30-10:45 AM
  Harnessing AI to Propel Pathology Research (Video Presentation)
  Liron Pantanowitz, MD, PhD ▪ University of Pittsburgh
- 10:45 -11:15 AM
  Machine Learning of Phenotype-Associated Subpopulations From Millions of Cells
  Zheng Xia, PhD ▪ Oregon Health and Science University

- 11:15- 11:45 AM
  Advancing Multiplex Tissue Imaging: 3D Reconstruction and Panel Reductionence Images for Colorectal Cancer
  Yong Hwan Chang, PhD ▪ Oregon Health and Science University
- 11:45 AM-12:15 PM
  Artificial Intelligence (AI)-Assisted Flow Cytometry Analysis for Hematologic Disorders
  Guang Fan, MD, PhD ▪ Oregon Health and Science University
- 12:15-12:30 PM
  ABSTRACT CANCER006 - Machine Learning-Enhanced MALDI Imaging Mass Spectrometry of N-Glycans for Hepatocellular Carcinoma Classification and Heterogeneity Analysis
  Muhammed Bayram ▪ Medical University of South Carolina

Session 033 – Vascular Inflammation in Atherosclerosis

10:30 AM-12:30 PM
Session Room: Pavilion Ballroom East
Sponsored by the Cardiac and Vascular Pathobiology Scientific Interest Group and the Società Italiana di Patologia e Medicina Traslazionale

Chair: Max Corsi Romanelli, MD, PhD ▪ University of Milan
Co-Chair: David Sullivan, PhD ▪ Northwestern University

Session Overview: Myocardial infarction is often seen by the public as the defining event of heart disease, but clinicians and researchers now appreciate that inflammation is at the root of all cardiovascular diseases (CVDs). Atherosclerosis is the leading cause CVD and, as a result, is a leading cause of morbidity and mortality in the developed world. In this session we will examine fundamental connection between atherosclerosis and vasculature inflammation. Various facets of the inflammatory process as they relate to CVD will be explored, including adaptive immune tolerance, therapeutic target identification, and the function of the inflammasome in atherosclerosis. The session will also highlight cutting edge research with shorter talks selected from submitted abstracts.

- **Chair - Welcome and Introductions**
- 10:30-11:00 AM
  Break of Tolerance to Self in Atherosclerosis
  Klaus Ley, MD ▪ Augusta University
- 11:00-11:30 AM
  Vascular Inflammation in Atherosclerosis: A Systems Approach to Target Discovery
  Masanori Aikawa, MD, PhD ▪ Harvard Medical School, Brigham and Women's Hospital
- 11:30 AM-12:00 PM
  The Inflammasome in Cardiovascular Disease
  Elena Vianello PhD ▪ University of Milan
- 12 :00-12 :15 PM
  ABSTRACT VASCUL003 - Circadian Factor Bmal1 Regulates Vascular Calcification
  Ming He, MD, PhD ▪ University of Alabama, Birmingham
- 12:15-12:30 PM
  ABSTRACT VASCUL004 - The T-Cell CXCR3-Endothelial Cell ICAM-1 Axis Drives T-Cell Cardiac Infiltration in Cardiometabolic Heart Failure with Preserved Ejection Fraction (HFpEF)
  Zachary Robbe ▪ Tufts University School of Medicine

Session 034 – Lunch and Career Development/Education Session - Clocking In Success: Turning Busy into Workplace Productivity

12:30-1:30 PM
Session Room: Skyline I/II (23rd Floor)
Sponsored by the ASIP Career Development Committee

Chair: Onur Kanisicak, PhD ▪ The Ohio State University
Co-Chair: Menglu Yang, MD, PhD ▪ Schepens Eye Research Institute, Harvard University

Session Overview: In today's demanding clinical and research environments, managing time effectively is essential for maintaining productivity and advancing one's career. This interactive session is designed to help clinicians, researchers, and trainees take control of their schedules, prioritize tasks efficiently, and utilize productivity tools specifically suited to the demands of scientific and clinical work. Featuring expert insights and actionable strategies, participants will learn practical methods for streamlining workflows, setting realistic goals, and maximizing output. Whether balancing patient care with research projects, managing teams, or enhancing personal efficiency, this session offers valuable approaches to boost productivity and achieve professional goals effectively.

- **Chair - Welcome and Introductions**
- 12:30-12:35 PM
  Session Introduction
  Menglu Yang, MD, PhD ▪ Schepens Eye Research Institute, Massachusetts Eye & Ear, Harvard Medical School
- 12:35-12:50 PM
  The Art of Harmonizing Professional and Personal Life: Achieving Balance for Intellectual and Emotional Well-Being
  Cecelia Yates, PhD ▪ University of Pittsburgh
- 12:50-1:20 PM
  Panel Discussions

  Satdarshan Paul Monga, MD ▪ University of Pittsburgh
  Traci Parry, PhD, ACSM-CEP ▪ University of North Carolina at Greensboro
  Daisy Shu, PhD, FAAO, FARVO ▪ University of New South Wales
  Cecelia Yates, PhD ▪ University of Pittsburgh
- 1:20-1:30 PM
  Session Conclusion
  Onur Kanisicak, PhD ▪ The Ohio State University

Session 035 – Young Scientist Leadership Award Lecture

1:30-2:30 PM
Session Room: Pavilion Ballroom West

- **Welcome and Introduction**
  William Muller, MD, PhD ▪ Northwestern University
- ***Leveraging the Frontline: Targeting Innate Immunity for Vaccines and Therapies***
  Nakisha Rutledge, PhD ▪ Temprian Oncology, Inc.

Session 036 – Minisymposium – Cardiovascular and Metabolic Homeostasis and Disease

2:30-4:30 PM

Session Room: Pavilion Ballroom West

Chair: Traci Parry, PhD ▪ University of North Carolina, Greensboro
Co-Chair: Maria Zambrano ▪ Tufts University School of Medicine

- **Chair - Welcome and Introductions**
- 2:30-2:45 PM
  ABSTRACT CARDIOVAS001 - AI-Assisted Drug Discovery for Early-Stage Type 2 Diabetes: Pathophysiology, Prognostics, and Risk Stratification
  Saifur Khan, PhD ▪ University of Pittsburgh
- 2:45-3:00 PM
  ABSTRACT CARDIOVAS002 - T-Cell-Released IFNγ Induces the Expression of Cardiac Fibroblast MHC-II Required for Doxorubicin Cardiotoxicity
  Maria Zambrano ▪ Tufts University School of Medicine
- 3:00-3:15 PM
  ABSTRACT CARDIOVAS003 - BMP7 Attenuates Fibrotic Response in a Murine Model of Proliferative Vitreoretinopathy
  Amy Li ▪ Boston University
- 3:15-3:30 PM
  ABSTRACT CARDIOVAS004 - Unraveling the Effects of Dchs1 on Cardiac Proliferation: A Study of Fibroblast-Cardiomyocyte Crosstalk in Cardiac Development
  Kathryn Byerly ▪ Medical University of South Carolina
- 3:30-3:45 PM
  ABSTRACT CARDIOVAS005 - Linking Ciliogenesis, Bromodomain Function, and Acetylation in Valvulogenesis
  Brian Loizzi ▪ Medical University of South Carolina
- 3:45-4:00 PM
  ABSTRACT CARDIOVAS006 - Synergistic Effects of Hemodynamic Forces and Genetics on Heart Development
  Makena Phillips ▪ Oregon Health and Science University
- 4:00-4:15 PM
  ABSTRACT CARDIOVAS007 - Differential Effects of Allulose, Glucose, and Fructose on Mitochondrial Respiration, Viability, and Invasion in Placental Trophoblast Cells
  Andrew Richardson ▪ Brigham Young University
- 4:15-4:30 PM
  ABSTRACT CARDIOVAS008 - Autoantibodies in Arrhythmogenic Cardiomyopathy Patients Activate GSK-3β Leading to Loss of Cardiomyocyte Cohesion
  Sunil Yeruva, PhD ▪ Ludwig Maximilian University Munich

Session 037 – Minisymposium – Cancer Biomarkers and Mechanisms

2:30-4:30 PM
Pavilion Ballroom East

Chair: Kari Nejak-Bowen, PhD ▪ University of Pittsburgh
Co-Chair: Selene Shore, PhD ▪ Medical University of South Carolina

- **Chair - Welcome and Introductions**
- 2:30-2:45 PM
  ABSTRACT CANCER007 - Nanoluciferase-CD63-Labeled Extracellular Vesicle Signaling and Biodistribution in Mouse Intraductal Model of Ductal Carcinoma in situ
  Cole Hladik ▪ University of Oklahoma Health Science Center
- 2:45-3:00 PM
  ABSTRACT CANCER008 - Pancreatic Tail Orthotopic Injections of Murine Pancreatic Adenocarcinoma Reveal Fetal Sex-Effects
  Terry Morgan, MD, PhD ▪ Oregon Health and Science University
- 3:00-3:15 PM
  ABSTRACT CANCER009 - Exploring the Role of Obesity-Associated Extracellular Matrix in Local Breast Cancer Progression
  Malika Sekhri ▪ University of Oklahoma Health Science Center
- 3:15-3:30 PM
  ABSTRACT CANCER010 - Histamine Receptor 1 is Expressed in Oropharyngeal Squamous Cell Carcinomas (OPSCC)
  Selene Shore, PhD ▪ Medical University of South Carolina
- 3:30-3:45 PM
  ABSTRACT CANCER011 - Improving Neuroendocrine Neoplasm Tissue Diagnostics Using Novel and Existing General Neuroendocrine Cell Markers
  Adrian Jones ▪ Queen's University
- 3:45-4:00 PM
  ABSTRACT CANCER013 - Loss of Hepatic Tuberous Sclerosis 1(TSC1) Promotes Liver Cystogenesis and Tumorigenesis Via a Non-Canonical mTORC1-TFEB-Dependent Mechanism
  Chen Zhang ▪ University of Kansas Medical Center

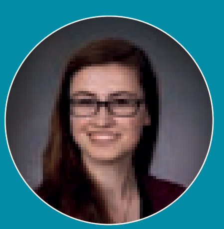
MEETING ADJOURNMENT 4:30 PM

ASIP 2025 Virtual Events

Young Investigator Spotlight Seminar
May 15 | 1 PM ET

Cardiac Fibroblast Specific PTBP1 Modulates the Profibrotic Response Through Alternative Splicing

Shea Ricketts, PhD Candidate (University of North Carolina)



Young Investigator Spotlight Seminar
July 17 | 1 PM ET

SARS-CoV-2 Infection Promotes Persistent Neuroinflammation in Non-human Primates

Meredith Mayer, PhD Candidate (Tulane University)

Young Investigator Spotlight Seminar
August 21 | 1 PM ET

Role of MET in Acetaminophen-Induced Acute Liver Injury and Liver Regeneration

Siddhi Jain, PhD (University of Pittsburgh)

Women in Pathology Special Event
September 18 | 1 PM ET

How to Delegate, Create Accountability, and Have More Productive Meetings

Deb Elbaum, MD, PCC (Deb Elbaum Coaching, LLC)

Young Investigator Spotlight Seminar
October 16 | 1 PM ET

The Role of Epidermal Growth Factor Receptor in Acetaminophen Hepatoxicity

Gillian Williams, PhD Candidate (University of Pittsburgh)

Young Investigator Spotlight Seminar
November 20 | 1 PM ET

Exploring the Effects of Antibiotics on the Gastrointestinal Epithelium

Anna Tingler, PhD Candidate (Medical University of South Carolina)

Learn more and register at ASIP.org/meetings/virtual-events or scan the QR code

Pathobiology 2025 Abstracts

Session 001 – Regenerative Medicine Innovations for Tissue Repair and Cancer Therapy

CANCER001

Novel Oncogenic Roles of Semaphorin 5B (Sema5B) in Renal Cancer Pathogenesis

Sagnik Giri[1], Chinaza Nnam[2], Alicia Thompson[1], Lucas A. Salas[2], and Anirban Kundu[1]*

[1]University of Arizona in Tucson, Tucson, AZ; [2]Dartmouth Geisel School of Medicine, Hanover, NH; *Corresponding author

Background: HIF stabilization via VHL inactivation is one of the major factors in >50% of renal cell carcinoma (RCC). Despite several lines of systemic therapies, advanced RCC portends a grave prognosis. Since HIF plays a major role in RCC progression and the recently approved HIF-2a inhibitor, Belzutifan, has variable response and therapy resistance, research on novel HIF-downstream targets is necessary. We discovered a novel HIF-targeted oncogene, semaphorin 5B (Sema5B), whose RNA expression is several folds upregulated in RCC compared to any normal and tumor tissues in the TCGA database. We demonstrated that Sema5B silencing compromises in vitro and in vivo tumor phenotypes of RCC cell lines. However, its mechanism of tumor progression is unknown. **Methods:** Confocal microscopy, Proteomics, gene silencing, CRISPR, and RNA-sequencing. **Results:** We demonstrate, for the first time, Sema5B protein expression in a human cancer, RCC. Sema5B protein was significantly upregulated in the tumors from patient-matched normal/tumor RCC samples. Sema5B's expression is upregulated by both HIF-1a and HIF-2a in RCC. Unlike other semaphorins, Sema5B lacks an N-terminal signal sequence, necessary for its membrane transport or secretion. We demonstrate that Sema5B is an intracellular protein, largely located in the perinuclear region, endoplasmic reticulum, and the nuclear membrane of RCC cells, and co-localize with p130Cas, calnexins, and lamin B1. Sema5B has a dedicated nuclear localization signal (NLS), and indeed, a fraction of Sema5B translocates to the RCC nucleus. However, mutation of the functional arginine residues within the NLS cannot stop its nuclear import, suggesting alternate mechanisms of Sema5B's nuclear localization. Next, RNA-seq of control and stable Sema5B knockdown OS-RC-2 cells (RCC cell line) followed by pathway analyses suggest that Sema5B promotes RCC progression via suppressing the inflammatory environment and stimulating angiogenesis. Moreover, we demonstrate that Sema5B may promote RCC cell proliferation via suppressing the expression of a putative tumor suppressor, FRMD3. FRMD3 is a member of the protein 4.1 superfamily, containing a highly conserved membrane-associated domain, FERM. FRMD3 overexpression in Sema5B-proficient RCC cells suppresses their proliferation, and its knockdown in Sema5B-depleted RCC cells rescues their proliferation. **Conclusion:** Unlike other semaphorins, Sema5B is an intracellular protein. It promotes RCC progression via suppressing a novel tumor suppressor, FRMD3.
Acknowledgments: The research was supported by grants to Dr. Anirban Kundu from the American Urological Association (Award 669279), the Department of Defense (Award HT9425-23-1-0339 and HT9425-23-1-0783), and the University of Arizona Cancer Center's startup fund.

CANCER002

Immune Checkpoint Inhibitor Enhances Response to RNAi-mediated β-catenin Inhibition in CTNNB1-mutated Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality globally with <18-month overall survival for unresectable disease. Response in first-line setting is suboptimal at ~30% with current immune checkpoint inhibitor (ICI) combinations. Patients with gain-of function mutations in β-catenin (encoded by *CTNNB1*; 26-38% of all HCC) demonstrate mixed benefit to ICI combinations, along with an overall dearth of precision therapies available. In the current study, we investigate the relevance of RNAi-mediated β-catenin inhibition in animal models of CTNNB1-mutated HCC and assess therapeutic efficacy in combination with ICI. **Methods**: We suppressed β-catenin mRNA in the S45Y-CTNNB1-hMet (β-M) and T41A-CTNNB1-G31A-NFE2L2 (β-N) mouse models of HCC, which represent around 12% and 10% of human HCC, respectively. Following hydrodynamic tail vein injection (HDTVi), mice were administered siRNAs

formulated in lipid nanoparticles (LNP) targeting either CTNNB1 (LNP-CTNNB1) or scrambled sequence (LNP-CTRL) once tumors were notably established, at 6-weeks or 8-weeks, in the β-M or β-N models, respectively. **Results**: We observed a heterogenous response to LNP-CTNNB1 treatment in advanced-stage disease in both models with 5/8 animals responding (responders; R) and 3/8 animals demonstrating minimal response (non-responders; NR) at 10.5-weeks and 13.5-weeks post-HDTVi, respectively. Spatial transcriptomics was performed with 10X Visium platform on both models, which revealed the R phenotype to be driven by restored adaptive immunity with active T and B cell infiltration and re-engaged interferon signaling within residual tumor nodules that were positive for a mutated-β-catenin gene signature (MBGS), previously shown by us to identify *CTNNB1* mutations. NR phenotype was driven in part by insufficient β-catenin suppression and expansion of secondary driver clones. Additionally, we observed increased *Cd274* (encoding PD-L1) and *Pdcd1* (encoding PD-1) expression in MBGS-positive nodules in NR and control compared to R phenotype, implying lack of lymphocyte effector function and increased exhaustion in NR phenotype. As a result, combining LNP-CTNNB1 with α-PD-1 enhanced efficacy with elimination of all NR phenotype which led to increased survival (p=0.02) compared to LNP-CTNNB1 + IgG treated animals. Enhanced response with ICI was associated with an increase in granzyme B (GZMB)-positive cells, a marker for cytotoxic T cells, and organized lymphoid aggregates (LA). Analysis of IMbrave150 trial demonstrated *CTNNB1* mutations inversely correlate with LA-like gene signature, which was associated with response and survival to combination-ICI. **Conclusions**: Overall, administration of α-PD-1 augmented LNP-CTNNB1 response through restoring global adaptive immunity, limiting T cell exhaustion via β-catenin/PD-L1 axis, and enhancing T cell cytotoxic function. **Acknowledgements**: This work was supported by NIH grants 1R01CA251155, 1R01CA250227 and Endowed Chair for Experimental Pathology to SPM. This work was also funded in part by T32EB001026 to TY and BML. This work was also funded in part by F30CA284540 to BML. This work was also supported in part by the University of Pittsburgh Center for Research Computing through the resources provided and by NIH grant 1P30DK120531 to Pittsburgh Liver Research Center (PLRC) for services provided by the Genomics and Systems Biology Core.

Session 004 – Gut-Liver Axis in Liver Pathobiology

LIVER001

CD47 Signaling Contributes to Liver Injury During Cholestatic Liver Injury

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Background: Cholestatic liver diseases are characterized by reduced bile flow, biliary injury, fibrosis, and, in severe cases, cirrhosis and Type C hepatic encephalopathy (HE). Thrombospondin-1 (TSP1) is a homotrimeric glycoprotein that is upregulated during various liver disease states and has been associated with promoting cellular senescence, inflammation, and fibrosis. TSP1 can induce downstream signaling via CD47 giving support that this may be a therapeutic target to alleviate aspects of pathology associated with cholestatic liver diseases. Therefore, the aim of this study was to antagonize CD47 signaling and investigate the resulting changes to pathology associated with BDL-induced cholestasis. **Methods:** Sham or bile duct ligation (BDL) surgery was performed in male Sprague Dawley rats and osmotic minipumps infusing anti-CD47 or anti-IgG2a (5 µg/day; intraperitoneal) were implanted at the time of surgery. Two weeks post-surgery, neurological and neuromuscular assessments were performed each week including rotarod and open field analyses. After 5 weeks, rats were euthanized, and tissue was collected. Liver injury was assessed by measuring serum alanine aminotransferase (ALT), total bilirubin, serum alkaline phosphatase (ALP), liver H&E imaging, and cytokeratin 19 (CK19) imaging. TSP1 and CD47 expression was measured by immunohistochemistry and real-time PCR analyses. Hepatic fibrosis was assessed using Sirius red staining and measuring collagen 1A1 (COL1A1) expression. Cellular senescence was determined by measuring p16 and p21 protein expression and immunohistochemistry. Interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNFα) mRNA expression was used to assess hepatic inflammation. **Results:** BDL rats had significant increases of both TSP1 and CD47 expression compared to sham controls. BDL + anti-IgG2a rats spent significantly less time on a rotating rotarod and engaged in less activity in an open field apparatus compared to BDL + anti-CD47 rats at 4 weeks after BDL. These data support that anti-CD47 infusion reduces neurological deficits observed following BDL surgery. Significant increases in ALT, total bilirubin, ALP, COL1A1, p16 and p21 expression, as well as IL-1β and TNFα, were observed in BDL + anti-IgG2a rats compared to sham controls. These same measures of pathology were significantly decreased in BDL + anti-CD47 compared to BDL + anti-IgG2a, demonstrating a reduction in BDL-induced liver injury following anti-CD47 infusion. **Conclusions:** These data demonstrate that BDL-rats infused with anti-CD47 had reduced neurobehavioral deficits and lessened biliary injury compared to BDL-rats infused with anti-IgG2a. This supports that CD47 may be a therapeutic target for cholestatic liver disease and its resulting complications including HE.

Abstract LIVER002

StarD10 as a Key Modulator of ErbB2-Driven Lipogenesis in Alcohol-Associated Liver Disease

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Background: In developed countries, one of the most important risk factors for liver cirrhosis and HCC is alcoholic steatohepatitis (ASH). Recently, ErbB2 (transmembrane receptors with tyrosine kinase activity) has been described in ASH and correlated with severity of inflammation and signs of longstanding liver injury like liver fibrosis. ErBb2 has the ability to translocate into the nucleus and mediates survival effects in injured hepatocytes. StAR-related lipid transfer domain containing 10 (StarD10) is a phosphorylated protein that positively regulates ErBb2 signaling pathway. We recently reported that ethanol administration lowers StarD10 phosphorylation leading to increased plasma membrane fluidity and induced ErbB2 activity in breast cancer. This study aims to investigate whether phosphorylation of StarD10 regulates lipogenesis via ErbB2 in alcohol associated liver disease (ALD). **Methods:** NIAAA mice livers and primary mouse hepatocytes. Western blot and immunoprecipitation. Duolink PLA and immunofluorescence. Lipids binding and membrane fluidity assays. **Results:** We found increased protein levels of StarD10 and ErbB2 in NIAAA livers as well as in ethanol-treated hepatocytes. In addition, ethanol promoted StarD10 and ErbB2 co-immunoprecipitation and co-localization in vitro and in vivo. StarD10 was found to bind all phosphatidylinositides species (PIPs) and ethanol treatment increased it further. Overexpression of StarD10 increased membrane fluidity in hepatocytes that presumably caused ErbB2 transactivation and nuclear translocation from plasma membrane. Also, StarD10 overexpression induced SERBP1 and its downstream target genes expression such as FASN and ACC, the de novo lipogenesis related gene. These findings suggest that StarD10 binds to, and may transport, PIPs influencing membrane lipid composition and lipids biosynthesis via ErbB2 pathway. **Conclusion:** This study suggests a potential role for StarD10-induced nuclear ErbB2 to cause liver steatosis upon alcohol treatment. It also opens a wide field of research about functional implications of StarD10 and nuclear ErbB2 cross-talk deducing possible therapeutic strategies in ALD.

Session 005 – Blood Vessel Club – Inflammation in Vascular and Retinal Pathologies: Of Cells and Cytokines

VASCUL001

The SH2 Domains of RASA1 Are Indispensable for Vascular Development

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Background: Loss-of-function mutations in genes encoding the Ephrin receptor B4 (EPHB4) receptor tyrosine kinase (RTK) and the p120 Ras GTPase-activating protein (RASA1) are genetically dominant and lead to a similar spectrum of blood and lymphatic vessel (LV) anomalies in humans. EPHB4 and RASA1 cooperate to negatively regulate the Ras-mitogen-activated protein kinase (MAPK) pathway in endothelial cells (EC). However, the precise mechanisms by which EPHB4 and RASA1 interact to regulate this signaling pathway remain poorly understood. We hypothesize that EPHB4 may phosphorylate an unknown membrane-resident protein, which could be recognized either directly or indirectly by the Src-homology-2 (SH2) domains of RASA1, facilitating RASA1's targeting to Ras-GTP at the plasma membrane. **Methods:** We utilized CRISPR/Cas9 gene editing to generate a RASA1 knock-in mutant (2SH2), introducing an R198A mutation into the N-SH2 domain and R389E and K391E mutations into the C-SH2 domain. These mutations completely abrogate RASA1's SH2 domain recognition of phosphotyrosine, while maintaining protein stability. Embryos from timed matings between Rasa1 fl/2SH2 heterozygous mice were harvested at various developmental stages. Additionally, we crossed Rasa1 fl/2SH2 and Rasa1 fl/fl mice with Cdh5ert2cre transgenic mice. Pregnant dams were administered tamoxifen (TM) at E11.5 to disrupt Rasa1 floxed alleles in EC, and embryos were collected at different times. Embryos were fixed in formalin, embedded in paraffin, sectioned (5 µm), and stained with antibodies for CD31. Stained sections were analyzed by fluorescence microscopy. **Results:** Our preliminary data showed that Rasa1 +/2SH2 mice were viable and fertile, but no Rasa1 2SH2/2SH2 pups were found in crosses of heterozygotes. Analysis of timed pregnancies revealed that Rasa1 2SH2/2SH2 embryos were present in progressively fewer numbers with increasing gestational age, with a complete absence by E14.5-E15.5. At E10.5, some Rasa1 2SH2/2SH2 embryos exhibited severe defects in developmental angiogenesis, similar to homozygous RASA1 null embryos. In contrast, embryos that survived to E13.5-14.5 showed an intact blood vascular system, but instead exhibited nuchal translucency and lymphedema, consistent with impaired development of lymphovenous valves. Notably, tamoxifen administration to Rasa1 fl/2SH2 Cdh5ert2cre embryos resulted in hemorrhagic phenotypes at E18.5, similar to those observed in some Rasa1 fl/fl Cdh5ert2cre embryos. **Conclusions:** The RASA1 2SH2 mutant is embryonically lethal, highlighting the essential role of RASA1's SH2 domains in blood vascular and lymphatic vessel development. These findings underscore the critical interaction between RASA1 and EPHB4 and undefined SH2

domain ligands in endothelial cell signaling and vascular development. **Acknowledgement:** This research was supported by NIH grants HL120888 and HL146352 to PDK.

VASCUL002

An Ex-Vivo Human Choroidal Explant Model to Investigate Angiogenesis and Fibrosis in Age-Related Macular Degeneration

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Introduction: Pathological angiogenesis and fibrosis are hallmark complications of neovascular age-related macular degeneration (nAMD), leading to irreversible vision loss. While anti-VEGF therapies have significantly improved patient outcomes, treatment resistance, and progressive fibrosis remain major clinical challenges. Studying these processes in human tissue has been limited by the constraints of 2D cell cultures and the scarcity of surgical specimens. To address this gap, we developed and characterized an ex-vivo human choroidal explant model embedded in a gel matrix, allowing for the investigation of angiogenic and fibrotic responses in a physiologically relevant 3D environment. **Methods:** Human donor eyes (<24 hours post-mortem) were obtained, and RPE/choroidal complexes were isolated into ~1 mm circular explants from central, macular, and peripheral regions of both healthy and wet AMD subjects. To assess RPE contributions to angiogenesis, RPE was mechanically removed from a subset of explants. Samples were either processed immediately for baseline analysis or embedded in a gel matrix and subjected to experimental conditions modulating (1) media composition, (2) cytokine/chemokine exposure (e.g., VEGF, TGF-β, PDGF), and (3) oxygen tension. Cellular responses were analyzed via phase-contrast and confocal microscopy, flow cytometry, and bulk RNA sequencing. The feasibility of explant storage, including cryopreservation, was also evaluated. **Results:** Choroidal explants exhibited dynamic, condition-dependent cellular outgrowth. Endothelial cells, pericytes, RPE, and fibroblasts contributed to distinct angiogenic and fibrotic responses. RPE removal enhanced endothelial outgrowth, suggesting an inhibitory role of RPE-derived factors in neovascularization. VEGF-rich conditions promoted angiogenesis, whereas VEGF inhibition led to fibroblast proliferation and extracellular matrix deposition. Hypoxia further exacerbated fibrosis, mirroring late-stage nAMD pathology. Wet AMD explants demonstrated significantly increased angiogenesis compared to healthy controls. Notably, explants remained viable and responsive under optimized storage conditions, including successful cryopreservation in liquid nitrogen for long-term use. **Conclusion:** This ex-vivo human choroidal explant model serves as a robust, translationally relevant platform for studying angiogenesis and fibrosis in nAMD. By preserving human-specific cellular interactions, it enables precise disease modeling, therapeutic target identification, and preclinical drug screening in a clinically relevant system. This model holds promise for advancing nAMD research and guiding the development of novel treatment strategies beyond VEGF inhibition.
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Session 009 – Chromatin Remodeling and Epigenetics in Cancer

CANCER003

Single Cell RNA and TCR Sequencing Reveals Novel Regulatory Mechanisms of CD8+ Memory T-Cell Formation and Identify a Marker of Response to Checkpoint Inhibitor Therapy in Melanoma

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Background: Melanoma is the deadliest form of skin cancer with 104,960 new cases and 8,430 deaths estimated for 2025 in the United States. Despite significant progress with immune-checkpoint inhibitors (ICI) targeting PD-1 and CTLA-4, many patients with advanced melanoma do not have a durable response to ICI and are at high risk for recurrence and death. Lack of a durable response is linked to failure of T-cell memory formation. However, (i) our understanding of memory T-cell formation during ICI therapy is limited and (ii) there are currently no markers to identify patients predisposed to impaired memory T-cell formation and poor response to ICI. **Methods:** Here, we use a BrafV600E driven mouse model of melanoma and patient samples to characterize the single-cell transcriptional and TCR profiles to identify CD8+ T cell clonal expansion with distinct transcriptomic and epigenetic signatures associated with response to CTLA-4/PD-1 blockade *in vivo*. **Results:** We uncover that expansion of a matching dominant tumor-specific CD8+ T clonotype emerges in the tumor microenvironment and lymph nodes (LN) in response to immunotherapy in melanoma. We performed single-cell regulatory network inference and clustering to analyze the transcriptional regulatory network in memory CD8+ T cells. Our results demonstrated that in addition to IL-7R pathway activation, cytotoxic and NK cell-like properties, expression of killer cell lectin receptor family members, cathepsin W, and interferon induced transmembrane proteins, but no exhaustion markers are

upregulated in this CD8+ T cell memory clonotype in responders to checkpoint therapy. In contrast, non-responders exhibit several non-dominant clonal CD8+ populations with features of exhaustion including Pdcd1, Lag3, Havcr2 and Tigit expression ($p<0.005$). Targeting IL-7R epigenetically increased immunogenicity and sensitivity of melanoma to ICI therapy. We integrate our findings with single-cell sequencing of melanoma patients to find that the memory signature is associated with response to therapy, longer overall survival ($p<0.0001$) and identify an epigenetic marker of therapy response. We investigate the marker in pre-treatment biopsies of 43 melanoma patients and found that hypomethylation of this memory locus is associated with a four-fold prolongation ($p<0.05$, Log-rank test) of response to ICI and could differentiate between responders and non-responders to therapy (HR=3.1). We measured marker expression by immunohistochemistry in a separate cohort of melanoma patients at Yale and found that high protein expression was associated with significantly longer progression free survival (HR= 8.2, $p<0.01$) and response to ICI therapy ($p<0.01$). **Conclusions:** These studies uncovered an IL-7R-dependent regulatory network that orchestrates multiple transcriptional programs and contributes to response to ICI therapy in melanoma and identify a marker which may be clinically useful to identify non-responders to ICI.

Session 010 – Guest Society Session – Functional Spatial Multi-Omics

LIVER003

Single-cell Spatial Molecular Imaging of Early and Advanced Fibrosis in Fontan Liver Disease (FALD)

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Background: Single-ventricle congenital heart disease is a rare and lethal cardiac abnormality that disrupts normal circulation in neonates. A series of palliative surgeries restores systemic oxygenation thereby extending life expectancy into adulthood. The final operation culminates in a “Fontan” circulation, where venous blood bypasses the subpulmonary ventricle to connect with the pulmonary circulation. The loss of venous pulsatility leads to chronic central venous congestion that leads to FALD. FALD is poorly understood and has metabolic and systemic sequelae, including the development of liver cancer and the need for heart-liver transplantation. **Methods**: Single-cell spatial transcriptomics (CosMx™ Spatial Molecular Imaging with in-situ hybridization of 6000 genes) was performed on liver explant tissue sections from FALD patients (n=2) identified at the University of Pittsburgh Medical Center (UPMC) with early and advanced fibrosis. Unbiased clustering and dimensionality reduction was performed to identify cell populations across normal liver, early, and advanced FALD. Gene set enrichment analysis (GSEA) identified pathways enriched in specific cell populations. **Results**: We analyzed 60,339 and 291,840 high-quality cells from normal and FALD livers, respectively. Unbiased clustering resulted in 12 cell types, including hepatic stellate cells, endothelial cells (ECs), immune subtypes, cholangiocytes, and hepatocyte subtypes, including pericentral hepatocytes, senescent-like hepatocytes, and cancer precursor cells. Senescent-like hepatocytes were unique to advanced FALD with a 4-fold enrichment and expressed cellular stress markers *JUN, HSPA1B,* and *ATF3.* Additionally, senescent-like hepatocytes expressed senescence-associated secretory phenotype chemokines CXCL1 and CXCL2 and demonstrated upregulation of pathways associated with TNFA signaling via NFKB, reactive oxygen species, hypoxia, and unfolded protein response. While senescent-like hepatocytes were distributed throughout the hepatic lobule from portal (zone 1) to central (zone 3) regions, metabolically, they demonstrated upregulation of Wnt/β-catenin signaling along with canonical zone 3 processes, including xenobiotic metabolism, bile acid biosynthesis, and glycolysis. This zone 3 phenotype was driven by ectopic EC zonation, whereby ECs in zone 1 aberrantly expressed zone 3 markers. Specifically, we identified cross-talk between zone 1 EC-derived WNT2 activating β-catenin signaling in hepatocytes, likely driven by chronic hypoxia throughout the hepatic lobule. **Conclusions**: We generated the first single-cell spatial transcriptomic profile in human FALD using the CosMx™ 6k platform to identify a unique population of senescent-like hepatocytes in advanced FALD. Cross-talk between ECs and senescent-like hepatocytes perturbed the metabolic zonation of the liver. Results from these studies will help identify FALD patients with detrimental physiologic sequelae. **Acknowledgements**: This work was supported by NIH grants R01DK062277 and SVC Endowed Chair in Pathobiology and Therapeutics to SPM. This work was supported in part by T32EB001026 and F30CA284540 to BML. AS was supported through K08 HL161440 and AHA CDA 852875. Support through The Center for Computational Immunogenetics and Drug Repurposing and the Heart Institute was also provided.

LIVER004
The Dynamic Role of β-catenin Activation in Early Liver Regeneration
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Background: The hepatic lobule demonstrates a functional polarity along the portal-central axis known as metabolic zonation (MZ), in which hepatocytes engage in distinct metabolic roles from the portal vein (zone 1; Z1) to the central vein (zone 3; Z3), with zone 2 (Z2) residing in the middle. During homeostasis, Wnt/β-catenin signaling regulates Z3 polarity by driving Z3 gene expression. This pathway has also been shown to be necessary for optimal proliferation following partial hepatectomy (PH), during which the liver regenerates to its original mass. This process of liver regeneration (LR) enables living-donor transplant and liver resection, which rely on regrowth of resected or transplanted tissue. The mechanistic role of Wnt/β-catenin signaling in MZ and during the acute regenerative response remains poorly understood. **Methods:** We utilized single cell spatial transcriptomics to investigate the spatiotemporal changes in expression of 100 zonated genes at baseline and 1, 3, 6, and 12 hours of LR following PH in a murine model. In situ hybridized imaging reads were assigned to cells using cell boundary tracing through QuPath. Uniform Manifold Approximation and Projection (UMAP) was performed on these cells and unbiased clustering was used to identify distinct expression profiles. Immunohistochemistry (IHC) was used to observe protein expression of key zonation markers at these time points. Immunofluorescence (IF) staining for β-galactosidase in Axin2-LacZ reporter mice was used to address β-catenin activity during early LR. **Results:** Unbiased clustering demonstrated a marked panzonal shift at 1h post-PH which persisted through 12h post-PH. These de novo clusters showed pan-zonal activation of the Z3 genes *Wnt2*, *Wnt9b,* and β-catenin target genes (e.g. *Glul, Axin2*). While Z1 and Z3 markers such as GLUL, CYP2E1, and CYP2F2 remained unchanged at the protein level via IHC, Z2 markers HAMP1/2 and CCND1 showed an expanded expression extending into Z1 and Z3 by 12h post-PH. IF of β-galactosidase in Axin2-LacZ reporter mice demonstrated ectopic expression extending towards the portal vein at 12h. **Conclusions:** Our study demonstrates early transcriptional changes during LR evident at 1h post-PH and maintained through 12h post-PH, suggesting activation of regenerative wave of Wnt/β-catenin activity extending transiently across the lobule. These changes occur alongside Z2 broadening with preservation of Z1 and Z3 markers, suggesting a role for ectopic β-catenin activation in promoting enhanced Z2, but not Z3, gene expression. This early Z2 expansion may be a prerequisite to subsequent entrance into the cell cycle by hepatocytes across the lobule. Our work underscores the significance of Wnt/β-catenin signaling and LZ during the immediate response to PH. **Acknowledgements:** This work was supported by NIH grants 1R01DK62277, 1R01DK103775, 1R01CA251155, 1R01CA250227 and SVC Chair in Pathobiology and Therapeutics to SPM. This work was also funded in part by T32EB001026 to TMY and BML. This work was also supported in part by the University of Pittsburgh Center for Research Computing through the resources provided and by NIH grant 1P30DK120531 to Pittsburgh Liver Research Center (PLRC) for services provided by the Genomics and Systems Biology Core.

Session 011 – Minisymposium – Inflammatory and Non-inflammatory Cellular Alterations in Organ Pathology

INFLAM003
***Clostridioides difficile* Reduces Chemosensory Tuft Cells in the Colonic Epithelium**
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Background: *Clostridioides difficile* is an important human pathogen that causes severe diarrhea and life-threatening colitis. *C. difficile* infection affects thousands of patients in the United States each year and represents an annual cost of over $1 billion. *C. difficile* colonizes the mucus layer of the colon, where it produces toxins that cause inflammation, diarrhea, epithelial damage, and pseudomembranous colitis. While previous studies have shown that *C. difficile* reduces goblet cells, its impact on other epithelial cell types remains poorly understood. One cell type of emerging interest is the tuft cell—a rare chemosensory cell that detects luminal signals and modulates the immune response. **Hypothesis**: We hypothesized that *C. difficile* infection decreases tuft cells. **Methods and Results**: Analysis of single cell RNAseq data revealed that tuft cells express multiple *C. difficile* toxin receptors, including LSR, FZD1, LRP1, LDL4 and SEMA6A; suggesting that tuft cells may be susceptible to *C. difficile* toxins. To create a murine *C. difficile* infection model, we treated adult C57BL/6 mice with a cocktail of antibiotics in the drinking water for four days, followed by a single intraperitoneal injection of clindamycin. We

then oral gavaged mice with PBS vehicle control or *C. difficile* strain R20291 and monitored the mice over time. As expected, mice lost significant weight, with the peak of weight loss occurring on day 3 post-infection and resolving at Day 10. Analysis of RNAseq of colonic tissue from mice from day 0-10 post-infection revealed a significant decrease in DLCK1 gene expression on day 3, 4 and 5 post-infection, with resolution on day 8 and 10. We also observed decreased levels of IL-25, a cytokine known to be secreted by tuft cells, as well other tuft cell markers SUCNR1, CKB, ALDH1A1, TRPM5, and BMX. At day 3 post-infection, we observed decreased levels of the tuft cell markers DLCK1, Pou2f3, cytokeratin 18 and phosphoEGFR in the colon of mice with *C. difficile* infection by immunostaining compared to PBS treated mice. **Conclusions**: These findings demonstrate that *C. difficile* infection significantly reduces chemosensory tuft cells, highlighting an underexplored mechanism by which *C. difficile* disrupts the intestinal epithelium and immune signaling. **Acknowledgements:** This was supported by funding from the 2024 Histochemical Society Capstone Research Grant and the 2024 American Society of Investigative Pathobiology Summer Research Opportunity Program.

INFLAM004

Investigating the Shrinking Colonic Mucus Layer: Antibiotic Impact on Goblet Cells and the Protective Role of Microbial Metabolites

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Background: Cystic fibrosis (CF) is a genetic disorder caused by mutations in CFTR, leading to thick mucus accumulation in the lungs, which causes frequent respiratory infections and necessitates antibiotic treatment. We hypothesized that antibiotics, while treating CF, could have unintended effects on the gut microbiome and intestinal architecture, potentially disrupting mucus production. **Methods:** To test this hypothesis, we conducted 16S RNA sequencing and non-targeted metabolomics using LC-MS/MS on fecal samples from pediatric CF patients and healthy children. Samples were collected from children who had not received antibiotics for 30 days and CF patients currently on antibiotics. We evaluated colonic mucus production by measuring mucin proteins MUC1 and MUC2 in biopsy specimens using immunostaining. MUC2 levels were further assessed in mucus-producing cells after antibiotic exposure using qPCR and immunostaining. Additionally, stool-derived bioreactors were cultivated from both groups and treated with antibiotics. Supernatants from these bioreactors were then applied to mucus-producing cells to observe MUC2 levels. **Results:** 16S RNA sequencing revealed that CF patients had significantly altered gut microbiota compared to non-CF children. In particular, stool samples from CF patients on antibiotics showed a reduction in Firmicutes (e.g., *Anaerostipes*, *Ruminococcus*, *Blautia*) and Actinobacteria (e.g., *Bifidobacterium*), while Bacteroidetes (e.g., *Bacteroides*, *Parabacteroides*) were elevated. Non-targeted metabolomics revealed a significant decrease in amino acids and metabolites promoting colonic mucus, especially in CF patients on antibiotics. Immunostaining of colonic biopsies from CF patients on antibiotics showed a reduction in mucus-filled goblet cells, decreased MUC2 levels, and lower sialic acid compared to non-CF controls and CF patients without recent antibiotic exposure. This mucus depletion was notably more pronounced with specific classes of antibiotics. In vitro experiments with human mucus-producing intestinal cells showed that antibiotics did not directly impact mucus production or MUC2 protein expression. However, metabolites from untreated stool bioreactors upregulated MUC2 production, while metabolites from antibiotic-treated bioreactors did not. **Conclusions:** Our findings suggest that antibiotics significantly alter the gut microbiome and deplete essential metabolites that promote mucus production, potentially exacerbating gastrointestinal complications in CF patients. Antibiotics, particularly certain classes, disrupt the microbial community and the associated metabolites necessary for maintaining the colonic mucus layer. These results underscore the importance of carefully considering antibiotic use in CF management, as the choice of therapy may worsen gut health and mucus-related complications. **Acknowledgements:** R35GM155451 and T32GM132055-01.

INFLAM005

Inflammatory Bowel Disease-Susceptibility Genes Display Altered Histone Modification Patterns in TWEAK-Primed Intestinal Inflammatory Fibroblasts

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Background: The ability of innate immune cells to acquire transient memory after an inflammatory insult is an immuno-regulatory mechanism associated to epigenetic reprogramming and known as trained immunity. Intestinal fibroblasts may acquire immunomodulatory properties and interact with immune cells in severe and therapy-resistant Inflammatory Bowel Disease (IBD) patients. However, whether inflammatory intestinal fibroblasts acquire trained immunity is unknown. Here we explore the role of TWEAK, a member of the TNF super family (TNFSF) of factors, as a mediator of inflammatory memory in colonic fibroblast. **Methods:** Human primary colonic fibroblasts were stimulated with TWEAK and treated with IL-1α either alone or sequentially, allowing stimuli-free period (2-5 days) in between. Bulk RNA-sequencing, Cleavage Under Targets & Tagmentation (CUT&Tag), qRT-PCR and ELISA were used for analysis. **Results:** Pretreatment with TWEAK followed by 2-5 days resting enhanced fibroblast response to IL-1α, including increased gene expression of *CCL5, CXCL6 or TSLP*, and CCL5 protein secretion, compared to cells only stimulated with IL-1α. RNAseq revealed a broad training effect with 213 genes enhanced in response to IL-1α after TWEAK pre-treatment, and predicted the involvement of TFs such as NFKB1 and JUNB in the activation of these genes. CUT&Tag analysis showed increased histone 3-lysine-4 trimethylation (H3K4me3), a transcription-permissive histone modification, at transcription start sites in fibroblasts trained with TWEAK and rested 2 days, compared with vehicle or TWEAK 24h. Specifically, promoters of IBD-susceptibility genes such as *ATG16L1, TNFAIP3, JAK2, BCL6* or *TSLP,* as well as *PDPN*, a marker of inflammatory stromal cells, had increased H3K4me3 compared to vehicle or TWEAK 24h. Additionally, other permissive (H3K27ac) and repressive (H3K27me3) histone modifications accumulated at inflammatory gene promoters 2 days post-training. **Conclusions:** TWEAK-mediated training enhances the expression of a selected cohort of inflammatory genes in response to secondary IL-1α stimulation. This training is associated to alterations in the pattern of H3K4me3, H3K27ac and H3K27me3 modifications at IBD-susceptibility genes, suggesting the involvement of chromatin remodeling in the acquisition of persistent inflammatory characteristics in human colonic fibroblasts. **Acknowledgements:** Project funded by Science Foundation Ireland / Irish Research Council Pathway Award (21/PATH-S/9621) and SBBS-SPARK UCD.

INFLAM006

Potential Use of V202, a Novel Small Molecular Bromodomain and End-terminal Inhibitor, in Mitigating Secondhand Smoke (SHS)-induced Pulmonary Inflammation

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Background: Inflammation underpins pulmonary disease progression during tobacco smoke exposure that may culminate in irreversible pulmonary disease. While primary smoke poses notable risk, nearly half of the US population is also susceptible due to exposure to secondhand smoke (SHS). In the present study, we assessed a potential role for V202, a novel small molecular bromodomain and end-terminal inhibitor, as a potential means of attenuating SHS-mediated inflammation. **Methods:** We exposed wild type mice to a 30-day time course of room air (RA), SHS five times weekly via a nose-only delivery system (Scireq Scientific, Montreal, Canada), or to both SHS and 10 mg/kg V202 via gavage three times a week. We evaluated leukocyte abundance and the secretion of inflammatory mediators in bronchoalveolar lavage fluid (BALF). We also assessed general morphology via histology staining and the activation of receptor tyrosine kinase (RTK) family members. **Results:** While morphology visualized by classic H&E staining was unremarkable, SHS-mediated increases in BALF protein abundance, total cellularity, and percent PMNs were attenuated with concomitant administration of V202. We also discovered SHS-induced activation of RTKs that were pro-inflammatory (JAK1, JAK3, ABL1, and ACK1), and related to endothelial and vascular remodeling (VEGFR3, VEGFR2, EphB4, EphB6, and FAK) RTKs. Furthermore, inflammatory cytokines including GCSF, IFN-γ, IL-12p70, IL-17A, LIX, and TNF-α were all augmented by SHS exposure. Importantly, each of these RTKs and cytokines/chemokines were significantly attenuated by V202 despite SHS exposure. **Conclusions:** In summary, inflammatory responses induced by SHS exposure were mitigated by V202. These data reveal fascinating potential for the utility of V202 in lessening smoke-induced pulmonary exacerbations. **Acknowledgements:** This work was supported by funding from the National Institutes of Health (1R15HL152257) and the Flight Attendant's Medical Research Institute (CIA2300003).

INFLAM007

Impact of Secondhand Smoke and E-Cigarette Aerosols on Maternal Lung Inflammation During Pregnancy

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Background: Maternal exposure to environmental pollutants, including secondhand smoke (SHS) and e-cigarette aerosols (eCigs), poses significant risks during pregnancy. This study examines the differential inflammatory responses in the lungs

of pregnant mice exposed to SHS or eCig aerosol. **Methods:** Pregnant C57BL/6 mice were exposed to SHS or eCig for four or six days during gestation. Bronchoalveolar lavage fluid (BALF) and lung tissues were analyzed for inflammatory mediators, including cytokines, chemokines, and oxidative stress markers. **Results:** SHS exposure elicited a pronounced inflammatory response characterized by elevated IL-1β, TNF-α, and IL-6, as well as increased MCP-1 and MIP-1α. Exposure also led to persistent oxidative stress and extracellular matrix remodeling. In contrast, eCig exposure induced a transient, milder inflammatory response, with moderate IL-6 and MCP-1 elevations and earlier activation of the anti-inflammatory IL-10 pathway. Both exposures disrupted key signaling pathways, including RAGE-mediated inflammation and mTOR-dependent growth regulation, potentially impairing maternal and fetal health. While SHS exposure results in sustained lung inflammation and tissue remodeling, eCigs elicit a less severe but still significant inflammatory response. **Conclusions:** These findings highlight the risks associated with both inhaled toxicants during pregnancy and emphasize the need for stricter regulations and preventive interventions to protect maternal and fetal health. **Acknowledgements:** This work was supported by funding from the National Institutes of Health (1R15HL152257) and the Flight Attendant's Medical Research Institute (CIA2300003).

INFLAM008

CoQ10 and Alpha-ketoglutarate Effect on Mitochondrial Function and Morphology in a DSS-Colitis Mice Model

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Background: Ulcerative colitis (UC) is an inflammatory bowel disease related to fragmented mitochondria, tricarboxylic acid cycle metabolites downregulation, and decreased electron transport chain (ETC) gene expression with also lower ETC complex activity. These features point out the colitis condition as a mitochondriopathy, highlighting the need for therapies that aim to improve mitochondrial pathological adaptation, especially for treatment-resistant patients. Alpha-ketoglutarate (α-KG) or CoenzymeQ10 (CoQ10) exhibit antioxidant and anti-inflammatory properties in *in-vivo* colitis models, however their effect on ETC activity, mitochondrial dynamics and morphology associated with colon healing is unexplored. To address this gap, we evaluated the impact of CoQ10 and a cell-permeable α-KG derivative (DM-αKG) on ETC complex activity, mitochondrial morphology, tissue architecture and colitis reversion in a DSS colitis mouse model. **Methods:** C57BL/6 male mice were treated w/wo DSS (2.5%) for 7 days and received CoQ10 (100 mg/kg), or DM-αKG (1%) or their combination (n=4 per group). Colitis severity was assessed via weight loss and colon length. Colon tissues were analyzed for oxygen consumption rates (OCR) using Seahorse technology. Fixed paraffin-embedded colon tissues were used for immunofluorescence using Tomm20 for mitochondrial morphology assessment, and E-cadherin and Iba1 as markers for epithelial cells and macrophages, respectively. Expansion microscopy with NHS staining was also performed for bulk labeling of the proteome (Pan-ExM) to explore the tissue architecture. Imaging was conducted using a Zeiss LSM900 with Airyscan 2 technology and analyzed with ImageJ software. **Results:** CoQ10 and DM- αKG prevented weight loss of DSS-treated mice, with the combined treatment providing the most significant protection. DSS-induced colitis resulted in decreased mitochondrial complex I and II activity, which was mainly restored by DM- αKG or the combination of CoQ10+ DM- αKG. Immunofluorescence revealed changes in mitochondria fragmentation levels in CoQ10+DM-αKG-treated DSS mice. Expansion microscopy yielded a ~4-fold expansion factor, improving resolution up to an estimate of ~50 nm, allowing visualization of colon crypt connections, enterocyte's granules, and interactions between immune lamina propria-infiltrating cells, which could further provide a clearer understanding of therapy mechanism. Our findings suggest that CoQ10 and αKG supplementation improve ETC activity and reverse pathologic mitochondrial morphological changes, potentially promoting colitis resolution and epithelial healing. Future studies should explore the beneficial impact of the combination of these supplements in translational medicine for UC patients. **Acknowledgements:** Fondecyt 3210367, 11201322, 1220702, HCS Keystone Grant, Anillo ACT210097.

INFLAM009

More Than Just Liver: *Foxa3*-Cre-mediated YAP Deletion Targets Genitourinary Tissues, Causing Polycystic Kidney Disease and Male Infertility

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Background: We previously showed that loss of Yes-associated protein 1 (YAP) in early liver development (*Foxa3*-Cre YAP KO) leads to an Alagille syndrome-like phenotype, with failure of intrahepatic bile duct development and severe cholestasis. However, these mice also develop severe cystic kidney injury. Here we investigate the renal pathology and differentiate the genetic defects caused by YAP deletion from long-term exposure to severe cholestasis. **Methods:** We deleted *Yap1* during early liver development using the *Foxa3* promoter to drive Cre expression. We also used a *Foxa3*-Cre TdTomato reporter mouse to investigate whether Cre recombinase is activated in genitourinary tissues. We evaluated these mice using microscopy, serum biochemistry, and RNA-sequencing. **Results:** We show that YAP KO mice have grossly normal renal development, with no visible abnormalities at 1 month of age. They develop polycystic kidney disease over the first three to six months of life, with cysts originating from distal tubules and glomeruli, accompanied by chronic inflammation and fibrosis. Since other mouse models of cholestasis do not share this phenotype, we examined whether *Foxa3*-Cre may affect genitourinary tissues. Using *Foxa3*-Cre TdTomato reporter mice, we found reporter expression not just in organs derived from foregut endoderm, but also throughout the genitourinary tract: urothelium of the ureter and bladder; testosterone-producing Leydig cells and maturing spermatids in the testes; uterine and ovarian stroma; and cells within the glomeruli, distal tubules, and collecting ducts. Notably, we found that YAP KO testes lack Leydig cells and fail to produce mature sperm, demonstrating a critical role for YAP in male fertility and raising the possibility that altered testosterone levels may be affecting other organs in this model. We next performed bulk RNA-sequencing on kidneys from 3 weeks and 4 months of age to identify potential mechanisms of disease progression. At 3 weeks, YAP KO kidneys show strong similarity to their WT counterparts, except for changes in the structure and function of primary cilia, bile salt metabolism, and steroid hormone signaling through RXR. Further study is ongoing to identify molecular mechanisms connecting YAP deficiency with polycystic kidney disease and male infertility in this model. **Conclusions:** Many genetic syndromes, like Alagille syndrome and autosomal dominant polycystic kidney disease, impact both liver and kidney function, while adults with liver injury often progress to multi-organ dysfunction. We demonstrate for the first time that the *Foxa3* promoter is active in genitourinary tissues, and thus the *Foxa3*-Cre YAP KO model represents a complex multi-organ pathology besides severe cholestasis. This model presents an opportunity to study the combined effects of genetics and cholestasis on kidney health, and presents YAP as a possible disease modifier in a variety of cholestatic and renal diseases.
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INFLAM010

Selective Ablation of FXR in Neurons Is Neuroprotective in a Mouse Model of Acute Liver Failure

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Background: Hepatic encephalopathy (HE) describes a spectrum of neurological and cognitive disturbances due to acute and chronic liver disease. Associated with HE is a buildup of ammonia, astrocyte swelling and neuroinflammation, however our understanding of the pathogenic mechanisms of HE is limited. We have demonstrated that the nuclear bile acid receptor, FXR, is activated in the frontal cortex in a mouse model of Type A HE and that strategies to block FXR in this region attenuated HE. However, whether FXR signaling is involved in other brain regions impaired during HE and whether neuron-specific ablation of FXR signaling is also protective is unclear and thus are the aims of this study. **Methods:** Male and female C57/Bl6 (WT), floxed FXR ($FXR^{fl/fl}$) and neuron-specific FXR knockout mice ($FXR^{\Delta neu}$) were injected with azoxymethane (AOM) to induce acute liver failure (ALF) and HE. Cognitive impairment was monitored by reflex response assessment at various time points. Neuromuscular deficits were assessed using a grip strength meter and balance was measured using rotarod testing. The expression and nuclear translocation of FXR was assessed in the frontal cortex, hippocampus and cerebellum by qPCR and immunofluorescence. Liver damage was assessed by hematoxylin and eosin staining and serum chemistry. Ammonia accumulation in the blood was assessed by colorimetric assay and in the brain by

Nessler staining. Immunohistochemical analysis of IBA1 for microglia was used to assess broad field count and cell morphology. Proinflammatory cytokine and chemokine expression was assessed by qPCR. **Results:** FXR nuclear translocation was increased in all brain regions studied in AOM-treated male and female WT and $FXR^{fl/fl}$ mice, which correlated with neurological and neuromuscular deficits associated with HE. While similar liver damage was observed in AOM-treated $FXR^{\Delta neu}$ mice, the neurological and neuromuscular deficits were attenuated when compared to AOM-treated $FXR^{fl/fl}$ control mice. This protective phenotype of $FXR^{\Delta neu}$ mice was not associated with alterations in ammonia, as brain ammonia increased to a similar degree in all mouse strains. However, neuron-specific FXR knockout mice displayed reduced microgliosis in all brain regions assessed and the reactive phenotype of microglia was attenuated. The expression of proinflammatory cytokines consistent with the activation of microglial morphological changes was also attenuated in AOM-treated $FXR^{\Delta neu}$ mice. **Conclusions**: These data suggest that neuron-specific ablation of FXR expression attenuated the neurological and neuromuscular deficits, and neuroinflammation associated with Type A HE, without altering the underlying liver damage and ammonia accumulation in the brain. **Acknowledgements:** This study was supported by an NIH R01 award (DK135995).

Session 013 – Healing Inflammation with Specialized Pro-Resolving Mediators

MUCOSAL001

Microbial Metabolites Regulate Restitution of Intestinal Epithelial Cells

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Background: Inflammatory bowel disease (IBD), surgical trauma, as well as enteric infections and environmental insults can cause intestinal damage. Tissue restitution is a coordinated but complex cellular process to restore damaged gut mucosa. Previously, we reported that specific gut symbionts promote intestinal epithelial repair by stimulating the proliferation and migration of the intestinal epithelial cells (IEC). We and others also demonstrated the impacts of probiotic bacteria on mucosal regeneration. However, we do not completely understand the mechanistic functions of how microbial small molecules and metabolic products enhance epithelial wound healing. In this current research study, we demonstrated the impacts of bacterial metabolites on intestinal epithelial cells. We found that the levels of spermidine, a member of the polyamine class of microbial metabolites, were significantly altered in a colitis model. We hypothesized that polyamine transporters expressed in IECs play a significant role in epithelial cell functions. **Methods:** We determined microbial and metabolite abundances. We also evaluated signature gene expression and signaling pathways important for IEC proliferation and migration. **Results:** The RNA sequencing and gene ontology analysis identified upregulated cellular pathways. We further examined the effects of polyamines on cell proliferation and migration, hallmark processes critical for the repair of damaged epithelia. Quantitative reverse transcription PCR and a proliferation assay demonstrated exogenous spermidine's ability to impact intestinal epithelial cell proliferation, even at a low dose. Further investigations via scratch assay showed that exogenous polyamines also modulate epithelial cell migration. We finally determined a potential mechanism of action for spermidine to affect IECs via novel epithelial transporters. **Conclusions:** Our investigations into both the mechanism and effects of exogenous, bacterially derived metabolite show the importance of the microbiome and its small molecules as the key factor in IBD and may eventually lead to a microbiome-based therapeutic for this disease.
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MUCOSAL002

Nanoparticle Encapsulation of Protectin D1 and DX: Advancing Colonic Repair Therapy with Pro-Resolving Mediators

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Background: Intestinal epithelial integrity and barrier function are compromised by poorly healing mucosal wounds in chronic diseases such as inflammatory bowel disease (IBD). Effective mucosal repair requires coordinated epithelial migration and proliferation, involving spatiotemporal interactions between epithelial cells and immune cells at wound sites. However, there is a significant gap in understanding the complex molecular mechanisms orchestrating epithelial restitution and mucosal repair in the gut. Specialized pro-resolving mediators (SPMs), derived from omega-3 polyunsaturated fatty acids, have emerged as crucial agents in resolving inflammation and promoting wound healing, including Protectin (PD) family. Our study explores the role of Protectin D1 (PD1) and its enantiomer, Protectin DX (PDX), in regulating epithelial

repair in the gut. **Methods:** Liquid chromatography-mass spectrometry (LC-MS) was used to analyze SPM profiles in healing biopsy-induced colonic wounds. To assess the functional effects of PD1 and PDX, we used *in vivo* models of colonic injury and repair, including DSS-induced colitis and biopsy-induced injury. To address the thermolability of PD1 and PDX and eliminate the need for ultracold storage, PDs were encapsulated in polylactic acid (PLA) nanoparticles, and their functional effects on colonic repair following biopsy wounding were evaluated. The effect of PDs on epithelial repair was also assessed *in vitro* using primary murine and human colonic epithelial cells. **Results:** LC-MS analyses of healing colonic wounds revealed significantly higher levels of PD1 and PDX compared to other SPMs. Importantly, the exogenous administration of PD1 and PDX significantly enhanced wound healing following biopsy-induced distal colon injury or DSS-induced colitis. Critically, PLA nanoparticle encapsulation of PDs retained bioactivity after extended storage at 4°C, exhibiting potent effects on *in vivo* colonic biopsy-induced wound repair. Mechanistically, PD1 and PDX were found to drive intestinal epithelial cell migration and proliferation *in vitro*. Finally, data revealed that PD1 and PDX regulate crucial epithelial repair processes by activating CREB (cAMP response element-binding protein) and focal adhesion kinase. **Conclusions:** Our findings demonstrate that PD1 and PDX are critical for intestinal mucosal wound repair in the colon. Furthermore, exciting data reveal the great potential of nanoparticle encapsulation to boost the therapeutic utility of PDs *in vivo*. This novel approach represents a targeted strategy to improve mucosal healing without compromising immune function in IBD and similar chronic mucosal diseases. **Acknowledgements:** We thank the Translational Tissue Modeling Laboratory at the University of Michigan for human colonoids. This research was supported by a Crohn's and Colitis Foundation Research Fellowship Award (934934, J.M.) and NIH grants (EB028840, A.H.M.; DK055679, A.N.; DK79392, C.A.P.).

Session 014 – Therapeutic Targeting of the Metastatic Niche in Cancer: Perspectives from Premalignant to Malignant Disease

CANCER004

Carcinoma-associated Fibroblasts Induce an Invasive Phenotype and a Preferentially lymphangiogenic Secretome in E6E7 Transfected Cancer Cells

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Introduction: The incidence of human papilloma virus positive (HPV+) mucosal cancers is increasing, with these cancers having high rates of lymph node metastasis. Metastasis is dependent on the tumor microenvironment (TME), and cancer-associated fibroblasts (CAFs) in the TME play a major role in the progression of many mucosal cancer types including head and neck, vulva, and penile cancers. Although recent studies have shed some light on the importance of CAFs in HPV-mucosal cancers, very little is known about the role played by CAFs in HPV+ cancers. In the present study, we aimed to identify particularities of the interactions between CAFs and HPV E6 and E7 transfected oral and vulvar cancer cells and whether it promotes lymph node metastasis. **Methods:** Oral (OSCC1) and vulvar (UMSCV4) cancer cells were transfected with HPV E6 and E7 oncoproteins and co-cultured with CAFs isolated from oral and vulvar mucosa, respectively. Bulk RNA sequencing and qPCR was performed to study the differentially expressed genes, whereas multiplex cytokine assay and western blotting was performed to assess the protein expression. Matrigel tube assay was used to study the formation of lymph-endothelial tubes *in vitro* when subjected to different conditioned media. Finally, the co-cultures of cancer cells and CAFs were implanted in nude mice to determine their *in vivo* growth characteristics. **Results:** CAFs co-cultured with E6E7 cancer cells showed increased gene and protein expression of hepatocyte growth factor (HGF), a known angiogenic protein in comparison to CAFs co-cultured with wild-type (WT) cancer cells. In response, E6E7 cancer cells co-cultured with CAFs showed increased gene and protein expression of vascular endothelial growth factor-C (VEGF-C) and Neuropilin-2, when compared to WT cells co-cultured with CAFs. Furthermore, E6E7 cells induced formation of more stable *in vitro* lymph-endothelial tubes in the presence of CAFs, became invasive into the underlying collagen matrix populated with CAFs in 3D organotypic cultures, and formed bigger and continuously growing tumors when co-injected with CAFs in vivo compared to WT cancer cells. **Conclusions:** Together, these results indicate that E6E7 cancer cells respond to CAF signaling by a preferentially lymphangiogenic secretome and a more invasive phenotype than their WT counterparts, pointing towards an important role of TME in HPV-related carcinogenesis. **Acknowledgements:** The authors acknowledge funding support from the Faculty of Medicine, University of Bergen, Norway (2022/15109-HIJO) (HD) and the Vascular Biology Program at Boston Children's Hospital, USA (DRB).

CANCER005

Infiltrating Plasma Cells Promote Glioblastoma Stem Cells Growth Through FCGR2A

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Background: Glioblastoma stem cells (GSCs) possess fundamental stem cell properties, including self-renewal and multipotency. GSCs have been demonstrated to promote tumor angiogenesis, brain invasion, and immune evasion in glioblastoma (GBM). Previous studies showed that plasma cells (PCs) derived soluble metabolites can promote colon carcinoma growth. However, the relationship between PCs and GSCs remains unclear and warrants further exploration. This study investigated single-cell RNA sequencing (scRNA-seq) and The Cancer Genome Atlas (TCGA) datasets for GBM patients. We then further characterized the functional relationship between PCs and GSCs. **Methods:** We analyzed scRNA-seq of 16 fresh GBM samples, TCGA, GSCs RNA-seq (n=41), Neural stem cells (NSCs) RNA-seq (n=5), and normal brain tissue (NBT) RNA-Seq (n=3) datasets. We used combined immunohistochemistry and immunofluorescent stains (CD138, MUM1, PRDM1, CD45, and CD19) for PCs in 20 GBM cases. To validate our results, we developed one control short hairpin RNA (shRNA) and two shRNA to knock down targeted genes in three GSCs in vitro. Cell viability was measured by CellTiter-Glo assay in four independent experiments. Two-sided unpaired Student's t-test assessed significance, and p values < 0.05 were significant. To perform in vivo experiments, immunocompromised mouse models (NCG mice) bearing co-implantation of PCs and GSCs or GSCs and non-PC B cells were used for Kaplan-Meier survival study (*n*=6 per group), p values were calculated using the log-rank test. **Results**: We identified that PCs were aberrantly enriched in the GBM-infiltrating B-lineage population; about 46.0% ~ 64.7% of B-lineage are PCs. Higher frequencies of PCs informed poorer overall survival among GBM patients (n=40, p=0.0025). Through in silico dataset analyses, we found FCGR2A is highly expressed in GSCs compared to NSCs and NBT. Notably, FCGR2A encodes the Fc receptor (FcγRIIA), a receptor for PC-derived soluble metabolites (immunoglobulin G). In TCGA datasets, high expression of FCGR2A is associated with poor prognosis in GBM patients (n=525, p=0.003). In shRNA experiments, FCGR2A knockdown decreased the cell viability and impaired self-renewal of GSCs compared to the control group (data from three independent experiments, p< 0.01). NCG mice bearing co-implantation of PCs and GSCs demonstrated poorer overall survival and increased tumor volume than those with GSCs co-implantation with non-PC B cells (n=6 per group, p<0.001). **Conclusions:** PCs aberrantly enriched within the tumor microenvironment (TME) of GBM, which stimulated tumor growth and informed poor prognosis. PCs infiltrated glioblastoma and interacted with GSCs through FcγRIIA. Our genetic approaches and animal experiments confirmed that FCGR2A is needed to maintain GSCs and GBM progression both in vitro and in vivo. Taken together, our data generate a framework for potential targeting therapy against FCGR2A in human GBM. **Acknowledgments:** Funded by NIH.

Session 015 – Regulation of Epithelial and Endothelial Junctions: Novel Molecules and Mechanisms

LIVER005

Complete Loss of β- and γ-catenin in Bile Ducts Leads to Cholestasis and Colonic Inflammation

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Background: Wnt/β-catenin signaling is essential for normal liver function. Loss of β-catenin in adherens junctions is compensated by γ-catenin, a homologous desmosomal protein. We have previously found that loss of both β- and γ-catenin in cholangiocytes disrupts bile duct structure leading to impaired bile flow and subsequent bile leakage damaging zone 1 hepatocytes. Expression of cholangiocyte markers in hepatocytes suggests hepatocyte transdifferentiation may help repairing β- and γ-catenin-deficient bile duct loss over time. Intestinal and gut microbiome response to bile duct loss or hepatocyte transdifferentiation has however, not been studied. In the present study we utilize our novel model of cholestasis via β- and γ-catenins deletion from the biliary epithelium to study pathophysiological implications of bile duct loss, in both liver and gut. **Methods:** We utilized Ctnnb fl/fl; Jup fl/fl mice with Opn-iCreERT2+/- to delete both β-catenin (Ctnnb1) and γ-catenin (Jup) from cholangiocytes. Littermate control with no Opn-iCreERT2+/+ were used as controls. All mice received tamoxifen (100 mg/kg) dissolved in corn oil injected intraperitoneally. Mice were randomly assigned to two groups: (1) low

dose group received 2 doses of tamoxifen and (2) high dose group received 4 doses of tamoxifen. Male and female mice were used in this study. **Results:** High dose DKO mice displayed increased morbidity with greater than 20% body weight loss by two weeks after first injection while low dose DKO mice displayed no appreciable morbidity or body weight loss compared to respective control mice. Low and high-dose DKO mice displayed elevated serum levels of ALT and AST compared to control mice. High dose DKO also presented with jaundice and elevated bilirubins compared to low dose DKO and control mice. We found elevated TBA levels in serum and liver of low dose and high dose DKO mice compared to control. Colon length is unchanged between DKO and control mice with the exception of high dose male DKO mice which displayed a significant increase in colon length. Histology revealed increased inflammation, stellate cell activation, and severe portal fibrosis in livers of high dose DKO mice. Bile duct mass was increased in high dose DKO and showed a trend towards an increase in low dose DKO mice. All DKO mice displayed reduced ileal and colonic mucin compared to control mice. However, high dose DKO group displayed increased colonic inflammation and fecal lipocalin 2 levels to low dose DKO and control mice. **Conclusions:** Dual loss of β- and γ-catenin from cholangiocytes causes severe intrahepatic cholestatic injury in mice with its severity dependent on healthy cholangiocyte population. Severe bile duct loss in high dose DKO mice leads to decreased mucin production and colonic inflammation, indicating bile duct health may impact gut function and bacteria clearance.

MUCOSAL003

Unconventional Myosin18A is a Novel Regulator of the Intestinal Epithelial Barrier and Mucosal Inflammation

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Background: Establishment of the intestinal epithelial barrier is a critical feature of healthy gut homeostasis, while disruption of this barrier is known to ignite and exaggerate mucosal inflammation. Gut barrier permeability is regulated by the assembly of adherens junctions (AJs) and tight junctions (TJs) in intestinal epithelial cells (IEC). This is dependent on junctional coupling to the underlying actin filaments enriched with non-muscle myosin II (NM II) motors. In addition to NM II, IEC express unconventional myosins, whose role in regulating epithelial junctions remain poorly understood. Unconventional myosin 18A (Myo18A) is a unique multifunctional scaffold protein lacking the myosin motor activity but possessing a PDZ domain, which may potentially bind to different TJ proteins. However, Myo18A function in controlling epithelial junctional integrity have not been previously addressed. In the present study, we investigated the roles of Myo18A in the regulation of gut barrier permeability, epithelial junction integrity, and mucosal inflammation. **Methods:** Loss of function experiments were performed by CRISPR/Cas9-mediated knock-out of Myo18A in model IEC. Barrier properties were determined by measuring transepithelial electrical resistance (TEER) and transepithelial flux of FITC dextran. Furthermore, we used an inducible IEC-specific Myo18A knockout mice strain to analyze intestinal permeability *in vivo* and mucosal responses during dextran sodium sulfate induced colitis. **Results:** Myo18A was significantly colocalized with epithelial apical junctions in healthy murine and human colonic mucosa. The junctional localization and expression of Myo18A protein was markedly decreased in inflamed colonic epithelium of ulcerative colitis patients. CRISPR/Cas9 mediated knock-out of Myo18A in DLD1 human IEC disrupted the epithelial barrier that was manifested by the decreased TEER, and increased transepithelial FITC-dextran flux. Such barrier disruption was driven by the increasing contractility of the prejunctional actomyosin belt leading to the distorted cell morphology in IEC monolayers. The increased actomyosin contractility in Myo18A-deficient cells was associated with enhanced phosphorylation of myosin light chains and recruitment of Rho-associated kinase to apical junctions. Consistently, mice with tamoxifen-inducible deletion of Myo18A in the intestinal epithelium showed increased gut permeability *in vivo* without developing obvious intestinal abnormalities. In despite of such gut leakiness, Myo18A conditional knockout animals were protected from dextran sodium sulfate induced colitis. **Conclusions:** Together, our findings revealed a novel cytoskeleton-dependent mechanism that regulates permeability of the intestinal epithelial barrier and epithelial responses to mucosal inflammation. **Acknowledgements:** This work was supported by research grants from NIH/NIDDK and Leona M. and Harry B. Helmsley Charitable Trust.

Session 016 – Intersection of the Vascular and Neural Systems

NEUROPATH001

Exploring Vascular Contributions to the Pathobiology of Cognitive Impairment: Focus on Small Vessel Disease of White Matter and Presence of Micro(nano)plastics

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Background: Cognitive impairment due to vascular pathology was recognized by Otto Binswanger on 1894, before Alois Alzheimer presented his findings in 1907. Unlike Alzheimer's disease, vascular causes of cognitive impairment are likely due to a range of underlying pathologies that are still, over a hundred years later, poorly defined. Recent epidemiological

studies of post-mortem human brain from multiple repositories report cases uniquely afflicted by either vascular or Alzheimer's, and others harboring both plaques and tangles as well as indicators of cerebrovascular pathologies: microhemorrhages and microinfarcts. While it is well-known that hypertension and diabetes affect the vasculature throughout the body as well as in brain, other types of vascular diseases that may uniquely affect cerebral vessels are understudied. Although consensus guidelines exist for the histopathological diagnosis of Alzheimer's disease, cerebrovascular diseases remain without guidelines for specific types of white matter vascular disease. **Methods:** First we consider the anatomy of brain vasculatures and then fine structure of the vessels. Then a categorization of vascular pathologies is proposed as a method to approach an understanding of vascular pathobiology. **Results:** A number of micrographs of examples are presented that illustrate structures and their pathologies with stains useful to detect them. Scoring of pathologies across many cases will improve diagnostic procedures to sub-divide vascular dementias into their underlying, treatable, categories. Identification of new biological targets will require new validated stains. Double or triple-labeling immunopathology by histochemistry with light microscopy, rather than immunofluorescence, will be necessary in order to correlate cellular structural detail with altered cellular molecular components. Serial sectioning is not optimal for correlation of cellular detail between different stains, and fluorescence poses the problem of formalin- and paraffin-dependent autofluorescence. Examples of recent discoveries of micro(nano)plastics in the brain are shown together with methods to image them and their associations with small vessel disease in white matter of both Binswanger's and Alzheimer's cases. **Conclusion:** This categorization and methodology will no doubt have a profound influence on how we diagnose vascular-pathology-associated cognitive impairment. The presence of abundant micro(nano)plastics in neurodegenerative diseases poses many as yet unanswered questions as to cause or consequence. Going forward, if anatomic pathologists apply this categorization schema to triage types of cerebrovascular pathology, we may begin to dissect underlying causes. **Acknowledgements:** Supported by NIA P30 AG086404 (GR, ELB), NIGMS P20 GM130422 (MJC), 3P30CA118100 (PI: Sanchez), and the Harvey Family (ELB)

NEUROPATH002

Microcurrent Electrical Stimulation Activates Small Ca2+-Induced K+ Channel (KCNN) to Promote Axons Growth in Trigeminal Ganglia Neurons and Restore Corneal Nerve Density in Diabetic Keratitis

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Background: Diabetic keratitis (DK) significantly impacts the quality of life for diabetic patients, leading to delayed corneal wound healing, reduced nerve density, and impaired corneal sensation. Very limited treatment has been proven effective for DK. Transpalpebral electrical stimulation (TpES) non-invasively delivers microcurrent electricity to the eye via orbital skin. This study investigates the neuroregenerative potential of TpES in a type I diabetes mellitus (DM)-induced DNK model and delves into the mechanism of electrical-induced neuroregeneration. **Methods:** Male C57BL6J mice were induced with type I DM through intraperitoneal streptozotocin (STZ) injection. Starting at week 15 post-STZ, daily 4-minute TpES sessions were administered for 14 days. Sham controls received electrode placement without current, while non-STZ mice served as blanks. Corneal wholemount analysis targeting beta-III-tubulin. **Results:** Following the 2-week TpES treatment, the TpES group exhibited significantly higher plexus density with an intact central whirl, contrasting with decreased density and disrupted structure in the sham control. Corneal fluorescein staining (CSF) at 17-week post-STZ showed significant epithelial defect in sham group, while minimal defect was observed in TpES group or blank. Isolated trigeminal ganglia (TG) neurons from the TpES group displayed significantly longer neurites than sham controls, indicative of direct neuronal stimulation. The membrane potential. **Conclusions:** TpES effectively restores corneal nerve density and protects the corneal epithelium in a type I DM model. The neuroregenerative effect is related to increased Ca^{2+} transport and increased expression of growth factors.

Session 022 – Diverse Models to Study Microbial-host Interactions

INFDIS001

Bacterial Polyphosphates Enhance Intestinal Inflammation Through Disruption of IL-27/STAT Signaling

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Background: Inflammatory bowel disease (IBD), affecting an estimated 3 million people in the United States, is characterized by dysbiosis of the normal intestinal microbiome and altered microbial interactions with the host immune cells, resulting in increased inflammation. The mechanisms that disrupt host-microbiome interactions in the gut are not fully

characterized. Bacteria produce polyphosphates, which are metabolites comprised of linear polymers of phosphate groups (≥700 Pi). Polyphosphates have been implicated in dysfunctional immune response during infection. Here, we explored the role of bacterial polyphosphates in mucosal immunity during homeostasis and inflammation. **Methods and Results:** Analysis of open-source metagenomics data from fecal samples of IBD patients compared to healthy controls revealed higher abundance of bacterial polyphosphate kinase (PPK), the critical the critical enzyme for polyphosphate synthesis. Next, germ-free mice were monocolonized with *Escherichia coli* that were either polyphosphate competent (WT *E. coli*) or deficient (∆ppk *E. coli*) due to deletion of polyphosphate kinase. Flow cytometry-based quantification of immune cells in the lamina propria indicated significantly higher numbers of $CD11c^+$, $CD103^+CD11b^-$ and $CD103^-CD11b^-$ dendritic cell populations, $ROR\gamma t^+FOXP3^-CD4^+$ TH17 cells and $CD8^+$ T cells in mice colonized by WT *E. coli* compared with ∆ppk *E. coli* mice. Similar differences were observed with intra-epithelial $TCR\gamma\delta^+CD8\alpha\beta^+$ cells. Adoptive transfer of lamina propria immune cells from the monocolonized mice into immunodeficient $RAG1^{-/-}$ mice resulted in greater weight loss, more severe colitis, and lowered survival in mice that received polyphosphate-exposed T cells. Bulk ATAC-sequencing of lamina propria $CD3^+CD4^+$ T cells showed lowered chromatin accessibility in *Stat1*, *Stat3* and *Il10* genes in mice monocolonized with WT *E. coli* compared with ∆ppk *E. coli* mice. Single-nucleus sequencing revealed that polyphosphates also mediated transcriptomic suppression of *Stat1* gene in tissue-resident large peritoneal macrophages and monocytes. Moreover, polyphosphates suppressed IL-27/IL-27Rα-induced STAT1/STAT3 phosphorylation in naïve $CD4^+$ and $CD8^+$ T cells. Lamina propria cells from IL-27Rα$^{-/-}$ mice induced severe colitis when transferred to $RAG2^{-/-}$ mice mimicking the severity observed with the adoptive transfer of WT *E. coli* cells. **Conclusions:** Our study demonstrates that bacterial polyphosphates promote a maladaptive immune response in the gut enhancing the severity of colitis. Polyphosphate-mediated dysfunctional IL-27/IL-27Rα and STAT1/STAT3 signaling disrupts homeostasis leading to unmitigated inflammation, which may contribute to the pathogenesis of inflammatory bowel disease. **Acknowledgments**: This work was financed by the National Institutes of Health (R01HL141513, R01HL166588, R01HL139641 to M.B.), the Federal Ministry of Education and Research (01EO1003, 01EO1503 to M.B.), and the Deutsche Forschungsgemeinschaft (BO3482/3-3, BO3482/4-1 to M.B.).

INFDIS002

A Two-Hit Sepsis Model Reveals JunB-Driven Endothelial Inflammatory Memory That Exacerbates Secondary Pneumonia

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Background: Sepsis patients requiring ICU care have a two- to five-fold higher risk of post-acute death than the general population. Sepsis survivors remain highly vulnerable to secondary infections, such as pneumonia. We developed a mouse model of sepsis followed by a secondary non-lethal Streptococcus pneumoniae (SP) challenge to investigate how prior inflammatory insults prime endothelial cells (ECs). Transcription factors such as STAT3 and JunB have emerged as critical mediators of this inflammatory memory. We hypothesize that an initial septic insult induces transient STAT3 activation but establishes persistent JunB-dependent transcriptional and chromatin remodeling in ECs, resulting in an exaggerated inflammatory response upon secondary infection. **Methods:** Mice underwent cecal ligation and puncture (CLP) or a sham procedure. 20 days later, survivors were challenged intranasally with SP. Blood, lungs, and kidneys were collected for flow cytometry and RNA-seq 2 days after SP. ECs were enriched via magnetic bead sorting. In vitro, HUVECs were treated with IL-6+R for 72 hours, washed for 48 hours, then challenged with LPS for 6 hours. Transcriptomic, chromatin accessibility and immunofluorescence analyses were performed. **Results:** CLP survivors clinically returned to normal by day 10 but displayed persistent kidney dysfunction at day 13, which worsened following the SP challenge. Notably, 100% of CLP+SP died within 4 days of SP exposure (day 24), while sham+SP mice survived. Blood inflammation profiling at day 22 revealed significantly elevated IL-6, TNF-α, and MCP-1 in the CLP+SP group. RNA-seq of lung and kidney ECs showed marked upregulation of pattern recognition receptors, NF-κB/IRF signaling, and multiple cytokines. Lung immunofluorescence confirmed endothelial dysfunction (increased P-selectin) and immune cell infiltration (CD45+). We observed 1,249 overlapping differentially expressed genes between the lung and kidney, indicating a shared inflammatory program and organ-specific signatures (1,527 unique to the lung and 1,730 to the kidney). In vitro, IL6-primed HUVECs increased ICAM1 and CCL2 protein, amplified upon secondary LPS challenge, supporting the concept of inflammatory memory. pSTAT3 rose only during the initial IL-6+R stimulus, indicating that sustained activation is driven by epigenetic and TF changes rather than ongoing STAT3 signaling. ATAC-seq revealed persistent chromatin accessibility at 27 inflammatory genes, which remained open post-washout and were upregulated by secondary LPS challenge. JunB emerged as a regulator, its knockdown significantly reduced inflammatory gene expression and chromatin remodeling. **Conclusions:** Our data demonstrate a correlation between transcript-level priming, chromatin remodeling, and protein-level amplification, underscoring how prior inflammatory signals establish a long-lasting molecular imprint in ECs that drives heightened responsiveness to subsequent insults.

Session 023 – The Beginning Affects the End: Developmental Origins of Metabolic Disease

Diabetes001

Impact of Gestational Diabetes Treatment Modalities on Placental Metabolic Adaptations: Differential Roles of PKM2, AMPK, and mTOR Pathways

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Background: Gestational diabetes mellitus (GDM) alters placental metabolism, influencing fetal growth and pregnancy outcomes. While insulin and dietary management are the main therapeutic approaches, their distinct effects on placental metabolic pathways remain unclear. This study investigates the differential roles of Pyruvate Kinase M2 (PKM2), AMP-activated protein kinase (AMPK), and the mechanistic target of rapamycin (mTOR) pathways in placental tissues from insulin-treated (GDM-I) and diet-managed (GDM-D) pregnancies. **Methods:** Placental samples from GDM-I, GDM-D, and normoglycemic pregnancies were analyzed using immunofluorescence and immunoblotting to quantify PKM2, AMPK, and mTOR pathway components. Statistical analyses were completed to discern protein expression differences among groups. **Results:** PKM2 expression was upregulated in both GDM-I and GDM-D placentas, with GDM-I showing greater localization in the syncytiotrophoblast. AMPK phosphorylation was significantly increased in GDM-I and moderately elevated in GDM-D, suggesting differential energy stress responses. mTOR signaling was activated in GDM-I, indicating enhanced nutrient transport, whereas GDM-D placentas exhibited decreased p70S6K and increased 4EBP1, suggesting a moderated mTOR response. **Conclusions:** These findings indicate that treatment modalities influence placental metabolic adaptations, with insulin therapy amplifying PKM2 and mTOR activation while dietary intervention promotes a more balanced metabolic response. Understanding these pathway alterations may guide tailored therapeutic strategies to optimize pregnancy outcomes in GDM. **Acknowledgements:** This work was supported by funding from the National Institutes of Health (1R15HL152257) and the Flight Attendant's Medical Research Institute (CIA2300003).

Session 028 – Minisymposium – Immune Cell Activation in Tissue Inflammation and Injury

IMMUN001

Phosphorylated UBC9 regulates RAB GTPase mediated cytokine secretion signaling in alcohol-associated liver disease

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Background: Alcohol metabolism-induced oxidative stresses result in hepatocyte injury, leading to the release of damage-associated molecular patterns (DAMPs) into the extracellular environment, which contributes to tissue inflammation. DAMPs are packaged in extracellular vesicles (EVs) and enable crosstalk between hepatocytes and non-parenchymal cells such as Kupffer cells (KCs). EVs are essentially regulated by Rab GTPase family (RABs), which are scaffold proteins that modulate immune responses by regulating the transport of immune receptors, endocytosis and phagocytosis as well as secretion of chemokines and cytokines. EVs are emerging as key players in the pathogenesis and progression of alcoholic-associated liver disease (ALD). We already reported that ubiquitin conjugating enzyme 9 (UBC9), the sole E2 enzyme essential for SUMOylation, plays a key role in inflammatory response and ALD development. In this study, we investigated the role of UBC9 and RABs interplay on KCs activation via cytokine secretion and EVs endocytosis in ALD. **Methods:** Primary mouse hepatocytes and KCs, NIAAA mouse model, phospho-peptide mapping, CRISPR/Cas9 UBC9 Y68 editing, co-immunoprecipitation, western blot, ELISA, RT-PCR, Oil red O, Bodipy, and H&E stain. **Results:** in vitro Lipopolysaccharide (LPS)-treated and in vivo KCs from NIAAA model revealed that Tyr68 (Y68) of UBC9 is phosphorylated. Levels of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) were curtailed by CRISPR/Cas9 editing of UBC9 Y68 to phenylalanine (F68) in KCs along with striking decline in TNF-α secretion. This finding pointed towards the potential role of phosphorylated UBC9 (pUBC9) in cytokine secretory signaling. Interestingly, interaction of pUBC9 with several RAB family proteins (RAB1A, RAB5A, and RAB14) was found in alcohol activated-KCs from NIAAA mouse livers as well as in LPS-activated KCs. Specifically, UBC9 Y68 gene editing led to elevation in RAB1A SUMOylation and prevention of IL-1β secretion in LPS-treated KCs. Further, blocking UBC9 Y68 phosphorylation in KCs prevented de novo lipogenesis in hepatocytes in vitro as well as in vivo. **Conclusions**: Our findings provide highly novel insights on the interplay between pUBC9 and RABs in context of EVs that could influence the crosstalk between KCs and hepatocytes in ALD thereby paving the way for future therapeutic strategies. **Acknowledgements:** This work was supported by NIH/NIAAA grants R01AA029723 (Role of phospho UBC9 in alcohol-associated liver disease).

IMMUN002

The Protein Tyrosine Phosphatase CD45 Regulates PMN Transepithelial Migration and Inflammatory Function in Mucosal Tissues.

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Background: Efficient and controlled recruitment of neutrophils (PMNs) is critical for host defense and initiation of mucosal wound repair processes. However excessive PMN influx and associated mucosal tissue damage are implicated in the pathogenesis of chronic inflammatory disorders including inflammatory bowel disease (IBD). Critically in IBD, disease flares requiring medical intervention are associated with migration of large numbers of PMNs across colonic epithelium resulting in mucosal injury/ulceration. While the migration of PMN from the vasculature has been extensively studied, the complex signaling mechanisms regulating PMN transepithelial migration (TEpM) are less well understood. **Methods and Results:** Here we investigated the role of the protein tyrosine phosphatase CD45/PTPRC in regulating PMN trafficking and inflammatory function in the gut. Using an inhibitor that blocks CD45 phosphatase activity we demonstrated reduced PMN colonic TEpM *in vitro* and *in vivo*. Decreased PMN intestinal trafficking was confirmed *in vivo* using novel tissue targeted transgenic mice with PMN specific deletion of CD45 (*MRP8-Cre;Ptprc$^{fl/fl}$*). In addition to limiting PMN intestinal trafficking, CD45 inhibition also reduced key effector functions including degranulation and superoxide release in both human and murine PMN. Finally, we demonstrate that PMN specific deletion of CD45 improves recovery from dextran sodium sulfate (DSS) induced colitis as assessed clinically and histologically. **Conclusions:** Taken together, data demonstrate that loss of CD45 signaling in PMN results in reduced transepithelial influx, decreased degranulation and superoxide release and improved mucosal repair in the gut. Therefore, CD45 represents a novel target for ameliorating PMN mediated intestinal tissue damage and reducing chronic inflammation in IBD and other mucosal disorders.

IMMUN003

Epithelial Immune Memory Imprinted by Transmigrating Neutrophils

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Background: A hallmark of mucosal inflammation is the accumulation of neutrophils (PMN) at sites of injury. Myeloperoxidase (MPO) is a primary antimicrobial enzyme in PMN. MPO generates hypochlorous acid via a reaction between hydrogen peroxide and chloride. Hypochlorous acid diffuses through the tissue and can indiscriminately react with the phenol moiety on tyrosine to generate chlorinated tyrosine. It is unknown what impact tyrosine chlorination might have on cellular function. We hypothesized that targeting MPO attenuates acute colitis and prevents the development of chronic colitis by limiting bystander tissue damage and imprinting of a pro-inflammatory memory in the tissue. **Results:** To define the role of MPO in murine colitis, we employed wild-type and MPO-deficient mice in combination with acute and chronic DSS-induced colitis. Initial studies revealed that MPO-KO mice experience less inflammation and more rapidly recover from acute colitis compared to wild-type controls. Next, we evaluated the impact of MPO on the development of chronic colitis. MPO KO mice exhibited significantly less inflammation when compared to WT mice. MPO mice had longer colons, lower cytokine levels, and decreased tissue damage as determined by histological scores. To better understand the mechanism(s) involved in MPO associated tissue damage and inflammation we extended these studies to include *in vitro* models. Analysis of the extracellular loops of occludin revealed a disproportionately high number of tyrosine residues (24% and 15% of amino acids in extracellular loops 1 and 2, respectively). Given the observation that MPO activation has the potential to chlorinate tyrosine, we examined tyrosine chlorination by HPLC analysis of occludin immunoprecipitants. Following PMN transepithelial migration or exposure to activated recombinant MPO, we observed prominent 3-chlorotyrosine in occludin. We further examined the functional role of tyrosine chlorination within the binding domain of occludin. Utilizing tyrosine chlorinated and non-chlorinated occludin blocking peptides in combination with functional and morphological analysis, in intestinal epithelial cells (IEC), we demonstrated that chlorination of occludin tyrosines results in abnormal tight junction morphology and barrier dysfunction. **Conclusions:** These results support a pathological role for the enzymatic action of MPO in bystander tissue damage during acute and chronic colonic inflammation. Activated MPO not only damages tissue and inhibits wound healing, but also disrupts IEC barrier function. This work suggests that barrier dysfunction associated with MPO activation results in tyrosine chlorination in the functional domain of occludin. Taken together these studies highlight the need to further study the impact of MPO on the inflammatory microenvironment during active mucosal inflammation. **Grant and Funding Support:** This work was supported by NIH grants DK104713, DK050189, DK095491, VA grants BX002182 and BX005710, and by the Crohn’s and Colitis Foundation.

IMMUN004

Noncanonical Inflammasome Caspase-11 Promotes Neutrophil Infiltration and Inflammatory Response in Severe Tissue Injury

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Background: Severe trauma releases damage-associated molecular patterns (DAMPs), which activate the immune system via pattern recognition receptors (PRRs). This triggers inflammatory cascades that can lead to systemic inflammatory response syndrome (SIRS), immunosuppression, and multiple organ dysfunction syndrome (MODS). Pyroptosis, a form of inflammatory cell death mediated by caspase-11 (casp-11) and gasdermin D (GsdmD), plays a critical role in these processes. This study examines caspase-11's effects on neutrophil infiltration and inflammation following tissue injury. **Methods:** Male C57BL/6J (WT), casp-11 knockout (casp11$^{-/-}$), and endothelial cell-specific and platelets cell-specific casp-11 knockout (casp11$^{EC-/-}$, casp11$^{plt-/-}$) mice, were subjected to polytrauma (n=8-12/gp), consisting of a blind cardiac puncture (25% of total blood volume taken), liver crush, and bilateral pseudofractures (hindlimb crush injury and injection of crushed bone solution from an age- and weight-matched syngeneic donor). Six hours post-polytrauma, plasma was collected and citrated. Activation of caspase-11 (casp-11) and GasderminD (GsdmD), were assessed in the lung and liver using Western blot. We examined neutrophil infiltration (Ly6G) in endothelial cells (CD31) and macrophages (F4/80) in the lung and liver 6h post trauma, using flow cytometry. Plasma IL6 and IL-10 (inflammation), syndecan-1 (endothelial dysfunction), CXCL1 (chemoattractant) levels were measured using ELISA. **Results:** Six hours following trauma, casp-11 activation is observed in the lung and liver of WT mice, with more gsdmD activation in WT mice compared to casp11$^{-/-}$ mice. Plasma CXCL1 levels were elevated in casp11$^{-/-}$ (676.04±111.98 pg/mL, $^{**}p < 0.01$) and casp11$^{EC-/-}$ mice (775.03±138.166 pg/mL, $^{**}p < 0.01$) compared to WT mice (407.36±44.49 pg/mL, $^{*}p < 0.05$) and, casp11$^{plt-/-}$ (320.89±47.19 pg/mL, $^{*}p < 0.05$, $^{**}p < 0.01$). Similarly, IL6 levels increased in casp11$^{-/-}$ (149.59±24.05 pg/mL, $^{*}p < 0.05$) and casp11$^{EC-/-}$ mice (134.45±14.78 pg/mL, $^{*}p < 0.05$) relative to WT (64.13±13.36 pg/mL) and casp11$^{plt-/-}$ (77.50±9.62 pg/mL). In the liver, CD45/Ly6G expression was significantly higher in casp11$^{-/-}$ mice ($^{*}p < 0.05$) compared to WT, indicating increased neutrophil presence. CD45/F4/80 expression was higher in casp11$^{-/-}$ ($p = 0.065$) compared to WT, suggesting more macrophages. Lastly, plasma syndecan-1 levels increased in casp11$^{EC-/-}$ mice (775.54±288.09 pg/mL, $^{*}p<0.05$), compared to casp-11$^{-/-}$ (468.00±106.80 pg/mL), WT mice (302.26±62.74 pg/mL) and casp11$^{plt-/-}$ (337.32±51.76 pg/mL). **Conclusion:** Our data suggests caspase-11 regulates neutrophil and macrophage infiltration in the liver post-trauma. Endothelial-specific caspase-11 deletion exacerbates inflammation in the polytrauma model. These findings highlight potential therapeutic role for casp-11 inhibition in endothelial cells in the treatment of inflammation in tissue injury. **Acknowledgements:** NIGMS R01-GM102146.

IMMUN005

Neutrophils Migrate Long Distances and Persist for Days Following Reperfusion in a Mouse Model of Ischemic Stroke

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Background: Polymorphonuclear leukocytes (PMNs) are among the first leukocytes recruited to the infarct following reperfusion in ischemic stroke. Although transendothelial migration (TEM) blockade in preclinical studies was found to reduce infarct size, TEM blockade in clinical trials has been unsuccessful. Studying PMN recruitment and lifespan in ischemic stroke may offer insight into these failures in clinical trials and advance therapeutic development. **Methods:** PMNs were briefly pulse-labeled with 5-Ethynyl-2'-deoxyuridine (EdU) to determine PMN lifespan and migration pattern following ischemia/reperfusion in stroke. Large vessel occlusion and reperfusion were simulated using the transient middle cerebral artery occlusion model (tMCAO, 90 minutes of ischemia followed by reperfusion). Infarct size was determined via triphenyl tetrazolium chloride staining. The percentage of EdU+ PMN in circulation was assessed each day following tMCAO and compared to the percentage of EdU+ PMNs in the ischemic hemisphere at sacrifice by flow cytometry. The position of PMN in coronal sections was examined by wide field fluorescence microscopy. **Results:** Large cortical ischemic strokes involving over 20% of cerebral volume were induced in mice. A majority of PMN recruitment and extravasation was localized to the cortical surface of the infarct at early timepoints (12 and 24 h post stroke), contrasting other vascular beds where there is PMN migration deep into the infarcted tissue by 24 h. EdU+ PMNs persisted in the ischemic hemisphere until 96 h post stroke despite their clearance from circulation at least 48 h prior, indicating PMN can survive for days in the infarct. Additionally, the percentage of EdU+ PMN in the ischemic hemisphere was similar to the percentage of EdU+ PMN in circulation at 24 h post stroke, suggesting PMN recruitment centers around 24 h. **Conclusions:** Our findings demonstrate

PMN recruitment and infiltration post stroke evolves over several days. This data suggests that the majority of PMNs are recruited to the infarct within the first 48 hours and likely can persist within the infarct for days. Modulating the timing of PMN labeling via EdU can lead to a better understanding of PMN spatiotemporal infiltration and persistence and will help inform future therapeutic interventions targeting leukocytes.

IMMUN006

An Extracellular Histone-Platelet-Macrophage Axis Drives Pathophysiology in Pulmonary Fibrosis

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Background: Pulmonary fibrosis, a progressively debilitating disease, is characterized by excessive extracellular matrix deposition and tissue remodeling leading to lung damage. The molecular mechanisms that drive the inflammatory pathophysiology during fibrosis have not been fully characterized. Externalized histones, released during cell death, have been implicated in various inflammatory diseases. This study explored the role of externalized histones in the pathogenesis of pulmonary fibrosis. **Methods and Results:** Bronchoalveolar lavage fluid (BALF) from patients with idiopathic pulmonary fibrosis (n=29) showed significantly higher concentrations of citrullinated histone H3 (citH3), a marker for neutrophil extracellular trap (NET) formation, compared with healthy controls (n=10). Similar findings were also observed in bleomycin-induced lung fibrosis in mice. Externalized citH3 was detectable on neutrophils at 2 days after bleomycin injection, confirming exacerbated NET formation through both conventional and imaging flow cytometry. Neutralizing anti-histone antibodies diminished extracellular matrix deposition and fibrosis in the lung after bleomycin-injury with significantly lowered expression of collagens I–V. Histone neutralization after bleomycin was accompanied by TGFβ1 downregulation and IL-27 upregulation. Bleomycin injury enhanced accumulation of platelets in the lung, with platelets serving as the primary source of TGFβ1 during fibrosis. Platelet-specific deletion of TGFβ1 was associated with reduced deposition of collagen in the lung and elevated IL-27 in BALF compared with $TGFβ1^{fl/fl}$ mice. Platelet supernatants from TGFβ1-competent mice suppressed IL-27 release from macrophages, while blockade of the TGFβ1 receptor, TGFβRII, reversed this effect. Additionally, TGFβRII deletion in myeloid cells reduced extracellular matrix and collagen deposition in the lungs. TGFβ1 modulated enhanced phosphorylation of SMAD2/SMAD3, while inhibition of SMAD3 using lentiviral shRNA subverted TGFβ1-mediated suppression of IL-27 in macrophages. Mechanistically, IL-27 downregulation may be driven by TGFβ1-induced enhanced binding of SMAD3 to IL-27 promoter regions in macrophages, observed using chromatin immunoprecipitation assays. Of note, $IL\text{-}27RA^{-/-}$ mice showed more severe fibrosis after bleomycin compared to wild type mice, with elevated collagen deposition. **Conclusions:** This study elucidates a novel mechanism by which externalized histones from neutrophil extracellular traps contribute to the development of pulmonary fibrosis through a cytokine-driven platelet-macrophage circuit. Targeting the histone-platelet-macrophage axis may offer potential therapeutic strategies for managing this debilitating disease. **Acknowledgments**: This work was supported by the NIH (1R01HL141513, 1R01HL139641, and 1R01HL166588 to M.B.), the Federal Ministry of Education and Research (01EO1503 to M.B., and K.S.), the Deutsche Forschungsgemeinschaft (BO3482/3-3, BO3482/4-1 to M.B., CRC829 to S.A.E., and a Marie Curie Career Integration Grant of the European Union (Project 334486 to M.B.). We thank Dr. Jürgen Roes for generously providing the $TbRII^{fl/fl}$ mouse strain.

IMMUN007

Role of Neutrophils in Pathogenesis of Primary Sclerosing Cholangitis

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Background: Primary Sclerosing Cholangitis (PSC) manifests with an inflammatory milieu that can contribute to fibrotic scarring of the liver. Human PSC bile ducts are enriched with myeloid cells suggesting a role for the innate immune system, which has not been rigorously explored. The stimulator of interferon genes (STING) signaling pathway regulates the innate immune responses via the release of cytokines, including neutrophil chemoattractants. We aim to investigate the mechanism and impact of peribiliary neutrophil infiltration observed in PSC. **Methods:** Primary cholangiocytes isolated from WT and mouse models of PSC (3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC)-fed mice and Mdr2-/- mice) were analyzed by RNA-sequencing. Immunofluorescence (IF) was performed on liver tissues from PSC patients and mouse models of PSC for markers of bile ducts and neutrophils (KRT19 and MPO). Intrahepatic leukocytes (IHL) isolated from WT and Mdr2-/- were evaluated for neutrophil abundance and activation state by flow cytometry and RT-PCR. Anti-Ly6G antibody mediated neutrophil depletion in Mdr2-/- mice was analyzed by immunofluorescence (IF), histology, and cytometry by time-of-flight (CyTOF). Cholangiocytes stimulated with LPS (to induce an inflammatory phenotype) were analyzed for neutrophil chemoattractants with STING interventions. **Results:** RNA-seq analysis of primary cholangiocytes from PSC mouse models demonstrated enrichment in inflammatory and neutrophil degranulation pathways. Increased presence of peri-portal neutrophils was observed from PSC patient and mouse liver tissues compared to controls (6.7±1.74-fold, $p<0.0005$, n=5). Congruently, flow cytometry analysis on $CD45^+$ IHL revealed an increase in the neutrophil population ($Ly6^+$ $Cd11b^+$) in Mdr2-/- mice compared to WT (8.7% vs 2.5%, FC=3.48, $p<0.0001$). These neutrophils displayed an activated phenotype with increased expression of Cxcr1 and Cxcr2. Correspondingly, anti-Ly6G-mediated peripheral depletion of neutrophils in Mdr2-/- mice alleviated liver injury and inflammation (57.6% and 59.2% reduction in ALT and AST, respectively). IF and histology revealed a substantial reduction in peribiliary neutrophil infiltration and reduced bridging fibrosis in neutrophil-depleted Mdr2-/- mice livers. Interestingly, CyTOF analysis of IHL revealed a significant reduction in CD8+ T cells upon neutrophil depletion, implying a role for neutrophils in attracting CD8+ T cells to the liver. Mechanistically, the LPS-mediated increase in neutrophil chemoattractant, CXCL1, in cholangiocytes was abrogated with both pharmacological and genetic inhibition of STING. **Conclusion:** Our findings suggest that activation of the STING pathway in cholangiocytes in cholestatic liver disease triggers an immune response resulting in peri-portal neutrophil infiltration. The sustained presence of these activated neutrophils engages the adaptive immune system to perpetuate the inflammation and fibrosis seen in PSC.

Session 030 – Immunity and Innate Environments in Inflammation

INFLAM001

The TNF Superfamily Factor TWEAK Promotes Inflammatory Stroma Expansion in Ulcerative Colitis and Modulates Fibroblast-Monocyte Crosstalk via STAT3

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Background: Colonic fibroblasts have emerged as key modulators of the immune response in inflammatory bowel disease (IBD). We have previously shown that TNFSF12/TWEAK can induce an inflammatory polarisation in colonic fibroblasts *in vitro.* Here, we investigate the correlation between TWEAK and the expansion of the inflammatory stroma in biopsies from ulcerative colitis (UC) patients and characterize the effect of TWEAK-differentiated inflammatory fibroblasts in the recruitment and activation of monocytes. **Methods:** Endoscopic biopsies from healthy donors or UC patients (active or in remission) were analyzed via flow cytometry and immunofluorescence microscopy. Human primary colonic fibroblasts were isolated from resections and stimulated with TWEAK for 24 or 48 hours. THP1 cells were co-cultured with fibroblasts pre-treated with TWEAK for 24 hours and analyzed via RNAseq. THP1 cells and primary monocytes were cultured with fibroblast conditioned medium for 24 hours an analyzed via immunoblotting. **Results:** The colonic mucosa of active UC patients showed an accumulation of TWEAK-expressing myeloid cells in inflamed areas compared to non-involved areas, biopsies from patients in remission, or samples from healthy donors. Concomitantly, inflamed areas showed increased frequency of $CD90^+/PDPN^+$ stromal cells, and this expansion correlated with the abundance of $TWEAK^+$ cells. Analysis of the mononuclear phagocytes ($CD11b^+/CD14^+$) in these biopsies revealed an increase in $CSF1R^+/CD163^+/TREM1^+/CD64^+$ cells

in active UC, consistent with infiltrating monocytes previously identified by scRNAseq. *In vitro*, co-culture with TWEAK-stimulated colonic fibroblasts polarized monocytes towards a phenotype that resembles their *in vivo* profile (FCGR1A, CD163, TREM1, OSM, IL1B) and presents increased expression of IBD susceptibility genes (NOD2, PTPN2, ATG16L1, TLR5). Enrichment analysis pointed towards STAT3 as a key driver of this monocyte priming by fibroblasts, and immunoblotting confirmed STAT3 phosphorylation in monocytes treated with conditioned media from TWEAK-stimulated fibroblast. **Conclusions:** Our work solidifies the role of TWEAK as a driver of inflammatory differentiation in colonic fibroblasts in UC and highlights their role in coordinating the immune response. Moreover, we identify STAT3 as a potential mediator in this stroma-immune crosstalk, which may be amenable to therapeutic intervention. **Acknowledgements:** This work is supported by funding from Science Foundation Ireland and The Irish Research Council under the Pathway program (21/PATH-S/9621) and the IRC/GOI postdoctoral fellowship scheme (GOIPD/2023/1118), as well as funding from Enterprise Ireland and the UCD Conway Institute Director's Strategic Fund.

INFLAM002

Exploring Neutrophil Contributions to Anti-TNFα Therapy Resistance in IBD

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Background: Inflammatory Bowel Disease (IBD) is a chronic and relapsing disease that features robust neutrophil infiltration and accumulation in the intestinal mucosa, which leads to exacerbated inflammation, epithelial barrier disruption and tissue injury. TNFα inhibition is a common therapy for IBD patients refractory to standard immunosuppressives, however, up to 40% of patients fail to respond to such treatment. Persistent presence of neutrophils in the intestinal mucosa has been correlated with disease severity, higher risk of relapse and resistance to therapy in UC patients. However, although much is known about neutrophil-associated pathology in IBD, how neutrophils promote therapeutic resistance remains unknown. **Methods:** To investigate neutrophil contributions to anti-TNFα resistance, we established a murine model of anti-TNFα resistance, using colitis susceptible IL10KO mice and low-dose DSS treatment (2.0%, 5 days). **Results:** In this model, animals consistently developed severe colitis at the time of treatment and failed to respond to anti-TNFα therapy. An antibody (Ab)-mediated neutrophil depletion ameliorated disease and rescued response to anti-TNFα treatment, confirming neutrophils as drivers of anti-TNFα resistance. Transcriptome analyses by single cell RNAseq of inflamed colon mucosa revealed the enrichment of a neutrophil subset differentiated by high expression of Oncosatin M (Osm), which has been previously implicated in therapeutic resistance in IBD. Osm-high neutrophils also featured heightened migratory and proinflammatory activation state defined by Cxcr2, Cxcl2, IL-1β and Mmp8. We established neutrophils as a major source of Osm in inflamed colon and found that Osm expression was increased in colon tissue infiltrating neutrophils via GMCSF activation of STAT-5. Finally, Ab-mediated inhibition of Osm ameliorated disease and showed gain of response to anti-TNFα treatment, consistent with the effect observed following neutrophil depletion. **Conclusions**: We identified a novel specialized neutrophil subset in inflamed colon mucosa enriched for Osm expression which serve to promote anti-TNFα resistance. These observations establish a new framework for neutrophil-targeted therapies in IBD. **Acknowledgements:** DK124199 and AI153568 NIH grants to Ronen Sumagin, and AWD00002608 Crohns and Colitis Foundation grant to Laura D. Manzanares.

Session 031 – Understanding Liver Cancer Pathogenesis

LIVER006

Deletion of Glutamine Synthetase from Hepatocellular Carcinoma-Associated Macrophages Reduces Glutamine Bioavailability and Disease Severity

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Background: Hepatocellular Carcinoma (HCC) impacts roughly 40,000 new patients annually in the United States with a fatality rate of at least 50%. Recent therapeutic strategies have leveraged immune checkpoint inhibitors (ICIs) to treat HCC with modest success. Patient response to ICI therapy is heterogeneous, and evidence suggests that while mutational burden may inform on a stable clinical response, some HCCs with classic indicators of poor ICI response (e.g., β-catenin-mutated/pathway activation) may respond given the right tumor immune microenvironment (TIME). β-catenin-mutated

HCCs can demonstrate robust Glutamine Synthetase (GS) expression, the enzyme responsible for converting Glutamate (Glu) to Glutamine (Gln). We and others have found that HCC patient survival is predicated on overall *GLUL* (the gene encoding GS) expression such that patients with lower *GLUL* expression have worse survival odds. Therefore, we asked whether the relative abundance of Glu/Gln impacts the TIME and immune cell function in β-catenin-mutated HCCs. **Methods:** GS enriched HCCs were modeled in *Glul$^{f/f}$-Lyz2^{creERT}* mice by co-delivering expression plasmids via hydrodynamic tail vein injections coding for mutant-*CTNNB1*-(T41A) and mutant nuclear factor erythroid 2–related factor 2 (*NFE2L2*-G31A) (B+N model) which models ~12% of HCCs. HCC and hepatocyte *Glul* elimination following tumorigenesis was achieved by delivering AAV8 carrying Cre-recombinase while macrophage elimination was achieved via biweekly administration of Tamoxifen (100 mg/kg). HCC burden was assessed in *Glul*-KO and controls. We conducted spatial transcriptomics via Molecular Cartography, as well as single-cell RNA-sequencing (scRNA-Seq) to identify changes in the TIME in B+N HCCs ± GS. Key results were validated by immunostaining. **Results:** scRNA-Seq and Molecular Cartography identified an increased accumulation of immunosuppressive myeloid cells in the TIME contributing to enhanced tumorigenesis in *Glul*-KO B+N HCCs. This was abrogated when mice were treated with clodronate liposomes. CLEC4F expressing macrophages in *Glul*-KO HCCs demonstrate enhanced GS expression via confocal microscopy. We observed a marked reduction in disease burden when *Glul* expression was deleted from both HCCs and myeloid cells with a concomitant increase in CD8 T-cell infiltration. **Conclusions:** Our work identifies a link between Gln bioavailability and functional shifts in the TIME of HCCs. Reducing Gln levels in HCCs enhances tumor growth with a subsequent immunosuppressive shift in the macrophages in the microenvironment. To compensate for a loss in Gln availability, macrophages begin expressing intrinsic GS, which, when deleted, suppresses HCC growth while concomitantly promoting intratumoral cytotoxic T-cell infiltration. Our studies indicate modulating Glu/Gln availability in HCCs may promote susceptibility to ICI and future work plans to test whether Gln loss sensitizes HCCs to ICI approaches. **Acknowledgements:** This work was funded by E.R.D. K22CA258677 and S.P.S.M. R01CA250227.

LIVER007

A Digital Pathology Approach to Predict Spatial Subtype Signatures of Hepatocellular Carcinoma from Histologic Images

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Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality. There are multiple classifications of HCC, including the Hoshida system, which stratifies HCC into 3 subtypes (S1, S2, and S3) with distinct pathomolecular features. Advances in precision medicine for HCC have been delayed by a scarcity of tissue biopsies due to historical concerns of safety risks. However, with the advancement of clinical trials for targeted therapies, tissue biopsies are being increasingly advocated for and even required. Thus, there is an emerging opportunity to pioneer the use of digital pathology approaches to advance precision medicine for HCC. **Methods:** We accessed publicly available spatial transcriptomics data, paired with corresponding hematoxylin and eosin (H&E) images, from 17 HCC slides. Analysis was performed to characterize the spatial distribution of S1, S2, and S3 subtype signatures. The H&E images were divided into tiles corresponding to the spatial transcriptomic spots. Using a deep learning foundation model for digital pathology, we extracted an embedding vector from each tile. Hoshida subtype signature scores were calculated for each spatial transcriptomic spot, and 2 spatial subtype clusters (HS1 and HS3) were derived using k-means clustering. A neural network was trained to predict these clusters from the H&E tile embeddings and was evaluated using leave-one-out cross-validation. External validation was performed by applying the trained model to H&E whole-slide images from HCC patients (n=352) in The Cancer Genome Atlas (TCGA). We assessed the correlation between the proportion of predicted HS1-positive tiles (HS1+) for each patient and bulk RNA-derived subtype signature scores, *CTNNB1* mutation status, and overall survival. **Results:** Among the 17 HCC slides, our neural network model achieved a mean prediction accuracy of 0.74 for spatial transcriptomics-derived Hoshida subtypes across spots, using corresponding H&E tiles. When applied to the TCGA data, the model demonstrated a significant positive correlation between HS1+ and bulk RNA-derived S1 signature scores ($p<0.0001$). Conversely, we observed a negative correlation between HS1+ and the bulk RNA-derived S3 signature ($p<0.0001$). *CTNNB1* mutation status was negatively associated with HS1+ ($p=0.0019$). In addition, patients with low HS1+ demonstrated improved overall survival compared to those with high HS1+ ($p<0.0001$). **Conclusions:** Our results suggest that deep learning can effectively predict the spatial distribution of gene expression-derived subtypes from H&E whole slide images. These predictions recapitulate established paradigms in HCC, while providing improved prognostic value compared

to bulk RNA-based signatures alone. This highlights a promising role for digital pathology in HCC precision medicine. **Acknowledgements:** This work was supported by NIH grants 1R01CA251155 to SPM, R00CA248944 & R35GM154967 to YCC, and T32EB001026 to TMY.

Session 032 – Advancing Cancer Research Through Artificial Intelligence

CANCER006

Machine Learning-Enhanced MALDI Imaging Mass Spectrometry of N-Glycans for Hepatocellular Carcinoma Classification and Heterogeneity Analysis

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Background: Hepatocellular carcinoma (HCC) represents a significant challenge in cancer diagnostics, with tumor heterogeneity playing a crucial role in disease outcomes. While N-glycosylation changes are associated with cancer development, their spatial distribution and relationship to tumor heterogeneity remain poorly understood. Mass spectrometry imaging of N-glycans offers a unique opportunity to investigate these changes at high spatial resolution while preserving tissue context, potentially providing new insights into tumor biology and classification. **Methods:** FFPE tissue sections from HCC patients underwent MALDI-TOF imaging mass spectrometry following PNGase F and sialidase treatment. Tissues were imaged at 80-150 µm resolution with 200 laser shots per pixel. Data processing included TIC normalization and relative intensity calculations for 300 annotated N-glycans. We developed an XGBoost classification model using mutual information-based feature selection, with strict patient-level separation between training and testing sets. Model validation was performed through five-fold cross-validation and external validation on an independent cohort. UMAP analysis was employed to visualize glycan profile distributions and assess tumor heterogeneity. **Results:** Analysis included two independent HCC patient cohorts: discovery (n=86) and validation (n=60), each containing matched tumor and adjacent non-tumor tissues. MALDI imaging mass spectrometry generated comprehensive N-glycan profiles, followed by H&E staining for pathological verification. The XGBoost classification model achieved a mean AUC of 96% in five-fold cross-validation during training and 87% in external validation. Key discriminative N-glycan species were identified at m/z 2231.793, 1282.454, and 2393.846. UMAP analysis revealed distinct clustering patterns between tumor and non-tumor tissues while highlighting significant inter-tumor heterogeneity. Despite analyzing spots independently, the model generated coherent tumor region probability maps through clustered high-confidence predictions. Individual patient analyses showed clear separation between tumor and adjacent non-tumor regions based on glycan profiles, while comparison of individual tumor spots revealed both patient-specific signatures and shared features across tumors. **Conclusions:** Our machine learning approach to MALDI imaging mass spectrometry successfully distinguished HCC tumor from non-tumor tissues based on N-glycan profiles, while revealing important aspects of tumor heterogeneity. The robust performance across independent cohorts demonstrates the potential of this approach for molecular-level tumor classification and heterogeneity analysis. **Acknowledgements:** This work was supported by National Cancer Institute grants U01 CA226052, R01 CA222900, R21 CA22547, R01 CA237659, and U01 CA242096.

Session 033 – Vascular Inflammation in Atherosclerosis

VASCUL003

Circadian Factor Bmal1 Regulates Vascular Calcification

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Background: Vascular calcification, a prevalent complication of diabetes, has been linked to the osteogenic differentiation of vascular smooth muscle cells (VSMC). Disrupted circadian rhythms have been associated with the progression of diabetes. Nevertheless, the mechanisms regulating circadian proteins in diabetes, particularly on vascular calcification, remain largely unexplored. **Methods**: In vitro mouse VSMC culture, Streptozotocin-induced diabetic mouse model, and VSMC-specific gene knockout mice were utilized. Alizarin Red staining was employed to assess cellular and vascular calcification. RNA-seq was utilized to characterize gene expression profiles. **Results**: We found that the Basic Helix-Loop-Helix ARNT-like protein 1 (Bmal1), a crucial regulator of the circadian clock, was elevated in VSMCs exposed to high glucose levels and in diabetic mice arteries. To investigate the function of Bmal1 in VSMCs, we generated a VSMC-Bmal1 deletion (Bmal1$^{\Delta/\Delta}$) mouse model. Our findings showed that SMC-specific Bmal1 deletion significantly reduced vascular

calcification in diabetic mice. RNA sequencing revealed that downregulated genes in Bmal1$^{\Delta/\Delta}$ VSMCs were involved in bone trabecula formation, osteoblast differentiation, and cellular responses to transforming growth factor-beta. These findings suggest a potential pro-osteogenic role for Bmal1 in VSMCs. Moreover, Bmal1$^{\Delta/\Delta}$ VSMCs exhibited reduced expression of the master osteogenic regulator, Runt-related transcription factor 2 (Runx2). To further explore this, we conducted mechanistic studies and identified Bmal1 DNA binding sites within the Runx2 promoter region, located close to peaks associated with Assay for transposase-accessible chromatin (ATAC), H3K4me3, H3K9ac, and H3K27ac signals, suggesting Bmal1 bind to the Runx2 promoter, leading to increased epigenetic activity that facilitates Runx2 gene expression. Consistently, overexpression of Bmal1 in VSMCs increased luciferase activity driven by the wild-type Runx2 promoter but not the Runx2 promoter containing a mutated Bmal1 binding site. To further confirm this, we used CRISPR-Cas9 to disrupt the Bmal1 binding site in the Runx2 promoter in VSMCs. Remarkably, this mutant VSMC exhibited the complete abolition of Bmal1 overexpression-induced Runx2 expression. **Conclusions**: These studies collectively show that elevated Bmal1 levels contribute to diabetes-induced vascular calcification by regulating Runx2 expression in VSMCs through direct binding to the Runx2 promoter. Our research has revealed a novel Bmal1-dependent, non-canonical circadian pathway that governs diabetes-induced vascular calcification. Consequently, maintaining appropriate levels of the clock regulator Bmal1 may enhance VSMC function and vascular health in individuals with diabetes. **Acknowledgements**: This work was supported by AHA CDA859625; NIH R21AG075450, R35GM138212, R01HL146103, R01HL158097, R01HL167201, R01AG082839; VA BX005800, BX004426, BX006321, and CX002706.

VASCUL004

The T-cell CXCR3-Endothelial cell ICAM-1 Axis Drives T-cell Cardiac Infiltration in Cardiometabolic Heart Failure with Preserved Ejection Fraction (HFpEF)

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Background: Heart disease remains the leading cause of mortality in the US, with heart failure (HF) representing a severe form of disease with a poor prognosis. Cardiometabolic Heart Failure with Preserved Ejection Fraction (HFpEF), associated with obesity and hypertension and characterized by diastolic dysfunction with preserved contractility, currently represents 50% of HF patients but is projected to lead HF diagnoses in the coming decade. T-cells are required for the development of pre-clinical cardiometabolic HFpEF in mice, but the mechanisms of T-cell cardiotropism and how cardiac endothelial cells (ECs) regulate this process are still emerging. HFpEF patient hearts demonstrate increased levels of intercellular adhesion molecule-1 (ICAM-1), a critical adhesion molecule that interfaces with CXC Motif Chemokine Receptor 3 (CXCR3) on infiltrating immune cells, but whether this increase is responsible for T-cell cardiotropism is unknown. We hypothesized that an inflamed cardiac endothelium orchestrates cardiotropism of peripheral T-cell subsets through specific chemokine-receptor signaling. **Methods**: We treated male C57BL/6J mice with high-fat diet (HFD) and N(ω)-nitro-L-arginine methyl ester (L-NAME) (H/L) for 5-weeks, mimicking obesity and hypertension combined, an established model of preclinical cardiometabolic HFpEF, and used STD-fed littermates as controls. We assessed diastolic and systolic function through noninvasive pulsed-wave doppler echocardiography. Flow cytometry and cellular indexing of transcriptomes and epitopes by sequencing (CITEseq) were utilized to characterize cardiac endothelial cell activation and T-cell chemokine receptor signaling in the spleen, blood, and heart. **Results:** We found that H/L induces the expansion of CXCR3+CD4+ T-cells in the spleen and blood compared to STD-mice, but not CXCR4+, CCR4+, or CCR6+ T-cells. Simultaneously, H/L induces higher expression of ICAM-1 on the surface of cardiac endothelial cells compared to STD-fed mice. Additionally, cardiac single-cell sequencing revealed ECs as the major source of *Cxcl9*, one of the predominant ligands for CXCR3 and a requirement for T-cell adhesion to ICAM-1 and subsequent extravasation into the heart. Within the heart, H/L induces increased infiltration of CXCR3+CD4+ T-cells compared to STD-fed mice, a trend not observed in cardiac CD8+ T-cells at this time point or with other CD4+ T-cell chemokine receptors. **Conclusions:** Taken together, we demonstrate that H/L induces an expansion of peripheral T-cells primed to infiltrate the heart through the T-cell CXCR3-endothelial cell ICAM-1 signaling axis, implicating this axis in T-cell cardiotropism in preclinical HFpEF. Ongoing studies will identify the requirement for EC ICAM-1 in T-cell cardiotropism using EC-specific knockout of ICAM-1 combined with H/L treatment. **Acknowledgements:** Funding Sources- R01HL165725 (PA), Tufts Student Enrichment Award (ZR).

Session 036 – Minisymposium – Cardiovascular and Metabolic Homeostasis and Disease

CARDIOVAS001

AI-Assisted Drug Discovery for Early-Stage Type 2 Diabetes: Pathophysiology, Prognostics, and Risk Stratification

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Background: Metabolic diseases present significant scientific challenges due to the intricate interplay of genetic predisposition, metabolic imbalances, inflammatory cascades, and environmental influences, making clinical trajectories highly unpredictable. This complexity necessitates advanced analytical tools to decode disease heterogeneity, refine risk assessment, and develop targeted therapeutics. Artificial intelligence (AI) has transformed disease stratification and the development of diagnostic and prognostic tools. However, its full potential depends on integrating comprehensive omics-derived molecular data to uncover fundamental pathophysiological mechanisms. Among various omics approaches, metabolomics stands out as a powerful tool for capturing the dynamic interactions between genetic and lifestyle factors, providing crucial insights into the initiation and progression of metabolic diseases. Despite its promise, investigating pathophysiology at advanced disease stages is challenging due to compensatory mechanisms and the complexity of metabolic networks, which can obscure primary pathological drivers. In contrast, early disease stages offer a clearer biological landscape, enabling precise identification of causal mechanisms and the discovery of predictive biomarkers for preventive interventions. Sophisticated AI methodologies alone are insufficient to uncover metabolic disease mechanisms. Instead, success depends on robust study design, rigorous data preprocessing, stringent quality control, and bias removal. The careful application of statistical and machine learning (ML) techniques is crucial to mitigate potential biases—including selection bias, confounding bias, and multiple testing bias—while preventing model overfitting and underfitting. To address these challenges, we have recently published methodological papers outlining key considerations in study design and data analysis, advocating for expert-driven standards to ensure unbiased AI-assisted decision-making in drug discovery (*Drug Discov Today*, 2021, **26**:982-992; *Drug Discov Today*, 2019, **24**:1735-1748; *Drug Discov Today*, 2014, **19**:562-578). Building on these principles, we developed an AI-assisted platform and applied it to a gestational diabetes mellitus (GDM) cohort, aiming to decode early-stage type 2 diabetes (T2D) pathophysiology, disease heterogeneity, and prognostic biomarker discovery. **Methods and Results:** We employed a comprehensive metabolomics and unbiased systems biology approach in a prospective cohort study on type 2 diabetes (T2D) progression, leading to a key discovery: downregulation of sphingolipid metabolism as an early-stage marker of generalized T2D pathophysiology (*Science Adv*, 2025, **11**:eadr1725; *Diabetologia*, 2019, **62**:687-703; *iScience*, 2020, **23**:101566). To investigate the genetic underpinnings of this dysregulation, we developed metGWAS 1.0, a novel bioinformatics tool integrating metabolomic and genomic data (*Bioinformatics*, 2023, **39**:btad523). Using metGWAS 1.0, we mapped downregulated sphingolipids to the GWAS database and identified ceramide synthase 2 (CERS2) as a key T2D risk gene, specifically through the SNP rs267738. This point mutation in exon 3 of the CERS2 enzyme leads to a partial loss of function. To elucidate the impact of CERS2 and its risk allele on glucose metabolism, we conducted in vivo and ex vivo studies using whole-body Cers2 knockout (KO) and rs267738 knock-in (KI) mice. Both models exhibited glucose intolerance and impaired insulin secretion, with isolated pancreatic islets demonstrating significantly reduced β-cell function and insulin secretion ex vivo (*Science Adv*, 2025, **11**:eadr1725). Leveraging machine learning (ML) approaches, we developed two prognostic models for predicting incident T2D: (A) Generalized T2D Model: Using a decision tree-based ML approach, we identified a seven-lipid metabolite signature with a discrimination power (AUC = 0.92, 87% sensitivity, 93% specificity, and 91% accuracy) (*Diabetologia*, 2019, **62**:687-703). (B) Race-Specific T2D Model: By integrating a random forest ranking algorithm with a univariate ranking system, followed by the application of the sMLR algorithm, we identified a three-analyte risk-score panel with a discrimination power (AUC = 0.95, 86% sensitivity, 90% specificity, and 88% accuracy) (*iScience*, 2020, **23**:101566). To explore early disease heterogeneity, we applied Mclust, a probabilistic Gaussian mixture model clustering approach, identifying three distinct clusters. These clusters were differentiated by postpartum blood parameters, including glucose tolerance, HOMA indices, fasting lipid profiles (triglycerides, leptin, HDL-c, and adiponectin), as well as age and BMI. Metabolomic integration further revealed unique molecular signatures for each cluster (*Diabetes/Metabolism Res Rev*, 2025, **41**:e70027). **Conclusion:** Our findings demonstrate the potential of AI-assisted platforms in every aspect of drug discovery, from pathophysiological decoding to prognostic model development, paving the way for precision medicine in metabolic diseases.

CARDIOVAS002

T-Cell-Released IFNγ Induces the Expression of Cardiac Fibroblast MHC-II Required for Doxorubicin Cardiotoxicity

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Background: Cancer patients receiving Doxorubicin (DOX), one of the most widely used chemotherapy agents, often develop cardiotoxicity and heart failure (HF) and have increased circulating numbers of IFNγ^{+} cytotoxic T cells. In mice, cardiac IFNγ^{+} cytotoxic T cells are necessary for pathological cardiac fibrosis through actions on cardiac fibroblasts (CFBs) as well as for contractile dysfunction. Whether CFBs adopt immune-like functions such as chemokine secretion and major histocompatibility complex II (MHC-II) expression to promote cardiac inflammation in DOX cardiotoxicity remains unknown.

We hypothesized that DOX induces a pro-inflammatory CFB state that is required for T cell immune responses and cardiotoxicity. **Methods:** Primary CFBs were treated with DOX (1.0ug/mL), IFNγ (100U/mL), or co-cultured with IFNγ-producing cytotoxic T cells (Tc1) in transwells, and analyzed for CFB expression of MHC-II, chemokines, and their ability to trigger Tc1 migration by flow cytometry, immunofluorescence, and qPCR. Wild-type (WT), T cell-deficient (*Tcra*$^{-/-}$), and *Tcf21*$^{iCre/+}$*MhcII*$^{fl/fl}$ mice, treated with Tamoxifen or not to induce CFB specific deletion of MHC-II (CFB-*MhcII*$^{-/-}$ and CFB-*MhcII*$^{+/+}$, respectively) were treated with PBS or cumulative DOX (5 mg/kg/week) intraperitoneally for 4 and 8 weeks. Contractile function was assessed by echocardiography. Flow cytometry was used to characterize CFBs and immune cell infiltrates in digested hearts, qPCR to analyze whole heart gene expression, and histology to determine cardiac atrophy and fibrosis by wheat-germ agglutinin and picrosirius red staining, respectively. **Results:** DOX induced CXCL9 expression in CFBs as well as Tc1 migration in a CXCL9 dependent manner, as shown by Tc1 migration inhibition in the presence of anti-CXCL9. However, DOX alone was not sufficient to induce MHC-II expression. DOX treatment of Tc1 cells enhanced IFNγ production, and induced surface MHC-II expression on CFBs. *In vivo*, 8 weeks of DOX induced the expression of MHC-II in a subset of CFBs in WT mice. This was T cell dependent, as it was not observed in *Tcra*$^{-/-}$ mice, and adoptive transfer of Tc1 cells into *Tcra*$^{-/-}$ restored CFB-MHC-II expression in the onset of DOX. Lastly, CFB-*MhcII*$^{-/-}$ mice developed less cardiac atrophy and fibrosis, showed improved systolic function, as well as reduced numbers of cardiac CD4^{+} T cells in response to DOX, compared to CFB-*MhcII*$^{+/+}$ littermate controls. **Conclusions:** Our results demonstrate that DOX induces a pro-inflammatory CFB state characterized by CXCL9 release which results in cytotoxic T cell migration. DOX enhancement of T cell IFNγ is necessary for CFB-MHC-II expression *in vitro* and *in vivo,* coinciding with increased pathology. Ultimately, our data positions CFB-MHC-II as a key mediator of fibrosis and contractile dysfunction in DOX cardiotoxicity. **Acknowledgments:** 1F31HL175911-01 (Zambrano).

CARDIOVAS003

BMP7 Attenuates Fibrotic Response in a Murine Model of Proliferative Vitreoretinopathy

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Background: Proliferative vitreoretinopathy (PVR) is a vision-threatening complication of retinal detachment repair, characterized by the formation of fibrotic membranes that lead to retinal traction and vision loss. Fibroblast activation, marked by α-smooth muscle actin (αSMA) expression, plays a central role in fibrosis. Bone morphogenetic protein 7 (BMP7) counteracts TGF-β–mediated fibrotic pathways and shows potential as an antifibrotic agent. This study qualitatively assessed BMP7's impact on fibrotic responses in a murine model of PVR. **Methods:** Male C57BL6J mice were used. Dispase II (0.4U/µL) was injected intravitreally to induce PVR, and PVR develops 7 days after the injection. The animals were assigned to three groups: naïve eyes with no PVR induction, PVR eye without treatment, and PVR eye with intravitreal BMP7 injection (0.04 µg/mL, 1µL). The eyes were collected 7 days after the injection, fixed in 4% PFA, and subject to cryosection. Immunofluorescence staining was conducted using primary antibodies against αSMA and glial fibrillary acidic protein (GFAP) followed by corresponding fluorescent secondary antibodies with DAPI counterstain for nuclei. In the negative control, naïve eye sections were incubated with 1% bovine serum albumin (BSA) without primary or secondary antibodies, yielding no specific staining. All sections were processed under standardized conditions, and imaging was performed. **Results:** The negative control exhibited no detectable staining, confirming the absence of non-specific antibody binding. Fundus images showed clear retinas in controls, but visible fibrocellular membranes in PVR eyes. OCT confirmed membrane thickening and retinal distortion in PVR eyes. BMP7-treated eyes exhibited fewer tractional changes and reduced membrane thickness, as well as improved retinal architecture. Naïve eye sections displayed baseline αSMA and GFAP staining, validating the immunostaining protocol. Eyes in the PVR group showed markedly increased αSMA staining with prominent fibrotic membrane formation alongside elevated GFAP expression, reflecting robust myofibroblast activation and glial response. Conversely, the PVR + BMP7 Group demonstrated a significant reduction in αSMA staining with less pronounced fibrotic membranes and GFAP expression remained comparable to the PVR Group. These results suggest that BMP7 selectively attenuates fibrosis without altering the glial activation profile. **Conclusion:** BMP7 treatment in the murine PVR model effectively reduced the fibrotic response, as evidenced by diminished αSMA-positive fibroblast accumulation and thinner fibrotic membranes, while the glial response remained intact. These findings support the potential of BMP7 as a targeted antifibrotic therapy in PVR, warranting further investigation. **Acknowledgements:** We gratefully acknowledge the support from ASIP SROPP for funding this research.

CARDIOVAS004

Unraveling the Effects of *Dchs1* on Cardiac Proliferation: A Study of Fibroblast—Cardiomyocyte Crosstalk in Cardiac Development

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Introduction: *Dchs1*, an atypical cadherin, was the first gene identified as causal to mitral valve prolapse (MVP). While haploinsufficiency partly explains the valve disease, complete knockout of the gene led to a reported high incidence of neonatal lethality in mice. Excised homozygous *Dchs1$^{-/-}$* (knockout) P0 hearts appeared grossly immature (smaller and rounder). Therefore, we hypothesize that *Dchs1* is critical for normal maturation and morphogenesis of the developing heart. **Methods:** To analyze normal expression of *Dchs1*, we engineered a mouse model with an HA-tagged *Dchs1* locus (referred to as *Dchs1-HA*) and analyzed it using immunohistochemistry (IHC) and western analyses. To investigate *Dchs1's* role during development, a global knockout mouse model (*Dchs1$^{-/-}$*) and mutant model in which the intracellular domain (ICD) was deleted and replaced with a V5-tag (referred to as *Dchs1-dICD-V5*) was used and analyzed with IHC, single-nuclei RNA sequencing (snRNAseq), transmission electron microscopy (TEM), and western analyses. **Results:** Analysis of the *Dchs1-HA* mice demonstrated DCHS1 protein localization to the cell membranes of embryonic and neonatal non-myocyte populations, including endothelial and epicardial cells, as well as fibroblasts. Notably, western analyses at embryonic and neonatal timepoints revealed bands corresponding to both the full-length DCHS1 protein and a smaller 50kDa band suggesting a potential cleavage of the intracellular domain that coincided with onset of observed intracellular DCHS1 protein in neonatal left ventricular myocardium via IHC. The presence of this smaller molecular weight band via western analyses was possibly dependent on cell density and was statistically increased during late gestation. Single-nuclei RNA sequencing (snRNAseq) of wild-type hearts corroborated non-myocyte expression, with a majority of transcripts identified in fibroblast and endothelial cell populations. It was found 100% of the homozygous *Dchs1$^{-/-}$* mice died during neonatal stages with evidence of impaired cardiomyocyte differentiation via TEM. Western analyses and snRNAseq datasets showed a statistically significant increase in cell cycle and cytokinetic genes across all cardiac lineages, including myocytes. *Dchs1-dICD-V5$^{-/-}$* mice exhibited a gross immature phenotype and increased proliferation staining implying the ICD may regulate the proliferative phenotype. **Conclusions:** These findings demonstrate a critical role for *Dchs1* in regulating myocyte and non-myocyte cell cycle in a cellular non-autonomous manner. This study is also novel in suggesting a role for the ICD as the effector domain of *Dchs1* in mammalian morphogenesis. Moreover, these data also suggest that cell density and the contribution of epicardial-derived fibroblasts in the heart likely exert a critical influence on myocyte cell-cycle exit through *Dchs1*. **Acknowledgements:** Supported by NIH R01HL131546 and R01HL149696.

CARDIOVAS005

Linking Ciliogenesis, Bromodomain Function, and Acetylation in Valvulogenesis

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Background: DZIP1, a zinc-finger protein essential for primary cilia assembly, was identified by our group as a key contributor to mitral valve prolapse (MVP). Mutations in *Dzip1* lead to a ciliopathy during cardiac development in mice, causing subtle but significant valve changes that ultimately result in MVP. **Methods:** To identify potential binding partners for DZIP1, an unbiased yeast two-hybrid (Y2H) screen was performed using a human heart cDNA library with human *DZIP1* as bait. We then confirmed identified interactions *in vitro* through co-transfection with *DZIP1* in embryonic cells via co-immunoprecipitation (co-IP), western blot, and immunocytochemistry (ICC). *In vivo* studies were performed on wild type C57/BL6, Dzip^{1em1Sasl}, and Dzip1$^{em2(flox)}$; Nfatc1$^{tm1.1(cre)}$Bz;RRID:IMSR_JAX:007676 /J mice. Hearts were excised at E13.5, E15.5, P0, and 6 months and immunohistochemistry, RNAscope, and *in-situ* hybridization were performed. **Results:** The Y2H screen identified BRD10, a bromodomain-containing protein, as a novel DZIP1 interactor, suggesting a mechanistic link between ciliogenesis and bromodomain function in regulating acetylation to direct cell behavior for proper valvulogenesis. This interaction was confirmed via co-IP and the DZIP1-binding region in BRD10 was further defined to lie within a highly conserved domain that serves as a putative nuclear localization sequence (NLS). *In vivo* experiments

demonstrated during development DZIP1 and BRD10 are co-expressed in valve fibroblasts, and co-transfection studies demonstrate that BRD10 subcellular localization and post-translational modifications are DZIP1-dependent, suggesting a regulatory role for DZIP1 in BRD10 trafficking and processing. Given BRD10's bromodomain function, its interaction with acetylated α-tubulin at the primary cilia was investigated and confirmed through co-IP and ICC. **Conclusions:** These findings establish a novel connection between DZIP1 and BRD10 in primary ciliogenesis, suggesting that bromodomain proteins coordinate both chromatin and cytoskeletal acetylation to regulate cell behavior during valvulogenesis. By linking DZIP1 function to BRD10-mediated nuclear trafficking and acetylated α-tubulin interactions, our study provides new insights into ciliary signaling disruptions in MVP and highlights bromodomain-mediated regulatory pathways as potential therapeutic targets for congenital valve disorders. **Acknowledgments:** NIH National Heart Lung and Blood Institute (NHLBI) grants RO1HL131546 and RO1HL149696 and AHA Established Investigator Award 24TPA1304545 to RN.

CARDIOVAS006

Synergistic Effects of Hemodynamic Forces and Genetics on Heart Development.

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Background: Heart development is guided by biophysical forces (e.g. forces exerted by blood flow) and signaling pathways (e.g. Sonic Hedgehog (Shh)) for precise synchronicity of heart development events. When either of these processes get disrupted, they can independently lead to various congenital heart defects (CHDs), such as Tetralogy of Fallot (TOF), Pulmonary Truncus Arteriosus (PTA), and ventricular septal defects (VSD). The Shh pathway is important in fetal development as it signals for second heart field contribution to the early developing heart tube. When Shh is disrupted in chicken embryos during early developmental stages, CHDs develop. Whether biophysical forces like blood flow are disrupted as well in these embryos has not yet been explored. Our goal is to determine whether treatment with cyclopamine (CPA), a substance known to block Shh signaling, alters blood flow velocities in the developing heart, before malformations are apparent. **Methods:** Chicken embryos were used to model heart development. Embryos were dosed with CPA at various concentrations (1.0μg/μL, 0.8μg/μL, 0.6μg/μL) or vehicle during the tubular heart stage (HH14). 24-hours after dosing (HH18), *in vivo* imaging using optical coherence tomography was done to obtain blood flow velocities through the developing heart outflow tract. Echocardiography imaging at the fully formed heart stage (HH38) was preformed to determine blood flow velocity through the aorta. HH38 dissected hearts were imaged using micro-computed tomography to phenotype hearts. **Results:** HH18 CPA-treated hearts had lower blood flow velocities compared to controls ($p<0.05$) in all concentration groups. CPA-treated HH38 hearts had distinct defects, such as PTA and TOF, but some had no visible defects and exhibited normal aortic velocities. Among malformed hearts, TOF hearts (n=11, 914.5 ± 243.56 mm/s) had higher blood flow velocities than the control hearts (n=38, 533.85 ± 118/52 mm/s) ($p<0.05$). **Conclusion:** Our work shows that early disruption of Shh affects blood flow velocities in the early developing heart, before CHDs are apparent. **Significance:** Abnormal blood flow precedes CHD after Shh inhibition. Further research is needed to determine interconnections between genetic regulatory networks and blood flow that affect heart development. **Acknowledgements:** This research was funded by the US National Science Foundation (NSF), grant number 2109918, and the US National Institutes of Health (NIH), grant number R01 HL170097.

CARDIOVAS007

Differential Effects of Allulose, Glucose, and Fructose on Mitochondrial Respiration, Viability, and Invasion in Placental Trophoblast Cells

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Background: Placental metabolism is crucial for fetal development, yet the impact of diverse carbohydrate types on mitochondrial function in trophoblast cells remains poorly understood. This study investigated the effects of allulose, glucose, and fructose on mitochondrial respiration, viability, and invasion capacity in placental SW71 (cytotrophoblast) and BeWo (syncytiotrophoblast) trophoblast cells. **Methods:** Cells were exposed to 25 mM of allulose, fructose, or glucose, and high-resolution oxygen consumption was assessed at 37°C using the Oroboros O2K Oxygraph. Cell number and cell viability were assessed to determine the functional consequences of sugar treatment on trophoblast metabolism and real time cell invasion was determined for the SW71 cytotrophoblasts. **Results:** SW71 mitochondrial respiration increased with allulose and glucose but decreased with fructose, suggesting distinct metabolic responses. Fructose decreased mitochondrial respiration in SW71 cells. BeWo cells revealed increased mitochondrial respiration with fructose, whereas allulose and glucose suppressed it. Cellular viability assays showed that allulose and fructose increased SW71 cell number with reduced cell viability with fructose in these cells. Bewo trophoblast cell number was significantly increased with all sugar treatments,

accompanied by reduced viability. Additionally, SW71 trophoblast invasion was impaired considerably by glucose or fructose, while allulose appeared to support invasion potential. These findings highlight sugar-specific effects on placental trophoblast metabolism. Glucose and fructose increased cell number but reduced viability and invasion, while allulose exhibited a protective role. Fructose enhanced mitochondrial respiration in BeWo cells, whereas allulose and glucose suppressed it. **Conclusions:** These results provide insights into the metabolic impact of maternal sugar intake on placental function and fetal development. **Acknowledgements:** This work was supported by funding from the National Institute of Health (1R15HL152257).

CARDIOVAS008

Autoantibodies in Arrhythmogenic Cardiomyopathy Patients Activate GSK-3β Leading to Loss of Cardiomyocyte Cohesion

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Background: Arrhythmogenic cardiomyopathy (ACM) is an inherited heart disease of the cardiac desmosome, as more than 50% of these patients carry mutations in desmosome protein-coding genes. Recent studies have demonstrated the presence of autoantibodies against intercalated disc (ICD) proteins, such as desmoglein 2 (DSG2), in ACM, which reduces cardiomyocyte cohesion. However, the underlying molecular mechanisms are still unclear, which is the focus of this study. **Methods:** IgG fractions from blood samples of five ACM patients (ACM-IgGs), three healthy relatives (HR), healthy controls (HC), and two different murine models of ACM were isolated. Dispase assays, immunostaining, and Western blots were performed in either murine cardiac slices, HL-1 cells (murine atrial cardiomyocytes), or induced pluripotent stem cells derived cardiomyocytes from a healthy donor (hiPSC-CMs) or ACM patient (ACM-iPSC-CMs). DSG2 cleavage assay was performed to determine the catalytic properties of ACM-IgGs. **Results:** ACM-IgGs but not IgGs from HR, HC, and murine models of ACM caused loss of cell cohesion in HL-1 cells and some ACM-IgG fractions cleaved DSG2. Immunostaining using human or murine cardiac slices revealed positive ICD staining by ACM-IgGs but not IgGs derived from murine ACM models. Investigation of signaling mechanisms revealed GSK-3β activation by ACM-IgGs. In line with this, GSK-3β inhibition rescued cell adhesion in HL-1 cells and murine ACM cardiac slices. Validation of ACM-IgGs effects in hiPSC-CMs and ACM-iPSC-CMs revealed that ACM-IgGs co-localize with DSG2 and cause loss of cardiomyocyte cohesion. **Conclusion:** Our study reveals that autoantibodies causing activation of GSK-3 β and, thereby, loss of cardiomyocyte cohesion are confined to ACM patients but are absent in HR and murine models of ACM.

Session 037 – Minisymposium – Cancer Biomarkers and Mechanisms

CANCER007

Nanoluciferase-CD63-Labeled Extracellular Vesicle Signaling and Biodistribution in Mouse Intraductal Model of Ductal Carcinoma in situ

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Background: Physiologically relevant models of in situ precancers that can accurately mimic human disease progression are limited. The Mouse INtraDuctal (MIND) model effectively recapitulates ductal carcinoma in situ (DCIS) within its native microenvironment. However, conventional MIND applications rely on histopathological analysis of excised tissues rather than non-invasive in vivo imaging. Furthermore, limited studies have investigated tumor-microenvironment interactions mediated by small extracellular vesicles (sEVs) in models of early cancer progression. Tumor-cell sEVs are implicated in numerous stages of cancer progression and metastasis, rendering them promising for early cancer detection and monitoring. Few studies have examined the temporal and spatial distribution of exosomes in precancerous states, partly due to limitations of sEV-specific labeling techniques. To address this, we utilized nanoluciferase (NLuc), which exhibits greater stability and sensitivity, and intensities approximately 150-fold higher, making it optimal for monitoring sEV dynamics in a model of in situ to invasive breast cancer progression. **Methods:** MCF10DCIS (DCIS) cells were engineered to express

a fusion of CD63, an sEV surface marker, with NLuc (CD63-NLuc). DCIS and CD63-NLuc cells were implanted into the inguinal glands of female mice. DCIS was monitored via bioluminescence imaging (BLI) began 3 days post-implantation (dpi) and continued for 28 days. CD63-NLuc BLI signal was quantified in whole-body and ex vivo imaged organs. The blood plasma and tissue lysates were harvested and assayed for CD63-NLuc activity. Histological assessment of the mammary tissue was performed following hematoxylin and eosin (H&E) and multiplex fluorescent immunohistochemical staining. **Results:** Whole-body BLI demonstrated a time-dependent increase in CD63-NLuc signal, further validated by ex vivo imaging of the mammary glands and proximal organs. Additionally, CD63-NLuc activity was elevated in whole blood plasma and organ extracts, showing a significant positive correlation with both whole-body and ex vivo BLI. Notably, NLuc mRNA was not detected in tissues, indicating that the observed signal originated from sEV-associated CD63-NLuc rather than from migrated CD63-NLuc-expressing cells. Histological analysis confirmed DCIS outgrowth as early as 7 dpi with focal invasion emerging at 14 dpi. **Conclusion:** Collectively, our findings demonstrate a proof-of-concept MIND model for monitoring in situ cancer and its interaction locally and systemically through the release of sEVs. Moreover, we demonstrated that the CD63-NLuc sEV signal exhibits an increase in the mammary glands, circulation, and peripheral and distant organs, correlating with the invasive progression of DCIS. **Acknowledgements:** Funded by a Team Science Grant from the Stephenson Cancer Center/Harold Hamm Diabetes Center and by the Tobacco Settlement Endowment Trust awarded to the Stephenson Cancer Center.

CANCER008

Pancreatic Tail Orthotopic Injections of Murine Pancreatic Adenocarcinoma Reveal Fetal Sex-Effects

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Background: Pancreatic ductal adenocarcinoma (PDAC) has genetic risks and environmental triggers. Variability in mutation penetrance between individuals is likely related to these environmental “hits” and the variance in the host response to cancer. In fact, it is unknown why certain individuals develop *Kras* mutations and why some people with *Kras* mutations never convert to PDAC. Our working hypothesis is the *in utero* environment has a significant impact on the fetal epigenetic programming of the pancreatic ductal epithelium and tumor microenvironment response making the individual more or less susceptible to PDAC during their lifetime. The objective of this study was to test for variance between male and female siblings. **Methods: W**e investigated pancreatic microenvironmental response to murine PDAC tumor cell injection (10^6/30ul) into the pancreatic tail of 12-week-old male and female C57Bl6 mice. Castrated males (performed at 6 weeks of age) served as a control. Animals were then autopsied at day 14 post-injection and tested for sex effects related to tumor size, distribution, and host response identified by review of H&E stained histologic sections post autopsy. **Results:** We observed that male mice grow orthotopic PDAC tumors that are 2x the size of those measured in their female siblings, independent of castration. Histologic analyses revealed more plasma cells in male pancreatic response and more neutrophils and lymphocytes in the female host response. **Conclusion**: This is the first step towards testing for fetal sex and fetal growth-related host responses to pancreatic cancer. The implication may be a new understanding about why males are more likely to develop PDAC than females in the human population.

CANCER009

Exploring the Role of Obesity-Associated Extracellular Matrix in Local Breast Cancer Progression

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Background: Breast cancer is the most prevalent invasive cancer in women. Obesity is a key risk factor implicated in the progression of ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC). Importantly, while breast cancer is often diagnosed as DCIS, it is unknown which lesions will progress to potentially lethal IDC, especially in obesity. The lack of relevant *in vitro* models hinders a mechanistic understanding of obesity's impact on early disease stages when cancer cells invade the local environment. In a retrospective study on human breast specimens, we analyzed gene expression in DCIS and IDC from women with varying BMI. Extracellular matrix (ECM) remodeling and epithelial-to-mesenchymal transition pathways were elevated in obesity associated DCIS compared to other groups, suggesting that obesity-induced ECM alterations promote DCIS progression. Many of the ECM-derived signals are relayed to cancer cells through the YAP pathway. Previous studies in our lab have focused on factors derived from the obese tumor microenvironment that promote breast cancer growth; specifically, estrogen and fibroblast growth factor 1 (Fgf1). Here, we developed both 2D and 3D in

vitro models to explore how obesity-associated extracellular matrix (ECM) remodeling is influenced by estrogen and Fgf1 and how this in turn promotes breast cancer. **Methods:** In the 3D model, ECM was isolated and decellularized from lean and obese mouse mammary glands. Human MCF7 breast cancer cells were cultured in this ECM, and spheroid size and growth rates were analyzed. We performed untargeted mass spectrometry to define the matrix proteome and pathway analysis of enriched networks in lean and obese conditions. In our 2D model, mouse adipose fibroblasts were treated with estrogen (E2) or Fgf1 and allowed to produce ECM for 2 days, followed by decellularization. Human breast cancer cells with and without YAP (MCF7 control or YAP-KO) were seeded on the fibroblast ECM and gene expression profiling as well as morphological analyses were performed. **Results:** Overall, tissue from obese mice had greater levels of most ECM proteins compared to lean mice, indicating a fibrotic environment. MCF7 spheroids grew larger in obese versus lean ECM, displayed a morphology consistent with invasion, and had greater levels of nuclear-localized YAP, which indicates pathway activation. MCF7 YAP KO cells upregulated ECM remodeling genes such as fibronectin (FN), vimentin (VIM), MMP2, MMP9, and LOX compared to control cells, potentially suggesting compensation for changes in ECM-related signaling through YAP. **Conclusion:** Our study highlights a crucial role for obesity-associated ECM remodeling in breast cancer cell growth and DCIS progression and suggests therapeutic potential for targeting ECM in obese breast cancer patients with obesity. **Acknowledgement:** Supported by R01 CA241156.

CANCER010

Histamine Receptor 1 is Expressed in Oropharyngeal Squamous Cell Carcinomas (OPSCC)

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Background: Histamine receptor 1 (HRH1) plays a pivotal role in mediating histamine signaling within cells and regulates several biological processes. While the function of HRH1 in normal physiology is well established, its involvement in cancer remains poorly understood. This study aims to investigate HRH1 expression and function in oropharyngeal squamous cell carcinomas (OPSCC), which ranked as the 7th most common cancer worldwide in 2020 and has a 5-year survival rate of 68.5%. **Methods:** We analyzed transcriptomic data from OPSCC samples in the TCGA database, which includes a diverse cohort of patients in terms of sex, age, and ethnicity (n=520 tumors, n=44 normal tissues). Additionally, we examined RNA sequencing data from individuals with tongue cancer, encompassing both whole tumors (n=26 tumors, n=12 normal tissues) and single-cell samples. An *in vitro* model for tongue cancer, Cal-27 cells, were utilized to measure the impact of histamine signaling on cancer cell phenotypes. Finally, cancer outcomes over time for patients with low and high HRH1 expressing tumors were analyzed. **Results:** Our findings reveal that HRH1 is significantly upregulated in OPSCC tumors compared to normal tissues, with consistent results across all demographics. Notably, HRH1 upregulation was observed in tumors at all stages. Methylation analysis showed decreased methylation of the HRH1 promoter, which correlated with increased HRH1 expression. Furthermore, we found reduced expression of genes involved in histamine degradation in tumors, while genes related to histamine synthesis and transport remained unaffected. HRH1 expression was particularly high in tongue tumors, and single-cell RNA sequencing indicated that HRH1 was primarily expressed in epithelial cells and fibroblasts within these tumors. *In vitro* inhibition of HRH1 signaling in Cal-27 cells with the inhibitor pyrilamine decreased the metabolic activity of the cancer cells, as assessed by resazurin conversion. Importantly, HRH1 expression levels were strongly associated with patient survival outcomes. Patients with low HRH1 expressing tumors had a 90% rate of survival, compared to just 50% for those with high HRH1 expressing tumors at 72 months. **Conclusions:** HRH1 upregulation is linked to poor prognosis in OPSCC, particularly in tongue cancer. HRH1 may serve as both a prognostic biomarker and a potential therapeutic target.
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CANCER011

Improving Neuroendocrine Neoplasm Tissue Diagnostics Using Novel and Existing General Neuroendocrine Cell Markers

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Background: Neuroendocrine neoplasms (NENs) are clinically diverse tumors and cancers that are sometimes challenging to diagnose. microRNAs (miRNAs) are small regulatory RNA molecules that are also excellent biomarkers due to their abundance, specificity, and stability in tissues and biofluids. Because miRNAs can be used to classify cancer, we examined their utility in 14 NEN pathological types and site-matched control tissues (Nanayakkara et al., NAR Cancer 2020). Based on this study, we hypothesized that miRs-375 and -7 are general neuroendocrine markers like Chromogranin A (CGA), Synaptophysin (SYP), Insulinoma-Associated Protein 1 (INSM1), and Yes-Associated Protein (YAP1). Additional general neuroendocrine markers could aid NEN tissue and liquid diagnostics. **Methods:** To assess the diagnostic utility of miRs-

375 and -7 in tissue and plasma, we (i) constructed a tissue microarray (TMA), comprising 122 non-diseased, 62 NEN, and 33 non-NEN cancer tissues from 25 different anatomic sites, and examined the distribution of miRs-375 and -7 and CGA, SYP, INSM1, and YAP1 using chromogenic *in situ* hybridization and immunohistochemical staining, respectively. **Results:** In our TMA study, we found miR-375 and miR-7 positive staining cells in 51 (42%) and 27 (23%) of 121 non-diseased tissues, and 48 (77%) and 38 (61%) of 62 NEN tissues, respectively; staining was absent in all 33 non-NEN cancer tissues. We are currently determining the number and distribution of CGA, SYP, INSM1, and YAP1 staining cells and their overlap with miR-375 and -7 stained cells. **Conclusions:** Our findings indicate that miR-375 and to a lesser extent miR-7 are general neuroendocrine markers that can be used to detect neuroendocrine cells and neoplasms in tissue. We are currently evaluating these microRNA markers in a wide range of other NEN and non-NEN tissues, including from persons with gastrointestinal and pancreatic NENs. This work will help to establish rapid, simple, and inexpensive NEN diagnostic tests that enable accurate diagnosis and accelerate time-to-treatment. **Acknowledgements:** Cancer Research Society.

CANCER012

Loss of Hepatic Tuberous Sclerosis 1(TSC1) Promotes Liver Cystogenesis and Tumorigenesis Via a Non-canonical mTORC1-TFEB-dependent Mechanism

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Introduction: The integration of various environmental cues by mTORC1 is crucial in regulating cell growth and metabolism. Activation of mTORC1 occurs when amino acids stimulate Rag GTPase heterodimeric complexes at the lysosomal surface. In mice, the loss of *Tsc1* leads to the activation of mTORC1, increased ductular reaction (DR), cystogenesis, and spontaneous liver tumorigenesis. While mTORC1-mediated phosphorylation inactivates Transcription factor EB (TFEB), a master transcription factor regulating gene expression of autophagy and lysosomal biogenesis, emerging evidence highlights that TFEB is dephosphorylated and hyper-activated in TSC deficiency-associated kidney cystogenesis and cancer. The purpose of this study was to investigate whether and how TFEB regulates liver cystogenesis and tumorigenesis in L-*Tsc1* KO mice. **Methods:** *Tsc1* Flox/Flox and *Tfeb* Flox/Flox mice were crossed with albumin Cre mice to generate liver-specific *Tsc1* knockout (L-*Tsc1* KO) and L-*Tsc1/Tfeb* double KO (DKO) mice. Additionally, a liver-specific *Tfeb* knockin (L-Tfeb-KI) mouse was created. These mice were housed for various time points up to 12 months, and biochemical and histological analysis was conducted on blood and liver tissues. We further investigated the mechanism and role of Rag-GTPase in TFEB activation by overexpressing RagC WT and Constative Active RagC in *Tsc1* KO mouse livers. **Results:** In L-*Tsc1* KO mice, levels of phosphorylated S6 and 4EBP1 increased, indicating the activation of mTORC1. Surprisingly, L-*Tsc1* KO mice had decreased levels of phosphorylated TFEB with increased TFEB nuclear translocation resulting in increased expression of lysosomal biogenesis genes. L-*Tsc1* KO had increased YAP1 activation, decreased HFN4α, and increased Rag A/C proteins in mice, which were all blunted by deletion of TFEB. L-*Tsc1* KO mice also developed liver cysts, hepatocellular carcinoma, and cholangiocarcinoma when they were aged 8-12-months old. Deletion of *Tfeb* in L-*Tsc1* KO mice markedly also improved liver cyst formation and decreased the tumor burden. Moreover, L-*Tfeb*-KI mice had increased DR, hepatocyte degeneration, fibrosis at 2-months-old, and developed polycystic liver diseases and cholangiocarcinoma-like phenotypes at 8-months-old, reminiscent but worsened phenotypes of L-Tsc1 KO mice. Mechanistically, overexpression of constitutive active form but not WT RagC inhibited TFEB nuclear translocation and activation. **Conclusions:** Our findings reveal a non-canonical mTORC1 pathway that promotes TFEB activation leading to hepatocyte degeneration, increased DR, cystogenesis, and liver tumorigenesis. Selective targeting of this non-canonical mTORC1-TFEB activation may be a promising approach for polycystic liver disease and cholangiocarcinoma mediated by *TSC1* mutations. **Acknowledgement:** Funding support: R01AA031230.

POSTER PRESENTATIONS

Cancer Pathobiology

Poster Board 1

CANCER013

Angiotensin II and Estrogen as Key Players in Sustaining Glioblastoma Stemness, Growth, and Progression

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Background: Glioblastoma (GBM) is an aggressive brain tumor with a poor prognosis (9-12 months), driven by high cellular heterogeneity, including a subpopulation of stemness cells, which contributes to the resistance to therapy. Recent evidence indicates how Angiotensin II/AGTR1 signaling, hallmark of different malignancies, is also involved in GBM progression. Our previous finding showed AngII promotes tumor progression by enhancing local estrogen production via aromatase

upregulation. Thus, the present study explores how AngII may influence estrogen signaling, and its interaction with key oncogenic pathways, including PI3K/AKT and Hedgehog (HH). This study aims to elucidate the molecular mechanisms linking AngII to GBM progression and evaluate the therapeutic potential of anti-estrogen therapy. **Methods:** Human GBM cell lines (U87MG) were treated with AngII, estrogen (E2), and the selective ER antagonist ICI 182,782 (ICI). Transcriptomic analysis and MetaCore software identified key KEGG pathways. Gene and protein expression analyses were assess using RT-PCR, western blot, and immunofluorescence (IF) assays to evaluate PI3K/AKT and HH (GLI1) activation. Neurosphere formation (NF) assays were conducted to assess Ang II's impact on GBM stemness. Clinical database was used to correlate ESR1 and GLI1 in patient samples. **Results:** Transcriptomic analysis of U-87MG cells treated with AngII revealed activation of the HH pathway, crucial for maintaining tumor stem phenotype, indicated by increased levels of its effector, GLI1, and other stemness markers. To evaluate the therapeutic potential of estrogen signaling inhibition, GBM cells were treated with ICI. The results showed inhibition of the PI3K/AKT pathway, involved in non-genomic estrogen signaling, confirmed by using the PI3K inhibitor, LY294002. These findings were supported by reduced GLI1 expression and AKT phosphorylation after AngII/ICI treatment, confirming the involvement of the non-canonical HH pathway. It was investigated AngII's intrinsic ability to induce NF, which was drastically increased. This effect was reversed by ICI, with a downregulation of HH pathway. IF revealed increased nuclear colocalization of GLI1 and ER, upon AngII treatment. In vivo studies confirmed downregulation of stem markers, and marked tumor size reduction, following combined AngII/ICI treatment. Kaplan-Meier of TCGA data showed that high GLI1 and ESR1 levels correlate with poorer survival in GBM patients. Both TCGA and CGGA clinical datasets displayed increased GLI1 expression with severity grade of astrocytoma malignancy. **Conclusion:** This study identifies a novel AngII-estrogen-PI3K/AKT-Hedgehog axis in GBM, where anti-estrogen therapy significantly reduces tumor aggressiveness, suggesting ICI as a potential repurposed drug for GBM adjuvant therapy. **Acknowledgements:** Associazione Italiana Ricerca sul Cancro, Investigator Grant IG-26246; University of Calabria and MIUR Excellence Department Project funds (L.232/2016).

Poster Board 2
CANCER014
The Impact of Tumor Burden on Skeletal Muscle Function in Tumor-Bearing Mice and the Therapeutic Role of Physical Activity
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Background: Cancer cachexia is a wasting disorder characterized by degradation of lean and fat mass. Muscle atrophy occurs as a result of excessive muscle protein degradation. Physical activity is a known regulator of protein metabolism and turnover and therefore serves a potential treatment strategy for cancer-induced muscle wasting. Cancer rehabilitation aims to improve quality of life by relieving and reducing cancer- and cancer treatment-related side effects. Therefore, the purpose of this study was to determine if physical activity slows muscle wasting to preserve muscle function, and to determine the underlying mechanisms responsible for muscle preservation. **Methods:** Male LC3Tg+ and wildtype mice were randomly divided into four groups: sedentary non-tumor bearing mice (SED + NT), sedentary tumor-bearing mice (SED + T), wheel-running non-tumor bearing mice (WR + NT), and wheel-running tumor-bearing mice (WR + T). Mice were implanted with 5x10^5 LLC tumor cells or remained non-tumor for four weeks. The WR groups had access to a running wheel and sedentary mice were restricted to standard mouse cage activity. Body metrics and skeletal muscle function (grip strength) were measured at baseline and at the end of the four-week study. Skeletal muscle tissue was collected to investigate muscle wasting mechanisms. **Results:** Tumor bearing resulted in significant muscle weakness. At the end of the four-week protocol, tumor-bearing groups (SED + T) exhibited significant skeletal muscle weakness (lower grip strength) compared to their non-tumor counterparts (SED+NT). This coincided with evidence of muscle atrophy (lower relative gastrocnemius mass) in tumor groups vs non-tumor groups. Similarly, MuRF1 and GDF-15 were elevated in the mixed fiber gastrocnemius skeletal muscle compared to non-tumor groups, indicating muscle atrophy signaling pathways are upregulated during tumor-bearing. Interestingly, muscle strength was rescued in the wheel-run group (WR + T), indicating physical activity concurrent with tumor-bearing is capable of preserving muscle function. This protective effect of physical activity appears to be related to its ability to downregulate muscle atrophy signaling since WR groups exhibited lower levels of MuRF1 and GDF-15. **Conclusion:** Tumor bearing results in significant muscle atrophy, weakness, and is driven by muscle atrophy signaling through GDF-15/ MuRF1 axis. Physical activity reduces excessive muscle protein catabolism via regulation of GDF-15/MuRF1 signaling, thus preserving muscle mass and function. Therefore, physical activity may play a crucial role in cancer rehabilitation, helping to maintain muscle mass and function and overall physical function.

Poster Board 3
CANCER015
Mechanistic Study of NIFK-mediated Modulation on Lung Tumor Microenvironment
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Background: Nucleolar protein interacting with the forkhead-associated (FHA) domain of pKi 67 (NIFK) encoded by MKI67IP gene is a nucleolar and cytoplasmic protein that interacts with Ki-67. We previously published NIFK's clinical significance and biological functions in promoting lung cancer cell proliferation and metastasis. However, NIFK's function and regulatory mechanism in lung cancer progression remains largely unknown, especially regarding to the induction of angiogenesis in tumor microenvironment. **Methods:** An animal model-based single cell RNA sequencing (scRNA-Seq) and RNA immunoprecipitation (RIP) analysis were established using A549 cells. Immunohistochemistry (IHC) staining was performed to explore the relative NIFK expression levels in lung cancer specimens and adjacent normal tissues. Transcriptomics data was retrieved from the public database The Cancer Genome Atlas (TCGA) to further confirm MKI67IP's clinical significances conducted via the analysis of medical informatics. Cell migration ability of cancer cells was evaluated by transwell assay. **Results:** An animal model-based scRNA-Seq using A549 cells showed the high vascular endothelial growth factor A, B and C (VEGFA, VEGFB and VEGFC) and SYNE2 expressions in the cell cluster with high NIFK level. Furthermore, the induction of tubular-like structure formation in endothelial cells *in vitro* and the increased CD31 and VEGFA levels *in vivo* were observed upon NIFK overexpression in A549 cells. NIFK has also been proposed as an RNA binding protein. However, the comprehensive analysis has not yet been reported. Several direct interactive RNA targets of NIFK were identified by RIP and RNA-Seq analysis. SYNE2 was one of interactive targets with prognostic significance in a transcriptomic analysis. scRNA-Seq analysis identified the concurrently high SYNE2 and NIFK expression levels in cell cluster 2 in tumor microenvironment mimic. **Conclusions:** Hence, the results indicate the modulatory role of NIFK in regulating lung cancer progression via inducing angiogenesis, and suggest the molecular mechanism of NIFK-Nesprin-2 (SYNE2)-VEGFA/B/C axis in A549 lung cancer cells. **Acknowledgements:** We thank for the travel fund supported by Chang Gung Memorial Hospital, Taiwan.

Poster Board 4
CANCER016
Monoclonal Antibody Inhibits 17β-Estradiol-induced Overexpression of α6 Integrin Subunit in ERα-positive Breast Cancer Cells
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Background: Breast cancer (BC) is one of the most prevalent malignancies worldwide, significantly affecting mortality rates. However, recent advancements in early detection and treatment are improving efficacy and survival outcomes. Targeted therapy, utilizing monoclonal antibodies (mAbs), has become a key strategy in breast cancer management, offering a precise approach that targets specific molecular alterations in cancer cells, thus enhancing treatment effectiveness and minimizing off-target effects on healthy tissues. Recent studies evidenced the pivotal role of integrins in cancer progression, driven by their ability to regulate cellular responses to the tumor microenvironment via "inside-out" and "outside-in" signaling. In BC, α6integrin (ITGA6) overexpression linked to poor prognosis and reduced survival, promoting increased motility and resistance to standard therapies. All of this makes ITGA6 a promising candidate for therapeutic targeting. Although ER is expressed in 70% of all BCs, it remains unclear if estrogen signaling may influence ITGA6 levels. Thus, the aim of this study was to assess the estrogens impact on ITGA6 expression and to explore the potential therapeutic role of mAbs targeting this protein in ERα-positive BC cells. **Methods:** MCF-7 cells were treated with 17β-Estradiol (E2), anti-estrogen ICI-182,780 (ICI) and mAb anti-ITGA6 (MAB) alone or in combination. qRT-PCR and Western Blotting assays to evaluate mRNA and protein expression. Luciferase assay to assess promoter activity. Quantitative cell adhesion assay to determine the MAB minimum effective dose. Cycloheximide (CHX)-chase analysis to evaluate ITGA6 turnover and stability. Metastatic potential of breast cancer cells was assessed *in vivo* by using a tail vein injection into female 6-week-old athymic nude mice. **Results:** Our findings revealed in MCF-7 cells a significant E2-induced increase in ITGA6 mRNA and protein levels, as supported by the enhanced ITGA6 promoter activation, which was reversed by ICI, highlighting the critical role of ERα in mediating ITGA6 upregulation. Interestingly, the strong ITGA6 increase induced by E2 was no longer noticeable in the presence of MAB. It was shown that the MAB downregulatory effect was abolished following CHX treatment, addressing the crucial role of the proteasomal activity on the complex MAB/ITGA6. The efficacy of MAB in counteract ITGA6 activity was confirmed by immunohistochemistry, showing a lower metastatic burden in mice injected with MCF-7 cells and treated with intraperitoneal

injection of MAB compared to those treated with E2 alone. **Conclusions:** Our data candidate ITGA6 as a potential target to reduce metastatic spread, exploitable in the novel therapeutic strategies for BC treatment. **Acknowledgements:** Associazione Italiana Ricerca sul Cancro, IG-26246; PON Salute ARS01_00568 SI.F.I.PA.CRO.DE.; Italian Minister of University and Research (D.D. n.407/2018)-PON R&I 2014-2020, AIM "Attraction and International Mobility."

Poster Board 5
CANCER017
The Anti-Inflammatory and Cardioprotective Effects of Exercise Preconditioning in the Tumor-Bearing Male Mouse
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Background: Cancer incidence continues to rise, reaching nearly 2 million new cases per year. Cancer cachexia, a muscle and fat wasting disorder, affects up to 80% of cancer patients. Severe muscle wasting from cancer cachexia decreases anti-cancer treatment efficacy, lowering long term survival. While exercise has been shown to be beneficial for cancer survivors, less is known about the most beneficial timing of exercise within the cancer continuum nor the underlying beneficial mechanisms of exercise. Therefore, the purpose of this study was to determine whether exercise *prior* to tumor bearing (i.e., preconditioning) could offer cardioprotection during tumor bearing. **Methods**: Male mice were separated into groups: sedentary non-tumor (SED+NT), sedentary tumor (SED+T), treadmill non-tumor (TM+NT), and treadmill tumor (TM+T). Mice underwent an 8-week progressive treadmill exercise preconditioning intervention (TM) or remained SED. At the end of the preconditioned TM intervention, all groups remained sedentary for 4 weeks. During this 4-week sedentary period, mice were implanted with tumor cells (T group; 5x10^5 LLC cells in flank) or remained non-tumor (NT). Cardiac function was followed throughout the 12-week study and hearts were probed for potential protective mechanisms. **Results**: Tumor-bearing resulted in significant cardiac dysfunction and wasting. Interestingly, aerobic exercise initiated *prior* to tumor-bearing protected against tumor-mediated cardiac dysfunction and inflammation. SED+T exhibited significantly worse cardiac function compared to preconditioned TM+T mice (fractional shortening – SED+T: 36% vs TM+T: 42%; $P<0.05$). This coincided with evidence of significant body wasting (decreased body mass and muscle mass) in SED+T that was not observed in TM+T. In addition, pathways associated with inflammation (GDF-8/11, GDF-15, TGFβ, p-NF-κB) and proteolysis (MuRF1) were significantly upregulated in SED+T hearts compared to TM+T, indicating a protective effect of preconditioning aerobic exercise. **Conclusion**: Exercise initiated *prior* to tumor bearing elicits cardioprotective effects – even after exercise has ceased – to protect against tumor-mediated cardiac dysfunction and proteolysis. These data make an important case for the long-term benefits of an active lifestyle that last even after exercise is stopped (i.e., upon cancer diagnosis and treatment). Such information is critical in determining the safe and effective implementation of exercise in the clinical setting.

Poster Board 6
CANCER018
Investigating the Neural Progression of Pancreatic Ductal Adenocarcinoma
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Background: Within the tumor microenvironment, cancer cells co-opt normal cells to aid in their progression and dissemination. As cancer cells grow, their demand for nutrients and oxygen follows suit and subsequent hypoxic conditions stimulate pro-angiogenic pathways to recruit new blood vessels toward the tumor microenvironment. These secreted factors similarly induce axonogenesis from surrounding ganglia. High axon density surrounding tumors (close proximity of tumor cells and neurons) has been linked to high metastatic potential and poor prognosis. The molecular mechanisms mediating paracrine relations between cancer cells and neurons remain largely unknown. Therefore, our primary goal is to investigate the mechanisms mediating axonogenesis with the aim of targeting and inhibiting the aggressive cancer phenotype in pancreatic ductal adenocarcinoma (PDAC). We hypothesize that PDAC-stimulated axonogenesis is dependent on Neuropilin-2 (Nrp2), a receptor expressed in endothelial cells and sympathetic and sensory neurons. Furthermore, we propose that Semaphorin-3F (SEMA3F) protein, a ligand of NRP2, may represent a novel therapeutic approach to repel Nrp2-expressing neurons away from PDAC tumors. **Methods:** To test our hypothesis, syngeneic PDAC and melanoma isografts were implanted in wildtype and *Nrp2*-null mice and tumorigenicity was compared. Tumor associated axonal density was analyzed by immunohistochemistry (IHC). Frozen and paraffin-embedded PDAC and melanoma samples were utilized in histologic analysis using several neuronal specific antibodies to determine the optimal markers for staining Nrp2-expressing axons in pancreatic tissue. The inhibitory and chemorepulsive effects of SEMA3F were tested *in vitro* in neuronal and endothelial cells co-cultured with cells stably transfected with SEMA3F or treated with exogenous SEMA3F. In vivo, human melanoma xenografts overexpressing SEMA3F were implanted in immunodeficient mice. Neurogenesis was compared histologically between control and SEMA3F-expressing tumors. **Results:** PDAC and melanoma tumorigenicity

was reduced in *Nrp2*-deficient mice compared to littermate controls. Using IHC staining with Protein Gene Product 9.5 (Pgp9.5), nerves were found within and surrounding PDAC tumors implanted orthotopically in the pancreas of syngeneic mice. In vitro, both neuronal and endothelial cells expressing NRP2 are repelled or inhibited in sprouting by the addition of SEMA3F. SEMA3F-secreting tumor xenografts showed reduced angiogenesis and axonogenesis compared to controls. **Conclusions:** Overall, this study suggests that PDAC tumors display significant axonal density in the tumor microenvironment and that SEMA3F diminishes NRP2-dependent angiogenesis and axonogenesis, thus reducing tumorigenicity in this aggressive cancer. **Acknowledgements:** Funding support from the Vascular Biology Program at Boston Children's Hospital (DRB), the American Society for Investigative Pathology Summer Research Opportunity Program in Pathology (CR), the Harvard College Research Program (CR), and the Dana-Farber Harvard Cancer Center CURE Program (AN).

Poster Board 7
CANCER019
Understanding the Crosstalk Between Neuropeptide Receptor RAMP1 in Tumor-infiltrating Pericytes in Melanoma Development
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Background: Melanoma is the most lethal form of skin cancer, and its incidence is rising. Current therapeutic approaches are often insufficient to achieve durable responses. Thus, efforts to develop more effective therapies are likely to increase. Recent reports from our laboratory revealed that cancer and the nervous system bear an entangled relationship. Melanoma-infiltrating nerves impact tumor progression, but the mechanisms remain poorly understood. Neuropeptides when released from sensory neuron endings communicate with other cell types via neuropeptide receptors. One of these neuropeptides is calcitonin gene-related peptide (CGRP) which binds to the receptor activity modifying protein 1 site (RAMP 1) selectively. We previously found that certain stromal cells, specifically the pericytes (expressing marker protein PDGFRβ), that infiltrate the melanoma metastatic microenvironment have elevated expression of the RAMP1 gene than normal pericytes. Here, we aim to evaluate the mechanism of CGRP-RAMP1 signaling in cancer-associated pericyte invitro. **Methods:** To evaluate the role of CGRP on pericyte invitro we cultured immortal lung pericytes in a conditioned media form D4M3 (cultured melanoma cells). We were treated with either CGRP, BIBN (a RAMP1 receptor antagonist) alone or BINB combined with CGRP. We performed FACS to quantify the proliferation (Ki67 staining) in these cells. Levels of cAMP by ELISA and % of phosphorylated-PKA by western blotting were measured to see the impact of CGRP action and antagonism on signaling pathways in cultured pericytes. Further to understand the functional impact of CGRP and blocking CGRP -RAMP1 signaling in cultured pericytes conditioned with melanoma cells we performed in vitro Matrigel angiogenesis assay in HUVEC cells using standard protocol. **Results:** The in vitro experiment revealed that pericytes cultured in melanoma cells conditioned media along with CGRP have higher cAMP levels leading to phosphorylation of PKA. This was reversed with the addition of RAMP1 antagonist, BIBN. CGRP increases the proliferation and angiogenic capacity of pericytes and pharmacological inhibition of CGRP/RAMP1 signaling by BIBN reduced tube formation as well in HUVEC cells. The preliminary data suggests that RAMP1 signaling is active in melanoma-conditioned pericytes. **Conclusions:** Our study has revealed the importance of RAMP1 signaling in melanoma-associated pericytes. Current and future work includes identifying mechanisms of RAMP1 signaling to identify novel strategies for the clinical management of melanoma modulating RAMP1 signaling to obtain superior and lasting anti-melanoma responses.

Poster Board 8
CANCER020
Myc Oncogenicity Varies Between FVB and C57BL/6 Mice
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Background: In our previous study, we found strain-specific differences in susceptibility to Myc-induced hepatocellular carcinoma (HCC), which became more pronounced when combined with secondary oncogenes, including NICD, Yap S127A, and NRas. Tumorigenesis was generally more robust in FVB mice than in C57BL/6 mice, though the phenotyping of tumors induced by different oncogene combinations remained consistent across both strains. We hypothesized that the genetic background of different mouse strains plays a critical role in Myc-driven tumorigenesis. **Methods:** To investigate how genetic background influences tumorigenesis across different models, we performed bulk RNA sequencing (RNA-seq) using normal mice of different strains as controls. Additionally, we conducted crossbreeding between FVB and C57BL/6

mice to explore genetic risk factors associated with Myc-induced tumor development. Hydrodynamic tail vein injection (HTDVI) with Sleeping Beauty transposons was used to deliver Myc alone or in combination with Yap S127A, NRas, or NICD into liver hepatocytes. **Results:** RNA-seq analysis revealed differentially expressed genes among models, associated with single nucleotide polymorphisms (SNPs) in different strains. However, no significant impact on tumorigenesis was observed in Myc-Yap S127A, Myc-NRas, or Myc-NICD models. Interestingly, in the F1 generation from C57BL/6 × FVB (male × female) crosses, Myc alone induced HCC in female mice but not in males, approximately 10 weeks post-HTDVI. In contrast, small tumor nodules were observed in 1 of 4 F1 males from FVB × C57BL/6 (male × female) breeding. In the F2 generation, Myc did not induce tumors in either males or females from C57BL/6 × FVB (male × female) crosses at 10 weeks post-injection. However, in F2 offspring from FVB × C57BL/6 (male × female) breeding, Myc-induced HCC was observed in 1 of 4 males and 2 of 5 females, suggesting a complex inheritance pattern affecting tumor susceptibility. These findings indicate that genetic background influences Myc-driven tumorigenesis and suggest the presence of strain-specific genetic modifiers. **Conclusions:** We are continuing crossbreeding to identify a Myc-alone-induced HCC-prone genetic program. Additionally, we will perform whole-genome sequencing and integrate RNA-seq data from our existing HCC models to pinpoint potential genetic factors involved in Myc-driven tumorigenesis.

Poster Board 9
CANCER021
Obesity and Breast Cancer – The Potential Role of Sulfatase 2
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Background: Women with obesity have a higher risk of developing invasive ductal carcinoma (IDC) after a ductal carcinoma in situ (DCIS) diagnosis. Current pathological and clinical parameters cannot accurately predict disease progression. Evidence suggests circulating biomolecules, tumor microenvironment modifications, and extracellular matrix remodeling contribute to this progression. Extracellular sulfatase 2 (SULF2) de-sulfates heparan sulfate proteoglycans, facilitating growth factor-receptor interactions that activate pro-tumorigenic signaling pathways in cancer. Gene expression datasets identified SULF2 as highly differentially expressed between DCIS and IDC in human tissues and genetic mouse models of breast cancer. Other studies show SULF2 is associated with diet-induced obesity in rodent models. This study aimed to characterize SULF2's role in DCIS to IDC progression and the impact of obesity. **Methods:** Spatial transcriptomics characterized molecular profiles linked to normal (<30kg/m^2) and high (>30kg/m^2) BMI in DCIS and IDC breast tissues in cytokeratin (CK), smooth muscle actin (SMA), and CD45 (immune) positive cells. DCIS progression was assessed in lean and obese female mice following intraductal mammary gland implantation of MCF10DCIS cells. Histological analysis used H&E and immunohistochemistry. SULF2 expression was modulated in MCF10A and DCIS cells in gain- and loss-of-function models. SULF2's effect on cytokine release was assessed by cytokine array and macrophage polarization by incubating RAW246.7 macrophages with conditioned medium from SULF2 models. **Results:** Elevated SULF2 expression was observed in CK+ luminal epithelial cells and increased M2 macrophages in tissues from high BMI patients with DCIS and IDC compared to normal BMI patients. Obese mice had a higher incidence of IDC compared to lean counterparts. SULF2 expression correlated with elevated levels of cytokines and growth factors involved in inflammatory responses, and the activation and polarization of macrophages to anti-inflammatory M2 phenotype. **Conclusion:** Our findings suggest obesity contributes to DCIS progression to IDC, potentially through increased SULF2 expression that may contribute to pro-tumor macrophage polarization. Elevated SULF2 levels in obese patients and mice correlated with increased inflammatory cytokines, growth factors, and macrophage polarization, highlighting its potential as a biomarker for disease progression. These results provide insights into molecular mechanisms linking obesity and breast cancer progression, emphasizing the need for further research into SULF2 as a therapeutic target. **Acknowledgements:** This project was supported by a Team Science Grant from the Stephenson Cancer Center/Harold Hamm Diabetes Center.

Cardiovascular Pathobiology

Poster Board 10
CARDIOVAS009
KLF2 Expression Regulates Human Endothelial Cell Size and Morphology
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Background: Atherosclerosis develops in regions of blood vessels exposed to disturbed flow, such as bifurcations or curves, where endothelial cells (ECs) exhibit altered gene expression and cuboidal morphology. Suppression of Kruppel-like factor 2 (KLF2), a transcription factor critical for EC physiology, is a hallmark of these regions. Endothelial cell size, which influences vessel diameter, perfusion, and vascular responses, is also affected by flow conditions. Although KLF2's role in regulating EC size has been demonstrated in zebrafish, its effects on human EC size and morphology, as well as its downstream targets, remain poorly understood. **Methods:** To investigate, we conducted a gain-of-function study using human umbilical vein endothelial cells (HUVECs), transduced with murine KLF2 (mKLF2) or GFP-only control adenoviral vectors and cultured in EGM-2 under static conditions. Cells were transduced for 48 hours before being either lysed for quantitative PCR (qPCR) or fixed for immunofluorescence imaging using antibodies against VE-cadherin. Images were processed and features quantified using iLastik and Fiji. **Results:** Post-transduction, Ad-mKLF2 cells were 22% larger in surface area than controls (95% CI: 15%-26%) and exhibited a more elongated morphology. While control cells proliferated, increasing in number by 29% (95% CI: 9%-48%), mKLF2 cells did not divide, instead prioritizing growth. Nuclear size remained unchanged between groups; thus, senescence or DNA damage were not reasons for lack of proliferation. Importantly, AQP1, a water channel protein and potential KLF2 downstream target, was significantly upregulated in Ad-mKLF2 cells, suggesting its involvement in size regulation. We further explored the relationship between KLF2 expression and EC size by correlating GFP fluorescence intensity with cell area in individual mKLF2-expressing cells. A positive correlation was observed, supporting the hypothesis that KLF2 directly influences cell size. Morphological analysis revealed that KLF2-expressing cells partially regained the elongated shape typical of ECs under laminar flow, suggesting restoration of vascular homeostasis. Notably, these elongated cells are associated with improved barrier function, better alignment to shear stress, and other hallmarks of healthy endothelium. **Conclusions:** These findings establish KLF2 as a key regulator of EC size and morphology, processes integral to vascular function and integrity. While AQP1 appears to contribute, further studies are needed to identify additional KLF2-regulated pathways involved in endothelial cell size determination and their therapeutic potential in combating atherosclerosis. This work provides foundational insights into the control of cell size in the context of vascular health. **Acknowledgements:** This was funded by the NIH grant 5R01HL161090-03. We thank Jesús Ortiz Urbina, Jules Allbritton-King, Adriana Blazeski, and Yuzhi Zhang for their feedback.

Poster Board 11
CARDIOVAS010
Myocardial Renin Expression in a Rat Model of Fat Embolism
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Introduction: Fat embolism was first identified in 1862, primarily caused by the release of fat droplets from bone marrow due to bone fractures. This condition leads to severe inflammatory reactions in the lungs, associated with a significant increase in renin levels. Various animal models have been utilized for studying fat embolism; notably, the intravenous injection of Triolein fat droplets is commonly used, especially in rats. In our recent study, we observed that unlike the lungs of Triolein-injected rats—where severe pulmonary vasculitis and septal inflammation, along with elevated renin levels, were evident—the hearts of the same animals did not exhibit severe vascular or septal inflammation, and the presence of fat droplets was limited. This study aims to evaluate the differential effects of Triolein on heart and lung damage, particularly regarding heart renin expression. **Methods:** Twelve male Sprague-Dawley rats (250-350 g) from Harlan Laboratories were randomly divided into two groups: six received 0.2 mL of saline and six received 0.2 mL of triolein. After ten weeks, the rats were euthanized using isoflurane anesthesia. Hearts and lungs were extracted, with 50% of each organ fixed in formaldehyde for histopathological analysis and the other 50% frozen for renin staining. The formaldehyde-fixed heart portions were embedded in paraffin and stained with hematoxylin and eosin (H&E). Lumen patency and media-adventitia ratios were measured in the heart and lung arteries. For renin staining, sections were blocked with 10% heat-inactivated goat serum, washed, and incubated with a biotinylated secondary antibody. After rinsing, they were treated with Vector ABC reagent and stained with 3,3'-diaminobenzidine. Finally, the slides were mounted in Permount and visualized using an upright light microscope. **Results:** The analysis of lumen patency revealed no significant statistical difference between saline-injected and Triolein-injected animals (p=0.4528). Similarly, media-adventitia ratios showed no significant differences (p=0.1237). The percentage of trichrome staining also did not show statistical significance between the two groups (p=0.453). Regarding renin staining, the average percentage for saline-injected rats was 44.1%, compared to 49.2% for Triolein-injected rats (p=0.294). The optical density for saline was 0.390, while for Triolein-injected rats it was 0.374 (p=0.5675). **Conclusion:** Experiments showed that Triolein-injected animals had no significant increase in fat droplets or renin staining in their hearts and no histopathological damage like vasculitis or fibrosis. In contrast, the lungs of these rats

exhibited severe histopathology and elevated renin levels. These results suggest that the renin-angiotensin system may contribute to organ damage due to fat embolism.

Poster Board 12
CARDIOVAS011
Investigating Associations Between the Gut and the Heart in PTSD-like Mice
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Background: Recent studies exhibit a strong connection between cardiovascular physiology and intestinal health, linking the gut microbiome to cardiovascular disease. Post-traumatic stress disorder (PTSD) is associated with increased risk for cardiovascular disease, but the mechanism is unclear. Furthermore, whether the gut plays a role in PTSD-induced cardiovascular pathology has largely not been studied. We hypothesize that PTSD-induced changes to the gut microbiome stimulate a systemic pro-inflammatory response and induce adverse cardiac remodeling**. Methods:** To induce experimental PTSD, male and female C57BL/6 mice (n=3-6/group) were placed in chambers where they were exposed to 5 separate foot-shock incidences (IFS; 1.0 mA, 1 sec duration) in 6 min. Behavioral testing designed to mimic the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) was performed to characterize mice as non-responders (NR; do not demonstrate PTSD-like characteristics) and PTSD-like. Control mice were also placed in IFS chambers for the same duration but without foot shocks. Echocardiography was performed at 8-weeks post-IFS to visualize cardiac function. Stool pellets were collected at baseline, 4-weeks and 8-weeks post-IFS, and gDNA was isolated and accessed via qPCR, to identify the contribution of the gut microbiota. Plasma and intestines were collected to measure circulating inflammation and changes in gut architecture, respectively. **Results:** Echocardiography demonstrated PTSD mice had elevated isovolumetric relaxation time compared to controls (p=0.0021) at 8 weeks post-IFS, suggestive of impaired filling. Additionally, female NR and PTSD mice had increased circulating inflammatory cytokines including interferon gamma (IFNy) and monocyte chemoattractant protein-1 (MCP-1) compared to controls. Using qpcr, no significant differences were observed at the phyla levels, but a trend towards decreased abundance of the commensal genera *Enterococcus* was observed in male PTSD mice compared to controls. These data indicate that there are subtle changes in the gut microbiome composition of male PTSD mice. To assess whether changes in the gut microbiome were reflected by changes in gut architecture, we examined histological staining of the small and large intestine. H&E staining revealed fewer mucus producing goblet cells in the colon of PTSD mice compared to controls and NR which was confirmed by alcian blue staining. **Conclusions:** These data indicate that PTSD mice have alterations in the gut structure which may be facilitating in impaired diastolic dysfunction. **Acknowledgements**: This work was supported by the National Institutes of Health R25GM072643, T32GM1523862, F31HL170740, T32GM123055, HL173273; the American Heart Association 24PRE1188095; the Biomedical Laboratory Research and Development Service of the Veterans Affairs Office of Research and Development Award I01BX00584; and South Carolina Translational Research Center UL1TR001450.

Poster Board 13
CARDIOVAS012
Unveiling the Dynamic Role of Cardiac Fibroblasts in Remodeling, Repair and Reverse Fibrosis
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Background: Cardiovascular disease inflicts a significant role on not only patients' health and wellbeing, but also healthcare costs worldwide. Following a cardiac event, many patients have long-lasting complications, one of which being an increase in extracellular matrix deposition which results in cardiac fibrosis. One in five people who have had a heart attack will suffer a second one within five years resulting in further cardiac remodeling with increased risk of mortality. A major component of cardiovascular disease is cardiac fibrosis which ultimately leads to decreased cardiac function. Chronic fibrosis in cardiac tissue is considered irreversible. Currently there are no therapeutic treatments to directly attenuate or reverse fibrosis. Recent studies using animal models and cell culture techniques have challenged the presumed irreversibility of cardiac

fibrosis. Activated cardiac fibroblasts have been identified as a potential therapeutic target in treating cardiac fibrosis. **Methods and Results:** Our lab has demonstrated that an activated fibroblast lineage is maintained after fibrosis resolution in the heart in a reversible ventricular fibrosis model of neuroendocrine stress. Increase tissue stiffness, a direct result of fibrotic tissue production, is a known key modulator of fibroblast activated phenotype. We have experimental evidence of phenotypic switching between an active and deactivated state when naïve fibroblasts are cultured on either rigid of soft substrates *in vivo*. **Conclusions:** By utilizing both experimental models, we can begin to unravel the molecular and cellular mechanisms defining the role of fibroblasts in chronic cardiac fibrosis, as well as reverse remodeling and resolution of cardiac fibrosis, which remain largely unknown.

Poster Board 14
CARDIOVAS013
***O*-GlcNAcylation of Runx2 Promotes Vascular Calcification**
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Background: Vascular calcification is prevalent in patients with diabetes mellitus, increasing the risk of cardiovascular morbidity and mortality. Our studies have demonstrated that elevated protein *O*-GlcNAcylation is associated with increased vascular calcification in diabetes. The present studies sought to uncover the causative link and the underlying molecular mechanisms. **Methods:** Human coronary artery specimens from diabetic subjects were used to monitor *O*-GlcNAcylation in vascular calcification by immunostaining. To determine a definitive role of *O*-GlcNAcylation in regulating vascular calcification, we generated a new mouse model with inducible smooth muscle cell (SMC)-specific deletion of *O*-GlcNAc transferase (OGT), the enzyme that adds *O*-GlcNAc onto proteins. Animals were exposed to low dose streptozotocin (STZ) to induce diabetes, resulting aortic calcification was quantified by Arsenazo III assay and aortic function was characterized by echocardiography. **Results:** Elevation of protein *O*-GlcNAcylation was evident in calcified lesions in human diabetic arteries, which was co-localized with increased immunostaining of Runx2, the master osteogenic transcription factor. SMC-specific OGT deletion did not affect STZ-induced elevation of serum glucose levels, but markedly inhibited STZ-induced vascular calcification and aortic stiffness in diabetic mice. Consistently, SMC-specific OGT deletion attenuated STZ-induced Runx2 upregulation in diabetic arteries. A causative effect of *O*-GlcNAcylation on calcification was further determined in OGT-defective VSMC, via its regulation on Runx2 osteogenic function. Immunoprecipitation analysis identified a direct *O*-GlcNAc modification of Runx2, which was essential for Runx2-induced VSMC calcification. With the use of a series of Runx2 site-directed mutants, we further identified that *O*-GlcNAc modification on Runx2 threonine (T) 412 residual was essential for Runx2 osteogenic transcriptional activity and Runx2-induced VSMC calcification. **Conclusions:** Our studies have provided the first genetic proof demonstrating a causative role for SMC-specific OGT in regulating protein *O*-GlcNAcylation and vascular calcification in diabetes; and further revealed a mechanism underlying post-translational modification of Runx2 by *O*-GlcNAc in regulating Runx2 transcriptional activity and VSMC calcification. These studies have elucidated a novel molecular mechanism underlying Runx2-governed vascular calcification, shedding lights on new strategies targeting vascular calcification in diabetes. **Acknowledgements:** YC is supported by grants from the National Institutes of Health (NIH HL146103, HL158097, HL167201 and AG082839) and the United States Department of Veterans Affairs Basic Sciences R&D Service (BX005800 and BX004426).

Poster Board 15
CARDIOVAS014
Immune Checkpoint Inhibitor-Induced Cardiotoxicity is Associated with Dysfunctional Metabolism, Muscle Wasting and Autophagy: An Exploratory Analysis of Pathological Signaling Pathways
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Background: Despite the recent clinical success of immune checkpoint inhibitors (ICIs) in fighting aggressive cancer types, a rapidly increasing number of patients suffers from ICI-induced cardiotoxicity with often fatal outcomes. Awareness of this severe side effect is low, clinical manifestations are nonspecific and underlying pathological cellular mechanisms have not been investigated. Therefore, the purpose of this study was to explore inflammatory, metabolic, muscle wasting, and autophagic pathways and their roles in ICI-induced cardiac remodeling and dysfunction in female mice. **Methods:** Female C57BL/6 wildtype and LC3 transgenic mice were randomly selected and separated into non-ICI controls (CON) and ICI-treated (ICI) groups. Additional tumor bearing groups (CON+T; ICI+T) were used to show clinical relevance and efficacy of the chosen ICI administration protocol. Mice underwent a 4-week ICI treatment protocol. During the 4 weeks, ICI mice

received ICI treatment (200ug/mouse) *via* i.p. injections twice/week, while control mice received saline placebo injections. Echocardiography was performed at baseline and sacrifice to determine changes in cardiac structure and function. At sacrifice, cardiac tissue was collected and cellular inflammatory, metabolic, and muscle wasting signaling pathways were analyzed *via* Western Blot. Autophagic flux was analyzed in cardiac tissue sections of LC3 transgenic mice *via* confocal fluorescent microscopy. **Results:** The administered ICI protocol successfully reduced tumor burden characterized by significantly smaller tumor masses (-48%) and tumor volumes (-45%) in ICI-treated tumor bearing mice compared to control tumors (P<0.05). ICI treatment led to significantly decreased cardiac function (-20%) and cardiac remodeling, measured as left ventricular dilation (+50%) and thinning of posterior cardiac walls at end-systole and end-diastole (P<0.05), indicative of dilated cardiomyopathy. Exploratory protein expression analysis revealed dysfunctional muscle wasting *via* Atrogin1 and MuRF1 expression (P<0.05) and an imbalance in protein homeostasis with dysfunctional AKT and Foxo1 pathways (P<0.05), and dysregulation of autophagic flux (P<0.05). **Conclusions:** In this model, ICI-induced cardiotoxicity was characterized by severe cardiac remodeling and dysfunction, and associated with dysfunctional metabolism, muscle wasting, and autophagy. To our knowledge, this is the first study to explore underlying pathological cellular mechanisms. Therefore, this study adds novel and impactful insight to the still unclear characteristics of ICI-induced cardiotoxicity and supports the critical need to further investigate underlying pathological mechanisms associated with side effects of immunotherapies to optimize clinical treatment of cancers.

Poster Board 16
CARDIOVAS015
RNA m6A Modification in Vascular Calcification
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Background: Vascular calcification is an actively regulated process wherein vascular smooth muscle cells (VSMC) undergo osteogenic differentiation. The N6-methyladenosine (m6A) mRNA modification determines the stability of target mRNAs and contributes to multiple biological processes. However, the role of mRNA m6A modification in VSMC calcification and its underlying mechanisms remain to be elucidated. **Methods:** RNA-sequencing and nucleotide-definition m6A microarray were employed to evaluate changes in genes and mRNA m6A modification during VSMC calcification. Simultaneous fluorescence *in situ* RNA hybridization (FISH) and immunofluorescence staining were used to demonstrate m6A modification and associated gene expression. STM2459, a methyltransferase inhibitor, and shRNA targeting the methyltransferase Mettl3 in VSMC were utilized to determine the regulation of mRNA m6A modification on VSMC calcification. **Results:** To investigate the role of m6A RNA modification in VSMC calcification, we first assessed the temporal dynamics of total RNA m6A modification in VSMCs exposed to osteogenic media. Dot blot and fluorescence in situ hybridization (FISH) analyses demonstrated a time-dependent increase in m6A modification, concurrent with increased expression of the m6A methyltransferase METTL3. To further elucidate the involvement of METTL3, we employed both pharmacological inhibition (STM2457) and genetic knockdown (shRNA) of METTL3. Both approaches attenuated VSMC calcification, suggesting a critical role for m6A modification in this process. Integration of RNA sequencing and m6A microarray data identified 54 genes that exhibit a concurrent increase in both mRNA expression and m6A modification under osteogenic conditions. Subsequent pathway analysis of these 54 genes revealed enrichment in pathways including angiogenesis regulation, vasculature development regulation, bone modulation, and osteogenic differentiation. **Conclusions:** Our studies have demonstrated an important role of RNA m6A modification in regulating VSMC calcification. Ongoing studies aim to identify key regulators in mediating the effects of RNA m6A modification in VSMC calcification *in vitro* and *in vivo*, which may provide molecular insights into novel regulatory mechanisms for vascular calcification. **Acknowledgements**: YC is supported by grants from the National Institutes of Health (NIH HL146103, HL158097, HL167201 and AG082839) and the United States Department of Veterans Affairs Basic Sciences R&D Service (BX005800 and BX004426). EVM is supported by a Diversity Supplement Award from the American Heart Association (24DIVSUP1281294).

Poster Board 17
CARDIOVAS016
Expression of *Dchs1*, a Critical Morphogenetic Gene, in Cardiac Tissue throughout Embryologic and Neonatal Development
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Background: *Dchs1*, an atypical cadherin, has been shown by the Norris Laboratory to contribute to the phenotypic manifestation of mitral valve prolapse. Through this discovery, it was noted homozygous knockout mice (*Dchs1$^{-/-}$*) were 100% neonatally lethal and exhibited abnormal heart morphology– leading to our laboratory's hypothesis *Dchs1* is critical for cardiac morphogenesis. *Dchs1* is known to be critical in brain, gut, vertebrae, and kidney development. Up to this point, the expression and effects of *Dchs1* in cardiac development has not been elucidated. This study characterized normal expression of *Dchs1* in cardiac tissue throughout mouse embryological and neonatal development, providing a foundation for future functional studies. **Methods:** To analyze expression of *Dchs1* in cardiac tissue, a mouse strain with a hemagglutinin (HA) tag in the *Dchs1* locus was designed, due to a lack of consistent antibodies against the cadherin. Hearts from heterozygous *Dchs1-HA$^{+/-}$* mice at embryologic days E11.5, E13.5, E18.5; and post-natal days P0, P7, and P21 were excised and analyzed by immunohistochemistry (IHC) with wild-type littermate controls. The tissue was co-stained for HA and cell markers for cardiomyocytes, endothelial cells, or fibroblasts. Lastly, *Dchs1* expression was analyzed using single-cell RNA sequencing (scRNA-seq). Publicly available datasets of wild-type mouse hearts were examined for *Dchs1* expression at both embryonic and adult time points. **Results:** IHC analysis revealed *DCHS1-HA* protein co-localization with only cell markers for endothelial cells and fibroblasts at all time points. The expression of *DCHS1-HA* throughout the myocardial free wall appeared to follow the intercalation of epicardial-derived cells through successive fetal timepoints. Additionally, during the embryonic period only membranous *DCHS1-HA* staining was observed and intracellular staining began to be observed in the neonatal period, suggesting a potential signaling and processing pathway for *DCHS1*. Single-cell RNA sequencing analysis showed that *Dchs1* RNA expression was detected exclusively in endocardial, endothelial, and fibroblast cell clusters. **Conclusion:** Understanding normal expression patterning of *Dchs1* is critical to clarifying its role in cardiac morphogenetic signaling. This study supports prioritization of cellular crosstalk involving fibroblasts and endothelial cells in ongoing function studies regarding the function of *Dchs1* in cardiac morphogenesis. **Acknowledgments:** Supported by NIH R01HL131546 and R01HL149696.

Poster Board 18
CARDIOVAS017
Vascular Dysfunction in Microgravity: A Meta-Analysis of Arterial Remodeling, Venous Thrombosis Risks, and Countermeasures in Astronauts
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Background: Microgravity induces vascular changes in astronauts, including arterial remodeling, endothelial dysfunction, and elevated venous thrombosis (VT) risks due to fluid redistribution and altered hemodynamics. Prolonged spaceflight is associated with carotid intima-media thickness (IMT) increases (~10-20%) and femoral stiffness, mirroring early atherosclerosis. Asymptomatic jugular VT cases on the International Space Station (ISS) highlight microgravity-specific risks like stagnant venous flow and hypercoagulability. This meta-analysis synthesizes data on vascular adaptations, VT mechanisms, and countermeasure efficacy for long-duration missions. **Methods:** A systematic review of PubMed, NASA Life Sciences Data Archive, and European Space Agency (ESA) publications (2015-2025) identified 25 studies (n=1100 subjects) evaluating arterial remodeling, VT, and countermeasures. Arterial remodeling was assessed through carotid/femoral IMT, compliance, and endothelial function (flow-mediated dilation, FMD) in astronauts and bed rest analogs. VT incidence, risk factors (e.g. hormonal contraception, microgravity-induced flow stagnation), and diagnostic challenges in space were examined. Countermeasures including exercise, lower-body negative pressure (LBNP), and nutritional interventions (e.g. resveratrol) were evaluated. Meta-analysis pooled odds ratios (ORs) for VT and standardized mean differences (SMDs) for arterial parameters using random-effects models. Heterogeneity was assessed via I^2 statistics. **Results:** Arterial changes included a 12.5% increase in carotid IMT post-6-month missions (SMD=0.62, 95% CI: 0.45-0.79, $p<0.001$), with greater stiffness in astronauts compared to controls. Femoral remodeling showed an inward diameter reduction (-8%) and compliance decline (-15%) in bed rest studies. VT incidence revealed 2 confirmed jugular VT cases

among 609 astronauts (0.33%), with retrograde flow in 54% of ISS crewmembers. Risk factors included hormonal contraception (OR=3.2, 95% CI: 1.5-6.8) and microgravity-induced cephalad fluid shifts (OR=4.1, 95% CI: 2.0-8.4). Countermeasures showed that exercise preserved femoral FMD (SMD=0.41, p=0.03) and attenuated carotid IMT progression. LBNP improved venous flow dynamics, reducing stagnant flow episodes by 60%. Resveratrol enhanced endothelial function (SMD=0.35, p=0.04) but had limited efficacy against VT. **Conclusion:** Microgravity drives vascular deconditioning through arterial stiffening and venous thrombogenesis. Exercise remains the cornerstone countermeasure, with LBNP and low-dose apixaban potentially mitigating VT risks. Future studies could prioritize sex-specific analyses (e.g. hormonal impacts) and validate NASA's VESGEN software for real-time vascular monitoring. These findings inform astronaut health protocols and terrestrial management of sedentary populations.

Cell Injury and Repair

Poster Board 19

CELL001

Role of Matrix Bound Nanovesicles on Macrophage Polarization and Myoblast Differentiation During Skeletal Muscle Repair

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Background: The spatiotemporal dynamics of immune cell populations and their crosstalk with myoblasts are crucial for skeletal muscle regeneration. An appropriately timed shift from a pro-inflammatory to a pro-healing immune response is essential for functional repair in acute muscle injuries. This regenerative capacity is lost following volumetric muscle loss (VML). We have shown that an Extracellular Matrix (ECM) scaffold can facilitate macrophage phenotype transition, leading to functional tissue deposition and myogenesis VML patients. More recently, we have identified matrix bound nanovesicles (MBV) as the major signaling moiety within ECM scaffolds that promotes an anti-inflammatory immune phenotype and downstream constructive remodeling. We showed that MBV are a rich source of polyunsaturated fatty acids (PUFA) crucial for macrophage polarization and stem cell differentiation. Thus, we hypothesize that the MBV phospholipid membrane may serve not only as a reservoir of oxidized and oxidizable esterified phospholipid species, but also as a hydrolytic platform of pro-resolving lipid mediators activated by different phospholipases dependent on the pro-/anti-inflammatory context of the extracellular environment. Herein, we evaluated the role of MBV lipids or their hydrolyzed lipid mediator products on macrophage polarization and myoblast differentiation. **Methods:** MBV were harvested from decellularized small intestinal submucosa (SIS) using a combination of enzymatic digestion, ultracentrifugation, and size exclusion chromatography. Purified MBV were hydrolyzed with platelet activating factor (PAF)-hydrolase to release free lipid mediators, followed by lipid purification using a modified Folch extraction method. The extracted MBV lipids were then used to stimulate murine bone marrow-derived macrophages to determine the effect on macrophage polarization, and to stimulate C2C12 cells to evaluate the role of MBV lipids on myoblast differentiation. **Results:** The results show that MBV lipid extracts that have been hydrolyzed with platelet activating factor (PAF)-hydrolase to release free lipid mediators from their esterified into phospholipids forms, resulted in a significant increase in the $F4/80^{+}CD206^{+}$ M2-like phenotype when exposed to macrophages. Interestingly, non-hydrolyzed MBV lipid extracts were shown to significantly upregulate the expression of levels of muscle differentiation markers. **Conclusions:** The lipid component of MBV has differential biologic effects on cell phenotype that is dependent on the catalytic hydrolysis of its phospholipid membrane. Whereas hydrolyzed lipids were able to activate an anti-inflammatory macrophage phenotype, the non-hydrolyzed MBV lipids were able to induce myoblast differentiation. Thus PUFA-enriched MBV are a critical source of pro-resolving lipid mediators activated by phospholipases and thus dependent on the pro-/anti-inflammatory context of the extracellular environment.

Experimental Therapeutics

Poster Board 20

EXPTHER001

Leveraging Immunohistochemistry to Assess Antisense Oligonucleotides Biodistribution

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Background: Oligonucleotide-based therapeutics are a promising and fast-growing treatment modality for a wide range of diseases due to their ability to precisely target RNA molecules and modulate protein expression. Among the various subsets

of oligonucleotides, antisense oligonucleotides (ASOs) bind specifically with high affinity to their RNA target through sequence complementarity and can target intronic regions as well as nuclear targets. However, ASOs delivery to specific tissues remains an important limitation due, in part, to their short half-lives. Chemical alterations to the sugar-phosphate backbone of ASOs, such as phosphorothioate (PS) incorporation, has been shown to increase resistance to degradation by nucleases and extend ASOs half-life, contributing to improved delivery to tissues. Detection of ASOs in tissues has traditionally utilized *in situ* hybridization assays, requiring a unique probe for each ASO. However, recent development of monoclonal antibodies targeting PS modifications offers the opportunity to detect PS-modified ASOs independently of their sequence. Here, we explored immunohistochemistry (IHC) using anti-PS antibodies as a method to assess ASOs biodistribution in mouse tissues. **Methods:** We used a panel of three anti-PS antibody clones PS03, PS04 and PS05 from the ModDetect™ Phosphorothioate panel (Rockland Immunochemicals Inc, Limerick, PA) to develop specific IHC assays using formalin-fixed paraffin-embedded (FFPE) tissues from mice treated with a targeted 16-mer ASO with a full PS backbone. **Results:** Anti-PS antibody screening by IHC showed positive immunoreactivity in tissues of mice treated with the PS-modified ASO with all three clones, but not in vehicle-treated mice. Interestingly, PS04 and PS05 displayed stronger immunoreactivity than PS03, indicating variability of sensitivity for each clone. Taken together, these data suggest that the anti-PS antibodies can specifically detect PS-modified ASOs with different levels of sensitivity. **Conclusions:** In summary, we successfully established a robust IHC assay to detect PS-modified ASOs in mouse tissues, providing a simplified and flexible tool for assessing ASO targeted delivery specificity and distribution. **Disclosures**: All authors are Sanofi employees and may hold shares and/or stock options in the company.

Gene Regulation in Disease

Poster Board 21

GENEREG001

Transcriptomic Insights into Gas6-Induced Placental Dysfunction: Gene Targets for Preeclampsia Therapy

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Background: Preeclampsia (PE) is a leading cause of maternal and fetal morbidity, characterized by hypertension, proteinuria, and systemic inflammation. Growth arrest-specific protein 6 (Gas6) is implicated in immune regulation, oxidative stress, and endothelial dysfunction—key features of PE pathophysiology. This study investigates the molecular mechanisms underlying Gas6-induced placental dysfunction using transcriptomic approaches. **Methods:** A PE mice model was established by administering recombinant Gas6 protein to pregnant mice. Placental tissues were analyzed using RNA sequencing, immunofluorescence, and enrichment analyses to identify differentially expressed genes, signaling pathways, and potential therapeutic targets. **Results:** Gas6 exposure significantly increased blood pressure and proteinuria in treated animals. Transcriptomic analysis revealed 98 differentially expressed genes, with upregulation of Fam111a, a marker of oxidative stress, and downregulation of Clca4, involved in ion transport and cellular homeostasis. Enrichment analysis identified disruptions in extracellular matrix remodeling, interleukin signaling, and metabolic pathways. Immunofluorescence confirmed increased FAM111A and decreased Clca4 expression in Gas6-treated placentas. Notably, pharmacologic inhibition of the Gas6/AXL pathway mitigated PE-like symptoms, highlighting its therapeutic potential. **Conclusions:** This study establishes Gas6 as a critical mediator of placental dysfunction in PE and identifies key molecular targets for intervention. Targeting the Gas6/AXL axis may provide novel therapeutic strategies for managing PE and improving maternal-fetal outcomes. **Acknowledgements:** This work was supported by funding from the National Institutes of Health (1R15HL152257) and the Flight Attendant's Medical Research Institute (CIA2300003).

Poster Board 22

GENEREG002

Investigation of Drosophila Spn42Dd Gene to Accurately Model Human SerpinE1 Diet and Stress-related Phenotypes

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Background: Diet and stress contribute to chronic inflammatory diseases, metabolic disorders, and cardiovascular disease, affecting over a billion people worldwide. Plasminogen activator inhibitor 1 (PAI-1) plays a critical role in these conditions by regulating fibrin breakdown and impacting lipid metabolism. High PAI-1 levels are linked to obesity, insulin resistance, and increased extracellular matrix protein deposition. Research on the Berne Amish community revealed that individuals with reduced PAI-1 levels exhibit improved metabolic health, lower fasting insulin levels, and reduced cardiovascular risk. To explore PAI-1's role in diet- and stress-related diseases, an in vivo model is necessary. *Drosophila melanogaster* serves

as an effective system due to its genetic similarity to humans and rapid life cycle. **Methods:** Genetic and protein analysis of Drosophila Serpin Spn42Dd shows structural and functional similarities with human PAI-1. Muscle-specific knockdown of spn42dd using RNAi technology was used and climbing and life span were assessed. Additionally, CRISPR-Cas9 gene editing facilitated the introduction of a mutation like human *SERPINE1* (c.699_700dupTA) into the *Drosophila* Spn42Dd gene to assess its impact on body mass, muscle strength, and metabolic health. **Results:** Preliminary data suggest that Spn42Dd knockdown in muscles presents with climbing ability changes and an extension of lifespan. These findings align with observations in the PAI-1-deficient Amish population. **Conclusion:** This study aims to determine whether *Drosophila* Spn42Dd can model human PAI-1 phenotypes. If validated, further research will investigate the molecular mechanisms linking Spn42Dd/PAI-1 to inflammation, metabolism, and longevity.

Infectious Disease

Poster Board 23

INFDIS003

Peromyscus Deer Mice as an Acute Rodent Model of Leptospirosis

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Background: Leptospirosis is a devastating global zoonotic disease affecting humans, companion animals, and domestic livestock. Wild rodents are the primary reservoir hosts of pathogen spread and laboratory rats and mice are largely asymptomatic. The Golden Syrian Hamster (*Mesocricetus auratus*) is the dominant model for acute disease, since they are susceptible to most pathogenic serovars and are heavily utilized to maintain laboratory strain virulence and test bacterin vaccine efficacy. *Peromyscus leucopus* (deer mice) are more closely related to hamsters than *Mus musculus* mice, historically have been alternative rodent models for other spirochete diseases such as Lyme disease, and are a wild rodent in the environment. This work reports on deer mice as an alternative model of acute leptospirosis. **Methods:** Male and female deer mice were challenged with strains of *Leptospira (L. interrogans)* serogroup Canicola and *L. borgpetersenii* serogroup Ballum serovar Arborea by intraperitoneal injection. Upon appearance of severe clinical signs of disease (weight loss, blood on nose/paws, bloody urine, lethargy, etc.) animals were humanely euthanized. Whole blood slides were Giemsa stained and manually evaluated for differential blood counts. Kidney and liver tissues were cultured and pathogen load determined by *lipL32* qPCR. Tissues including kidney, spleen, liver, and lung were formalin-fixed and sectioned for histopathologic analysis. **Results:** Challenged deer mice developed severe clinical signs and were positive for *Leptospira* by culture and qPCR. Males were more susceptible than females to acute disease and contained higher bacterial burdens in tissues. Additionally, deer mice produced circulating foamy macrophages, which are a marker of disease severity characterized in hamsters, in response to *Leptospira* challenge. Histopathologic evaluation documented differentiating lesion severity, especially in the liver and kidneys, with the two *Leptospira sp*. challenges. **Conclusions:** This work describes the first time that deer mice are susceptible to acute leptospirosis and offer an alternative rodent model for acute leptospirosis. Infected deer mice produce foamy macrophages, display susceptibility differences between sexes, and produce differing histologic severity of acute nephritis and hepatitis between serovars. **Acknowledgements:** The authors extend their profound appreciation to Denise Chapman and the NADC vivarium animal care staff for the care and handling of animals. USDA was the sole funder for this research.

Liver Pathobiology

Poster Board 24

LIVER008

Ataxia-telangiectasia Mutated Mediates Transforming Growth Factor Beta Response in Acetaminophen-induced Liver Injury in Mice

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Background. Acetaminophen (APAP) is a common analgesic/antipyretic that is widely used throughout the world. APAP overdose is a leading cause of drug-induced acute liver injury. APAP's toxicity is associated with increased tissue growth factor beta (TGFβ) signaling and elevated oxidative stress which in turn lead to DNA damage. Ataxia-telangiectasia mutated (ATM) is recruited to initiate DNA double-strand break (DSB) repair. ATM activation regulates transcription factor p53 activity

which effects cell cycle arrest, cellular senescence, and apoptosis. TGFβ has previously been shown to drive the ATM-mediated DNA repair and ATM-mediated p53 signaling, suggesting TGFβ could be providing additional driving factors for ATM signaling and response from repair to cellular death. In this study we sought to investigate ATM signaling following an *in vivo* APAP administration and to demonstrate the role ATM plays on APAP-induced TGFβ signaling. **Methods.** Male C57Bl/6 mice were injected with 300 mg/kg APAP. The treatment group was pretreated with 5 mg/kg of the ATM inhibitor KU55933 1hr prior to APAP administration. *In vitro* studies were performed in FL83B cells treated with APAP, KU55933, and/or the neddylation inhibitor MLN4924. Characterization of liver injury via histological and biochemical measures were performed. DNA repair and TGFβ signaling were assessed via phospho-H2AX, ATM, Chk2, activated TGFβ and SMAD2/3 levels. TGFβRII stabilization was evaluated via immunoblotting, co-precipitation with NEDD8, and IHC for p-c-cbl. The effects of neddylation inhibition were assessed using MLN4924 and evaluated via immunoblotting for TGFβR2 and pSMAD2/3. **Results.** APAP induces DNA damage and ATM signaling both *in vivo* and *in vitro*, which was observed via an accumulation of phospho-H2AX and phosphorylation of ATM and Chk2. KU55933 inhibits the ATM response to APAP and results in attenuated serum ALT/AST levels and tissue necrosis. Furthermore, ATM inhibition attenuated SMAD2/3 signaling with no changes in activated TGFβ levels. APAP induces TGFβRII stabilization through p-c-cbl and subsequent neddylation that was attenuated with ATM inhibition. Therefore, ATM signaling plays a crucial role in APAP-induced TGFβ signaling through the stabilization of TGFβRII and loss of ATM results in reduced hepatic injury to APAP. **Conclusion.** APAP-induced hepatotoxicity results from an accumulation of DNA damage in the form of DSBs which in turn leads to ATM-mediated DNA repair and ATM-mediated TGFβ signaling, ultimately resulting in hepatocellular death. The use of an ATM inhibitor allows us to attenuate the damage caused from APAP-induced liver injury and an attenuation of TGFβ-mediated SMAD signaling. Strategies to inhibit the DNA damage response or ATM signaling may help in the regenerative response to APAP and may be a viable target for the development of adjunct therapy for the management of APAP overdose.

Poster Board 25
LIVER009
Caspase-11 and GasderminD Deletion Exacerbates Coagulopathy in APAP-induced Acute Liver Failure
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Background: The noncanonical inflammasome caspase-11 and gasderminD, known for their role in inflammatory cell death, is significant in APAP-induced inflammation (*Livers*, 2022 2:425-435). However, their role in coagulopathy during APAP-induced acute liver failure (ALF) has not been investigated. This study examines the role of caspase-11 and gasderminD-mediated pyroptosis in coagulopathy in APAP-induced ALF. **Methods:** Male C57BL/6J wild-type (WT), caspase-11 knockout (casp11$^{-/-}$), and gasderminD knockout (GsdmD$^{-/-}$) mice were injected with 600 mg/kg of sterile acetaminophen (n=2-4/group). Blood samples were collected 24h post-injection and anticoagulated with sodium citrate. Hemostasis was assessed using rotational thromboelastometry (ROTEM), and platelet counts were determined via complete blood count. Western blotting was used evaluate caspase-11 and gasderminD activation in the liver. **Results:** Caspase-11(casp) and gasderminD (GsdmD) were activated in the liver of APAP-induced acute liver failure (ALF) mice. APAP-induced ALF casp$^{-/-}$ and gsdmD$^{-/-}$ mice prolonged initiation of clot formation (clotting time), compared to WT. Casp11$^{-/-}$ significantly increased clot formation time (time to clot firming), and reduced maximum clot firmness and fibrin buildup (alpha angle) (****p<0.0001). APAP-induced ALF GsdmD mice exhibited complete absence of blood clotting. These knockout (KO) mice also exhibited significantly lower platelet counts compared to wild-type (WT) mice (p = 0.078). APAP-induced ALF casp11$^{-/-}$ mice increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were in compared to WT ($p < 0.05$). **Conclusion:** Our findings highlight an important role of caspase-11 and gasderminD in exacerbating coagulopathy in APAP-induced acute liver failure. Further investigation of this pathway may reveal novel therapeutic targets for managing coagulopathy associated with acute liver failure. **Acknowledgements:** P30 DK120531.

Poster Board 26
LIVER010
Maternal Obesogenic Diet Exposure Increases Severity of Offspring Cholestatic Liver Disease
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Background: Cholestatic liver disease involves the obstruction of bile flow through the biliary tract. The use of 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) in mouse models induces cholestasis by the formation of porphyrin plugs in bile ducts. Prior work in our lab shows that maternal obesogenic diet exposure (MODE) worsens cholestatic liver disease

in offspring. In accordance with the developmental origins of health and disease hypothesis, an early life event known as the weaning reaction is crucial for the development of immunity and can exacerbate chronic pathologies later in life if impaired. We hypothesized that shifts during the lactation period would be associated with changes in cholestatic liver disease severity markers such as fibrosis and bile infarcts in offspring fed a DDC diet. **Methods:** Female mice were fed a normal (CHOW), high fat-fructose-cholesterol (HFFC), or high fat (HFD) diet starting at 4 weeks of age for 6 weeks and bred with lean males fed a CHOW diet. For cross-fostering (CF), offspring were switched on their day of birth to dams of opposing diets (i.e. CHOW offspring were switched to MODE dams and vice versa). At 10 weeks of age, offspring were fed a DDC diet for 2 weeks, and tissues were collected at 12 weeks. Histology was performed to assess fibrosis, ductular reaction, and bile infarct. Total liver bile acids were measured. Reverse transcription polymerase chain reaction (RT-qPCR) was performed to measure markers of fibrosis. **Results:** Histological analysis showed increased Sirius red staining in both male and female HFFC and HFD offspring compared to CHOW offspring. Cytokeratin 19 (CK-19) staining showed increased ductular reaction in both male and female HFFC and HFD offspring. HFFC and HFD offspring also had the presence of bile infarcts. CF offspring showed similar degrees of fibrosis and ductular reaction. However, the frequency of bile infarcts in CF offspring was increased in offspring fostered by an HFFC or HFD dam compared to a CHOW dam. Liver bile acid results showed similar levels of bile acids across all groups. **Conclusions:** These results support that MODE programs increased susceptibility to cholestatic liver disease including worse fibrosis, ductular reaction, and bile infarcts. Cross-fostering seems to have a varying effect on the cholestatic liver disease phenotype with notably increased bile infarct frequency in offspring fostered by a dam on an obesogenic diet. Future work will further investigate the mechanisms driving susceptibility to DDC-induced cholestatic liver disease in MODE offspring.

Poster Board 27
LIVER011
Central Administration of Recombinant Insulin-like Growth Factor 1 Dampens the Neuroinflammatory Response and Attenuates the Cognitive Deficits Observed in a Rodent Model of Hepatic Encephalopathy
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Background: Hepatic encephalopathy (HE) is a neurological complication that arises due to loss of liver function and is associated with neuroinflammation and subsequent onset of cognitive decline. Insulin-like growth factor (IGF1) is a neuroprotective peptide that has anti-inflammatory properties in the brain. We have previously demonstrated that IGF1 expression is decreased in the brains of mice with HE. However, little is known about the role of IGF1 on cognitive deficits and neuroinflammation during HE. **Methods:** C57Bl/6 mice were injected with azoxymethane (AOM) to induce acute liver failure and HE. In parallel, mice were given an intracerebroventricular infusion of recombinant IGF1 (rIGF1) for 3 days prior to AOM injection. Cognitive impairment was monitored by reflex response assessment at various time points. Neuromuscular deficits were assessed using a grip strength meter. Liver damage was assessed by hematoxylin and eosin staining and serum chemistry. Microglia were stained by IBA1, and cortex field staining and cell morphology were assessed. Proinflammatory cytokines were assessed by immunoblotting, immunohistochemistry, and/or qPCR. *In vitro*, a mouse microglial cell line (EOC-20) was activated with recombinant TNFα treatment and then treated with rIGF1 for various time points. In parallel, a mouse neuronal cell line was treated with rIGF1, and the supernatants were collected and incubated with EOC-20 cells. The expression and secretion of proinflammatory cytokines were measured by qPCR and EIA, and phagocytic activity was assessed using a commercially available kit. **Results:** Central infusion of rIGF1 attenuated the neurological and neuromuscular deficits observed after AOM injection without altering the degree of liver damage. Furthermore, IGF1 treatment reduced the degree of microgliosis in the frontal cortex and altered the cell morphology of the microglia from an activated ameboid phenotype (reactive) to a ramified morphology (quiescent) as well as attenuated the AOM-induced upregulation in proinflammatory cytokine expression. Treatment of EOC-20 cells with rIGF1, as well as the conditioned media from rIGF1-treated neurons, attenuated the phagocytic activity and expression and secretion of proinflammatory cytokines induced by TNFα, indicating both direct and indirect actions of IGF1 on microglia reactivity. **Conclusion:** Taken together, these data suggest that treatment of IGF1 signaling can dampen the neuroinflammatory processes and attenuate cognitive deficits observed during HE. **Acknowledgments:** This work was supported by NIH funding (DK112803 and DK135995) to SD and the use of equipment and resources from the Central Texas Veterans Healthcare System.

Lung Pathobiology

Poster Board 28

LUNG001

Senescence Induced Changes in ECM Proteins Contribute to Healthy Lung Aging

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Introduction: Aging is characterized by several hallmarks, including senescence, which has been implicated in chronic lung diseases. Senescence is a stress-induced mechanism leading to permanent cell cycle arrest and release of chemokines and cytokines, known as the senescence-associated secretory phenotype (SASP). Previous studies have focused largely on cellular outcomes of senescence, however, effects on the extracellular matrix (ECM), which is altered upon lung aging and injury, remain elusive. Here, we aim to decipher changes in the ECM proteome in the aging lung and investigate whether and how trigger- and cell-specific senescence is sufficient to drive ECM changes. **Methods:** Twenty healthy human donor lung tissues were obtained (4mo-90yrs) for label-free liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis completed in the Schilling Lab. Immunohistochemistry slides were prepared with human cohort tissue embedded in paraffin. Precision cut lung slices (PCLSs), an *ex vivo* model for multicellular interactions and ECM in the native lung, were prepared from human lung tissue to 300um thick and 1cm diameter slices. Samples were treated every two days with bleomycin (15ug/mL) for 7 days to induce senescence. **Results:** LC-MS/MS was performed on 20 human lung tissue to identify a list of proteins differentially expressed with age. Samples were divided into three age groups—infant (4mo to 22mo, n=5), young adult (31-50yrs, n=5) and old adult (75-90yrs, n=10). Statistical analysis, including age vs. abundance, demonstrated that abundance of proteins did change with age in our cohort. ECM proteins were identified through the MatrisomeDB, a database of ECM and ECM-associated proteins. To study senescence on proteomic changes in the lung, PCLSs samples were induced with the DNA damage mediated senescence inducer, bleomycin. LC-MS/MS was performed on treated human PCLS samples (n=9) that generated a list of over 200 significantly dysregulated proteins. Senescence was confirmed by assessing p21/p16 levels at the gene/protein level and □-galactosidase staining. To identify the potential effect of senescence on healthy aging, 15 key ECM proteins that were significantly altered with similar regulation in both lists were identified. Localization of the key proteins, which included top hits like S100A9, FBLN2, and SERPINA3, were identified through immunohistochemistry slides of the aging cohort. These proteins were found to be associated with activated fibroblasts (Pdgfra/aSMA), alveolar type 2 epithelial cells (Sftpc/HTII-280), and infiltrating immune cell populations (CD68/CD177). **Conclusion:** Proteomic analysis of a healthy aging lung tissue cohort revealed significant changes in the ECM with age. Further analysis with senescence-induced proteomics revealed 15 key ECM proteins that were altered in both aging and senescence, which may elucidate the effect of senescence on ECM dysregulation with age. **Acknowledgements:** NIA U54 AG075931.

Poster Board 29

LUNG002

Pulmonary Protective Effects by Drugs Interacting with the Renin-Angiotensin System on the Damage Induced by Fat Embolism and Other Injuries

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Background: Fat embolism was first identified in 1862. The primary cause of fat embolization is the release of bone marrow fat droplets into the venous system, often occurring after fractures. This condition can lead to acute chest syndrome, especially in patients with sickle cell disease. Researchers have utilized various animal models, particularly rats, to study fat embolism. **Methods:** Studies have shown that infusing bone marrow fat can deliver fat to the lungs, while subcutaneous fat injections in humans can also trigger this condition. Triolein, a neutral fat, is frequently used in experimental settings to study fat embolism. Our research group used rats for the experimental study. Histopathological evaluation in the course of lung injury induced by Triolein injections revealed changes as early as 12-24 hours, which included damage to the small caliber arteries and the arterial media, with increased number of myofibroblasts, and septal inflammation, bronchial inflammation, and the presence of many macrophages in lung parenchyma. Triolein was also present as droplets of various sizes, mostly in the small caliber arteries and septa. Inflammation was still present 11 days after the injection. After a partial resolution during post-injection interval time, there were persistent and progressive inflammatory and fibrotic changes up to 6 weeks after Triolein injections. These changes were associated with an increase in angiotensin peptides, implicating the

role of the renin-angiotensin system (RAS) in the pathogenesis of pulmonary damage. We found that three different agents that interfere with the RAS were found to ameliorate the pulmonary damage 48 hours after rats were injected with Triolein. They were, Angiotensin I converting enzyme inhibitor Captopril, the Angiotensin 2 type I receptor blockers, Losartan, and the renin inhibitor, Aliskiren. **Results and Conclusion:** The renin-angiotensin system (RAS) has been linked to pulmonary damage in lung injury models. Our research indicates that agents like Captopril and Losartan, which inhibit the RAS, can reduce both pulmonary damage and inflammation after Triolein injections. Moreover, mast cells and activated macrophages may play a role in the inflammatory response, as Triolein increases mast cell accumulation in the lungs.

Poster Board 30
LUNG003
Microgravity-Inspired Insights into Pulmonary Fibrosis: Integrating Advanced Imaging and Tissue Remodeling Biomarkers
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Background: Pulmonary fibrosis (PF) and microgravity-induced tissue remodeling share mechanisms like dysregulated extracellular matrix (ECM) turnover and fibrosis. Microgravity accelerates tissue atrophy and fluid redistribution, while PF causes alveolar collapse and collagen deposition, leading to lung stiffening. Aerospace-derived imaging, such as high-resolution computed tomography (HRCT) and molecular probes for denatured collagen, offer tools to map fibrosis progression and therapeutic responses. This meta-analysis synthesizes evidence on collagen remodeling biomarkers and aerospace-inspired imaging, bridging insights between PF and microgravity adaptations. **Methods:** A systematic review of PubMed-indexed studies (2015-2025) and NASA reports was conducted using keywords: Pulmonary fibrosis, collagen remodeling, microgravity, SPECT/CT, and radiomics. Inclusion criteria included studies quantifying ECM dynamics in PF or microgravity analogs (e.g. bed rest) and imaging innovations with aerospace applications. Meta-analysis pooled data from 15 studies (n=1200 patients / animal models) evaluating collagen-targeted PET/SPECT probes, automated CT tools, and spatial transcriptomics. Heterogeneity was assessed via I^2 statistics; fixed-effects models were applied for imaging accuracy metrics. **Results:** Collagen remodeling biomarkers, highlighted by denatured collagen detected via collagen hybridizing peptides (CHPs) in PF lungs, correlated with disease activity (r=0.80, p<0.001) and predicted antifibrotic therapy efficacy. Similar ECM breakdown was observed in microgravity-induced muscle atrophy, suggesting shared pathways. SPECT/CT imaging with $^{99m}Tc\text{-}(HE)_3\text{-}(GPO)_9$ probes demonstrated 96% specificity in murine PF models, highlighting potential for early fibrosis detection. Imaging innovations revealed that automated CT tools (e.g. CALIPER) showed comparable prognostic value to manual scoring for PF extent (HR=1.03, p=0.02) while reducing analysis time by 50%. Spatial transcriptomics identified fibroblast foci and alveolar epithelial dysregulation as key PF niches, with gene signatures (e.g. COL1A1, SFTPC) mirroring microgravity-induced mechanotransduction pathways. Meta-analysis outcomes indicated a pooled sensitivity of collagen-targeted probes for PF detection at 89% (95% CI: 82-94%) and specificity at 91% (95% CI: 85-95%). Automated CT quantification correlated strongly with spirometry (r=-0.50, p=0.03) and survival (HR=1.03, p<0.01), validating its clinical utility. **Conclusion:** Microgravity research offers a unique perspective on PF mechanisms, highlighting collagen catabolism and mechanical stress as targets. Aerospace-inspired imaging allows precise fibrosis staging and personalized monitoring. Future studies could explore microgravity analogs for PF modeling and antifibrotic countermeasures, integrating spatial transcriptomics with imaging biomarkers for early intervention.

Poster Board 31
LUNG004
The Hippo Pathway Component AMOTL2 Impacts Lung Epithelial Cell Reprogramming in Pulmonary Fibrosis
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Background: Idiopathic pulmonary fibrosis (IPF) is a serious and often fatal disease with limited treatment options. It leads to lung scarring, tissue stiffening, and alveolar damage, partly due to the failure of alveolar stem cells to mature into gas-exchanging cells. Alveolar type 2 (AT2) cells, which aid in lung repair, struggle to regenerate properly, contributing to disease progression. Single-cell RNA sequencing reveals abnormal AT2 cells in IPF, called basaloid cells, and similar alveolar differentiation intermediate (ADI) cells in mouse models. Their origins and accumulation remain unclear. We and others have recently demonstrated that the Hippo pathway effector protein and mechanotransducer Yes-associated protein 1 (YAP) is upregulated in AT2 cells in IPF and contributes to ADI cells and ECM crosslinking. The mechanisms that lead to YAP upregulation in fibrosis, however, are largely unknown. Here, we focus on the YAP regulator, AMOTL2 (a scaffolding protein called angiomotin-like protein 2), which is known to act by inhibiting the nuclearization of YAP. We hypothesize that AMOTL2 downregulation in AT2 cells leads to increased nuclearization of YAP where it upregulates genes related to ECM

crosslinking and reprogramming of AT2 cells. It is currently unknown if loss of AMOTL2 helps drive a fibrotic phenotype in IPF via dysregulation of YAP in AT2 cells. **Methods:** We examined localization of AMOTL2 in human lung tissue from healthy and IPF donors (n=10/group -IPF vs control) via immunofluorescent (IF) staining of AMOTL2 alongside specific AT2 cell-markers. We additionally assessed AMOTL2 expression and localization in AT2 cell lines, primary mouse AT2 cells as well as whole lung tissue from bleomycin-treated mice compared to control (n=5 each group). Using primary mouse AT2 cells, we temporally defined AMOTL2 expression and their ADI phenotypes using RT-qPCR and Western blotting. We performed AMOTL2 loss-of-function studies in AT2 cells using AMOTL2 siRNA and analyzed fibrotic and ADI phenotypes using RT-qPCR (n=2). **Results:** We found decreased expression of AMOTL2 in AT2 cells from IPF patients and experimental mouse models of lung fibrosis compared to their respective controls. We see an increased expression of ADI genes in AT2 cells from bleomycin treated mice, such as Claudin 4 and Keratin 8, and fibrosis-related genes such as Col1a1, confirming the presence of a fibrotic-ADI phenotype. Our *in vitro* knockdown results show successful inhibition of AMOTL2 expression and a decreased expression of ADI marker Krt8. **Conclusion:** AMOTL2 reduction leads to AT2 cell reprogramming and thus might contribute to the development and progression of pulmonary fibrosis. **Acknowledgements:** Three Lakes Foundation.

Metabolomics

Poster Board 32

METABOLOMICS001

Semaglutide Impairs C2C12 Myoblast Viability and Attenuates Key Anabolic Signaling Pathways

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Background: Semaglutide, a glucagon-like peptide-1 receptor agonist (GLP-1 RA) used in the management of metabolic disorders, has emerged as a promising agent for weight control. However, its potential adverse effects on skeletal muscle raise significant concerns regarding lean mass preservation, particularly in aging populations at risk for sarcopenia. **Methods:** In this study, we investigated the impact of semaglutide on C2C12 myoblast viability and its modulation of key intracellular signaling pathways critical for muscle maintenance and growth. C2C12 myoblasts were treated with semaglutide across a range of concentrations that simulate clinical dosing, and cell viability and cell number were assessed using standard cytotoxicity assays. **Results:** Our results demonstrated a significant, dose-dependent reduction in both myoblast viability and overall cell number, suggesting a cytotoxic effect of semaglutide on muscle cells. Mechanistic analyses revealed a pronounced decrease in the phosphorylation levels of ERK (extracellular signal-regulated kinase) and Akt, two major signaling proteins that drive anabolic processes and cell survival in muscle tissue. Interestingly, the mTOR pathway, a well-established regulator of protein synthesis and cell growth, remained unchanged despite semaglutide exposure. **Conclusions:** These findings imply that the detrimental impact on muscle cell viability is primarily mediated through the downregulation of pERK and pAkt signaling rather than alterations in mTOR activity. Collectively, our study underscores a potential mechanistic link between semaglutide treatment and lean mass loss, warranting further investigation into the long-term effects of GLP-1 RA therapy on skeletal muscle health, particularly among elderly patients.
Acknowledgements: This work was supported by funding from the National Institutes of Health (1R15HL152257) and the Flight Attendant's Medical Research Institute (CIA2300003).

Poster Board 33

METABOLOMICS003

Leveraging Lysosomal Calcium Flux as a Strategy to Induce Autophagy-Lysosomal Biogenesis in Macrophages

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Background: Atherosclerosis, characterized by the development of fatty plaques in arterial vessel walls, is the underlying cause of the majority of cardiovascular diseases, such as myocardial infarction, strokes, and heart failure, leading to tremendous morbidity and mortality worldwide. Dysfunction in macrophage lysosomes results in the improper handling of atherogenic lipids, apoptotic cells, and other cytotoxic debris, leading to progression of plaque size and complexity. Stimulation of TFEB, the master transcriptional regulator of autophagy-lysosomal biogenesis, in macrophages has been shown to promote the degradative capacity of plaque macrophages and reduce atherosclerosis. There is significant interest in harnessing this pathway pharmacologically to treat atherosclerosis and other debilitating cardiometabolic diseases. One

mechanism by which TFEB can be activated to translocate to the nucleus is through the release of calcium from the lysosomal lumen into the cytosol via specialized channels, such as mucolipin 1 (MCOLN1/TRPML1), located at the lysosomal membrane. We therefore sought to harness an agonist of the MCOLN1/TRPML1 channel, known as mucolipin synthetic agonist 1 (ML-SA1), to induce lysosomal calcium release and robustly trigger TFEB nuclear translocation in isolated macrophages to enhance the degradative capacity of dysfunctional macrophages. **Methods:** Peritoneal macrophages (PMACs) were isolated from 10-week-old C57BL/6J and cultured in complete DMEM. PMACs were treated with 20uM ML-SA1 for the designated amount of time and harvested for qPCR, western blotting, immunofluorescence, FURA-2 staining, and IL-1beta ELISA per well-established protocols. Immunofluorescence intensities were quantified using ImageJ. **Results:** We found that MCOLN1/TRPML1 is highly expressed in macrophages and is involved in a feedback loop by which TFEB positively regulates TRPML1 mRNA expression to further support long-term autophagy-lysosomal biogenesis. We confirmed that ML-SA1 induces lysosomal calcium release to trigger TFEB nuclear translocation in macrophages. TFEB translocation via ML-SA1 is time dependent, with the most robust TFEB nuclear localization at 4 hours post treatment. ML-SA1 also induces transcription of various autophagy-lysosomal genes, clearance of cytotoxic p62-enriched protein aggregates, and blunts pro-inflammatory IL-1beta production and inflammasome activation. **Conclusions:** Taken together, our data support the idea that pharmacological stimulation of lysosomal calcium flux can be a novel strategy of enhancing TFEB and macrophage degradative capacity in atherosclerosis. **Acknowledgements:** We thank all members of the Razani laboratory and collaborators: Dr. Joel D. Schilling (WashU), Dr. Irfan Lodhi (WashU), and Dr. Bettina Mittendorfer (UMissouri). Funding for this project was provided by: NIH R01 HL1255838, VA MERIT I01 BX003415, ADA #1-18-IBS-029, Washington University ICTS, and NIH T32GM133332.

Poster Board 34
METABOLOMICS002
The Incretin Effect of Verba Mate *(/lex paraguariensis)* Is Partially Dependent on Gut- Mediated Metabolism of Ferulic Acid
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Background: Yerba mate (YM), a traditional herbal beverage made from */lex paraguariensis,* contains bioactive compounds like polyphenols and alkaloids known for their metabolic benefits. This study investigates YM's incretin *effects,* focusing on glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). **Methods:** Male and female C57BL/6 mice were supplemented with YM for four weeks. Post-supplementation, GLP-1 and GIP gene expression levels were analyzed in jejuna, mucosa, and plasma hormone concentrations were measured. Additionally, in vitro experiments were conducted using GLUTag L-cells to evaluate the direct *effects* of YM and its metabolites, including ferulic acid and dihydroferulic acid, on GLP-1 secretion. Gene expression analysis involved quantitative real-time PCR, while hormone levels were assessed via ELISA. **Results:** YM supplementation significantly increased GLP-1 gene expression and plasma GLP-1 levels compared to controls, with no changes observed in GIP expression or plasma levels. Direct treatment of GLUTag L-cells with YM did not enhance GLP-1 secretion. However, dihydroferulic acid, a microbial metabolite of ferulic acid, markedly stimulated GLP-1 production in L-cells, highlighting a role of gut- mediated metabolism in YM's incretin *effects.* **Conclusions:** YM selectively upregulates GLP-1 pathways without affecting GIP, likely through gut- mediated mechanisms. These findings suggest YM as a promising nutraceutical for incretin modulation and metabolic disorder management. Further studies should explore the interplay between YM, the gut microbiota, and incretin pathways to fully realize its therapeutic potential.

Poster Board 35
METABOLOMICS005
Effects of Semaglutide on Trophoblast Cell Function: A Comparative Study
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Background: Trophoblast cells play a crucial role in placental development and maternal-fetal exchange. Semaglutide, a glucagon-like peptide-1 receptor agonist (GLP-1 RA), has been explored for its metabolic effects, but its impact on trophoblast function remains unclear. This study investigates the effects of semaglutide on BeWo and SW71 trophoblast cell lines to assess its potential implications in placental biology. BeWo and SW71 trophoblast cell lines were treated with semaglutide at varying concentrations. Cell viability, proliferation, and functional assays were conducted to evaluate semaglutide's effects. **Results:** Quantitative analyses assessed differentiation, invasion, and metabolic activity, while mechanistic insights were explored through mTOR pathway signaling and mitochondrial function assays. BeWo cells

exhibited a dose-dependent reduction in proliferation and differentiation. Conversely, SW71 cells demonstrated enhanced migratory capacity and invasion potential, indicating an upregulation of extravillous trophoblast function without affecting overall viability. Mechanistic insights revealed significant involvement of the mTOR pathway, where semaglutide led to a downregulation of mTOR signaling in BeWo cells, correlating with decreased cellular proliferation and metabolic activity. In contrast, SW71 cells maintained stable mTOR activity, potentially facilitating their invasive properties. Mitochondrial function studies indicated a reduction in mitochondrial respiration efficiency in BeWo cells, further supporting impaired differentiation, while SW71 cells showed no significant mitochondrial dysfunction. These findings highlight a differential response to semaglutide between trophoblast subtypes. The inhibitory effects on BeWo cells raise concerns regarding placental development, whereas the enhanced invasion of SW71 cells suggests a distinct, potentially adaptive response. **Conclusions:** This study underscores the importance of evaluating the safety and physiological consequences of GLP-1 RAs in pregnancy, as semaglutide may differentially impact trophoblast function through mTOR-mediated mechanisms and mitochondrial dynamics. Further investigations are necessary to elucidate the long-term implications on placental health and maternal-fetal outcomes. **Acknowledgements:** This work was supported by funding from the National Institutes of Health (1R15HL152257) and the Flight Attendant's Medical Research Institute (CIA2300003).

Poster Board 36
METABOLOMICS004
Bile Acid Supplementation in Mice Reestablishes the Weaning Reaction in Maternal Obesogenic Diet Offspring
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Background: Maternal obesogenic diet exposure (MODE) leads to increased susceptibility of steatotic liver disease (SLD) in offspring. One contributing factor is the vertical transmission of an altered microbiome to offspring, but the mechanism for how this increases susceptibility to SLD is unclear. A crucial early life event termed the 'weaning reaction' is dependent on the early microbiome. Disruptions during this period of development worsens pathologic inflammation in the offspring. Our research has demonstrated that MODE attenuates the weaning reaction and immune cell population, promoting worse SLD in mice. Along with the weaning reaction, we have also shown changes in bile acid populations among MODE offspring. We sought to define an intermediate between the early microbiome and the impaired weaning reaction. We hypothesized that an early intervention of bile acid supplementation may restore the weaning reaction and immune cell populations in MODE offspring. **Methods:** Starting at four weeks of age, female mice were fed either a chow (CON) or high fat, fructose, cholesterol (HFFC) diet for 6 weeks before being bred with lean males. Tissues were collected from offspring between 2 and 4 weeks of age. Some mice were gavaged with ursodeoxycholic acid (UDCA) daily between 2 and 3 weeks of age to assess the role of bile acids in this period of development. Ileal and liver expressions of inflammation and bile acid metabolism were measured. Flow cytometry was performed on ileal and liver tissues at 4 weeks of age to assess changes in immune cell populations. **Results:** Ileal Tnfa and Ifng expression peaked at 3 weeks in CON offspring consistent with a normal weaning reaction, while Tnfa and Ifng expression was impaired in HFFC offspring. BA profiling on 3-week-old cecal contents and liver identified an increase in abundance of primary bile acids and a decrease in abundance of the secondary bile acid, UDCA, in HFFC offspring. Supplementation of UDCA in offspring restored Tnfa and Ifng expression and immune cell populations in HFFC offspring. HFFC offspring had a reduction in CD45+ lymphocytes and CD4+Foxp3+ regulatory T cells in the ileum. UDCA supplementation reestablished the weaning reaction and increased CD45+ lymphocytes and CD4+Foxp3+ regulatory T cells in the ileum of HFFC offspring. **Conclusions:** These findings suggest that MODE alters early developmental programming in offspring intestine with modifications in bile acid metabolism. Supplementing MODE offspring with bile acids may restore the weaning reaction and immune cell populations that are otherwise changed in these offspring. MODE affects bile acid homeostasis in offspring including shifts in the cecal and hepatic BA profile toward more primary and conjugated BAs. These findings could indicate a shift in microbial BA metabolism. Shifts in early microbial BA metabolism could be a potential mechanism for attenuation of the weaning reaction in offspring after MODE.

Poster Board 37
METABOLOMICS006
Semaglutide Impairs Mitochondrial Bioenergetics and Elevates Oxidative Stress in C2C12 Myoblasts
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Background: Semaglutide, a glucagon-like peptide-1 receptor agonist (GLP-1 RA), is widely used for the management of metabolic disorders, yet its potential adverse effects on skeletal muscle and lean mass preservation remain a significant concern, especially in aging populations vulnerable to sarcopenia. **Methods:** In this study, we explored the impact of

semaglutide on mitochondrial bioenergetics and oxidative stress in C2C12 myoblasts, building on previous investigations by the Bikman laboratory. Myoblasts were treated with semaglutide at a range of concentrations, and assessments of cell viability and cell number demonstrated a significant, dose-dependent reduction compared to untreated controls. High-resolution respirometry was employed using substrates glutamate plus malate, ADP, and succinate, which revealed an approximate 50% decline in mitochondrial respiration, indicative of a substantial impairment in cellular energy production. Concurrently, oxidative stress levels, measured via glutathione redox status, were markedly increased following semaglutide treatment. **Results:** When normalized to oxygen consumption, our data further revealed a significant rise in oxidative stress per unit of respiration. These findings suggest that semaglutide compromises mitochondrial function while exacerbating oxidative damage in muscle cells, mechanisms that may collectively contribute to lean mass loss. **Conclusions:** Given the critical role of skeletal muscle in overall metabolic health and physical function, especially during aging, our results underscore the need for caution in the clinical use of GLP-1 RAs. Further mechanistic studies are warranted to fully elucidate the pathways underlying these effects and to evaluate the long-term implications of semaglutide therapy on muscle health in patients, particularly those at risk for age-related sarcopenia. **Acknowledgements:** This work was supported by funding from the National Institutes of Health (1R15HL152257) and the Flight Attendant's Medical Research Institute (CIA2300003).

Poster Board 38
METABOLOMICS007
Impact of High Fat Diet on Heart Proteome Reprogramming and Cardiac Remodeling in Diet-Induce Obesity (DIO) Mice: The PPARs Regulators

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Background: The impact of a high-fat diet (HFD) on the heart proteome and cardiac remodeling in the context of diet-induced obesity is a complex process that involves multiple molecular pathways. Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors that play a crucial role in regulating lipid metabolism, inflammation, and energy homeostasis. In the context of obesity, PPARs, particularly PPARα, PPARβ/δ, and PPARγ, serve as key regulators of cardiac response to metabolic stress. The aim of this work is to a detailed breakdown of how a high-fat diet might influence cardiac proteome reprogramming and remodeling via PPARs in diet-induced obesity (DIO) mice model. **Methods:** 14 six-week-old male C57BL/6N mice (Charles River Laboratories, Calco, Italy) were divided into two groups and fed for 20 weeks as follows: (1) normal chow diet (10% fat, CTR) and (2) high-fat (HFD) diet (60% fat). At the age of 26 weeks, the mice were sacrificed through exposure to atmosphere saturation of carbon dioxide for 15 min. Hearts were collected, immediately snap-frozen in liquid nitrogen, and stored at −80° until proteomic analyses. Bioinformatic analyses were made using Cluego software (Cytoskape release 3.8.2) and IPA software. The Italian Ministry of Health approved all animal procedures (Number 5AD83.N.G1Q). **Results:** Proteomic data clearly show an increase in mitochondrial fatty acid β-Oxidation and a concomitant alteration in glucose metabolism, a decrease in oxidative phosphorylation and an increase in lipid synthesis. Moreover, there is an important involvement of the PPAR signaling, especially α and γ, and the sirtuin signaling pathway important in cell survival under stressful conditions. From the IPA analysis a clear picture of cardiotoxicity emerges which also involves morphological aspects linked to alterations of the cytoskeleton. Cardiac dilatation and left ventricular fibrosis are among the disorders most likely caused by the alteration of the FHD proteome. Interesting, IL-33 is the possible up-stream regulator of cardiac damage (whose target molecules in the data set are ACAT1, APOE, EPHX2, IGHM, ITIH1, Marcks, MYLK, UCP1), produced from polarized T-helper 2 cells, upon interaction of IL-33 with its receptors and the subsequent activation of intracellular molecules in the NF-κB pathways. The induction of type 2 cytokines by IL-33 in vivo is considered to induce severe pathological changes in different organs including heart. **Conclusions:** Proteome network analysis demonstrated that PPARα and PPARγ driven heart remodeling through deregulation of cardiac lipid metabolism and cytokines activation, especially of IL-33 network. **Acknowledgment**: the study was supported by the fund of Italian Ministry of Health (PSR2023_VIANELLO) and by Italian Ministry of Health-Ricerca Corrente (IRCCS Istituto Auxologico Italiano, Milan, Italy).

Poster Board 39
METABOLOMICS008
Adipocyte-Specific HuR Overexpression Impairs Lipid Storage and Dramatically Decreases Adiposity Under Conditions of Control and High Fat Diet
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Introduction: Recent work from our lab and others has established a functional role for the RNA binding protein Human antigen R (HuR) in adipose tissue. Li et al suggested that HuR regulates lipolysis in white adipose tissue (WAT), while we demonstrated that adipocyte-specific deletion of HuR (Adipo-HuR$^{-/-}$) results in an impairment of brown adipose tissue (BAT)-mediated thermogenesis through HuR-dependent regulation of calcium cycling. HuR expression in adipose tissue has also been shown to decrease with obesity and age, and our results have additionally linked the loss of HuR expression in adipose tissue to cardiac pathology. The objective of this work is to determine the functional consequence of adipocyte-specific overexpression of HuR (Adipo-HuR^{OE}). **Methods:** Adipo-HuR^{OE} mice and littermate controls were fed either a 45% kcal/fat high fat diet (HFD; Research Diets) or chow control, followed by assessment of body mass distribution, food intake, activity, and energy expenditure via comprehensive lab animal monitoring system (CLAMS). Serum and hepatic triglycerides were measured using commercial kits from Wako Diagnostics according to the manufacturer's protocol. **Results:** Despite no changes in total food intake, activity, or body weight, Adipo-HuR^{OE} mice show a significant decrease in fat mass driven predominately by a loss of WAT mass, with a concomitant increase in muscle mass. When placed on HFD, Adipo-HuR^{OE} mice exhibit minimal adipose tissue and lack a HFD diet-induced increase in WAT. Indirect calorimetry reveals an increase in respiratory exchange ratio along with a decrease in energy expenditure, suggesting an impairment in fatty acid oxidation in HFD-fed Adipo-HuR^{OE} mice. Consistently, Adipo-HuR^{OE} mice have an exacerbated increase in both serum and hepatic triglycerides following 16-weeks on a HFD, though no differences were observed between Adipo-HuR^{OE} mice and littermate controls on chow. **Conclusion:** Taken together, these results suggest that adipocyte-specific overexpression of HuR impairs lipid storage in WAT leading to ectopic lipid accumulation in other tissues, such as the liver, but the underlying mechanisms remain unknown.

Mucosal Pathobiology
Poster Board 40
MUCOSAL004
Proteomic Analysis of Human Colon Organoids: Impact of Multi-Mineral Intervention Alone and with LPS-Cytokines and Mesalamine
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Background: Gut barrier dysfunction and inflammation are key characteristics of Ulcerative Colitis (UC). This study aimed to investigate how Aquamin and Mesalamine influence proteins associated with barrier integrity and inflammation, assessing their combined therapeutic potential in treating UC. **Methods:** Aquamin, a multi-mineral product derived from calcified red marine algae that contains calcium, magnesium, and 72 additional trace elements, has been shown to improve barrier structure and function in UC patients. Mesalamine, an approved drug for treating UC, was studied alongside Aquamin in this in-vitro trial. Human colon organoids (derived from human colon samples), maintained in a control culture medium or exposed to a pro-inflammatory stimulus (lipopolysaccharide [LPS] combined with three pro-inflammatory cytokines: tumor necrosis factor-α, interleukin-1β, and interferon-γ) to mimic mild-to-moderate UC conditions, were treated with Aquamin, Mesalamine, or their combination for 14 days. Proteomic analysis was conducted on the organoids to evaluate protein changes induced by the treatments, both individually and combined, under normal and pro-inflammatory conditions. **Results:** Colon organoids treated with Aquamin or Mesalamine, either alone or in combination, exhibited distinct effects on protein expression profiles. Both Aquamin and Mesalamine independently up-regulated numerous proteins. Aquamin enhanced barrier integrity by up-regulating cadherins and desmosomal proteins, while Mesalamine had minimal impact on these structures. However, the combination of treatments led to an up-regulation of basement membrane proteins. Inflammatory response proteins were differentially modulated: Aquamin down-regulated complement cascade and clotting-related proteins, whereas Mesalamine up-regulated them. Additionally, mineral transporter proteins were upregulated with Aquamin, except for metallothioneins (detoxifying moieties), ceruloplasmin and light-chain ferritin (required for iron storage), which were downregulated. When combined, the up-regulation of barrier-related proteins (cadherins, desmosomal, and basement membrane proteins) was similar to the individual treatments. On its own, Mesalamine's effects predominated in

inflammation-related pathways. The presence of a pro-inflammatory stimulus (LPS-cytokines) further highlighted unique responses to Mesalamine and Aquamin, with Mesalamine (under both conditions) aligning more closely with the pro-inflammatory stimulus in altering protein expression. **Conclusion:** These proteomic profiles offer intriguing insights into new mechanisms underlying Mesalamine's action in colon inflammation. These findings also suggest that Aquamin, a multi-mineral supplement, could serve as a beneficial adjuvant therapy with Mesalamine for treating UC. It enhances the expression of proteins related to gut barrier integrity while reducing the expression of specific pro-inflammatory proteins.

Poster Board 41
MUCOSAL005
***Clostridioides difficile* Stimulates IL-22 Which Increases Adherent Mucins**
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Background: *Clostridioides difficile* (*C. difficile*) is a pathogen that colonizes the mammalian intestine and leads to diarrhea and inflammation of the gut. Adherent mucins such as MUC1, MUC4, and MUC13 are important for the protection of the intestinal epithelium and promote wound repair after injury. Studies with *Citrobacter rodentium* have shown that the anti-inflammatory cytokine IL-22 can influence adherent mucin expression, but the relationship between IL-22 and adherent mucins during *C. difficile* infection is not well understood. We hypothesized that *C. difficile* stimulated IL-22 activates receptors on epithelial cells and promotes the transcription of adherent mucins. **Methods and Results**: To model *C. difficile* infection, a cocktail of antibiotics was delivered in the drinking water to mice and then the antibiotic clindamycin was delivered IP. Mice were then gavaged with PBS vehicle control or *C. difficile* and monitored for weight and disease activity for 10 days post gavage. As expected*, C. difficile* mice lost significant weight, with the peak of weight loss occurring at Day 3 post-infection. We examined bulk RNAseq data from the colonic tissue from mice infected with *C. difficile* or given a PBS vehicle control at day 3, 4, 5, 8 and 10 post-treatment. We found that IL-22 was significantly elevated on Day 3 post infection and then returned to normal levels by Day 8 and Day 10. Likewise, we observed a significant increase in MUC1, MUC4 and MUC13 at Day 3, which similarly declined in expression by Day 10. Immunostaining of colonic tissue confirmed elevated MUC1, MUC4 and MUC13 levels in the *C. difficile* infected animals compared to control animals. To confirm that IL-22 alone was able to drive the changes in adherent mucins, we treated human colonic T84 cells, as well as human and mouse colonic organoids with IL-22 and examined mucins at the mRNA and protein levels. We found that IL-22 was sufficient to elevate adherent mucins in all these models. To identify whether these changes were conserved in patients, we obtained 13 surgical resections of patients with *C. difficile* infection and compared them to tissue collected from healthy individuals undergoing routine colonoscopy. Mirroring the findings in our mice, we observed increased MUC1 and MUC4 in the *C. difficile* infected patients by immunostaining. Serum analysis further revealed elevated IL-22 in the blood of *C. difficile* patients compared to controls. **Conclusions**: Our findings highlight a critical role for IL-22 in modulating the protective functions of adherent mucins during *C. difficile* infection and provide insights into potential therapeutic strategies targeting mucosal immunity to combat *C. difficile*-associated disease.

Poster Board 42
MUCOSAL006
***Akkermansia muciniphila* Alters Small Intestine Specialized Cell Populations**
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Background: The gastrointestinal tract harbors millions of microbes that interact with the intestinal epithelium directly and indirectly. Among the many bacteria in the gut microbiota, *Akkermansia muciniphila* has become a microbe of interest due to its reported impacts in both health and disease. *A. muciniphila* is known for its ability to degrade host generated mucins and its interactions with the intestinal epithelium have been well characterized in the colon, but little research on its effects in the small intestine has been done. This mucin-degrading microbe is often studied using *in vivo* models with a complete gut microbiome, but these models are limited in their ability to specifically elucidate the role of *A. muciniphila*. We utilized a germ-free model to evaluate the impact of *A. muciniphila* alone on small intestine epithelial cells. **Methods:** Adult germ-free mice were inoculated with either vehicle control Brain Heart Infusion (BHI) bacterial growth media or 10^9 viable *A. muciniphila* in BHI. Small intestine from BHI and *A. mucin* treated mice were collected after 21 days. Bacterial Fluorescence in Situ Hybridization (FISH) staining confirmed the colonization of *A. mucin* in mono-associated mice and absence of bacteria in controls. Immunofluorescent (IF) staining was performed to examine changes in abundance and function of

specialized intestinal cell types. Tuft cells, enteroendocrine cells, paneth cells, stem cells, and goblet cells were assessed and quantified using well characterized IF markers. **Results:** In our germ-free model, *A. muciniphila* alone was not found to affect stem cell or paneth cell populations compared to vehicle control BHI treated mice. Interestingly, mice mono-associated with *A. muciniphila* had decreased numbers of enteroendocrine cells compared to germ-free control mice. While populations of mucus-producing goblet cells were not affected by *A. muciniphila*, changes in mucus composition were observed. Mono-associated mice exhibited decreased sialic acid and fucose residues compared to germ-free controls. Most notably, we found a significant increase in tuft cell number in *A. muciniphila* mice compared to controls. Mass spectrometry of conditioned media identified succinate as a byproduct of *A. muciniphila*. Succinate has previously been shown to activate tuft cells and to expand tuft cell numbers. Analysis of the *A. muciniphila* genome further confirmed its capability to produce succinate; suggesting that succinate production may be responsible for elevating tuft cells *in vivo*. **Conclusions:** Our data demonstrates that *A. muciniphila* alone can increase tuft cell populations and we speculate that this is likely to occur through succinate production. Since tuft cells signal to both immune cells and the enteric nervous system, elevated tuft cell with A. muciniphila could represent a mechanism linking microbial sensing to immune modulation and gut-brain communication.

Poster Board 43
MUCOSAL007
Mucus-Producing Gastric Metaplasia in Mice Driven by High-Fat Diet Consumption
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Background: High fat diets are becoming increasingly common worldwide and are associated with obesity, diabetes, and cancer. Previous studies have shown that a high-fat diet affects the intestine in mice, but its impact on the stomach remains poorly understood. The stomach is lined with specialized epithelial cells that promote the secretion of acid, digestion of food, and other key aspects of gastrointestinal health. Damage and inflammation can cause a loss of the acid secreting parietal cells and reprogramming of the proliferative chief cells to replace the normal epithelial cells with metaplastic cells that express several mucus-associated markers. This process is known as gastric metaplasia, and it represents the efforts of the epithelium to try to protect itself from a hostile environment. We hypothesize that a high-fat diet induces loss of parietal cells in the gastric epithelium and causes gastric metaplasia. **Methods & Results:** To generate a model of a Westernized diet, we fed adult male and female wildtype C57BL/6 mice either a standard chow diet consisting of 10kcal% or a high fat diet of 45kcal% fat (lard) for 25 weeks. Weight was monitored weekly during the dietary treatment and as expected the high fat diet treated animals gained substantial weight over time. Ater 25 weeks, we fixed and processed the stomach to examine morphological changes that resulted from a long-term high fat diet. Hematoxylin and eosin (H&E) staining showed normal gastric gland architecture in the corpus of chow fed mice. As expected, in these control animals we observed with the presence of zymogen containing chief cells at the base of the gland, numerous acid secreting parietal cells, and the proper proportion of surface mucous pit cells and mucus neck cells. In contrast, high fat diet treated mice had a loss of parietal cells and the presence of metaplastic glands, as denoted by a mucus secreting cell type. To identify the presence of mucus generating metaplasia in the mouse stomach, we immunostained the tissue for the mucus markers GSII and UEA-1, proliferation marker Ki67, parietal cell markers HK-ATPase, y-actin, P-ERM, chemosensory tuft cell marker DLCK1, and tight junction marker Ecadherin. Our immunostaining revealed robust gastric metaplasia in the mice on a high fat diet, with loss of parietal cells, an expansion of mucus cells and tuft cell, and increased proliferation compared to control diet treated mice. **Conclusion**: These data indicate that a high fat diet is sufficient to induce gastric metaplasia. Metaplasia has been reported following medication induced injury, chronic infection with the pathogen *Helicobacter pylori*, gastric ulcers and in genetically modified mice, but this is among the first reports of this cellular reprogramming occurring in normal mice in response to diet. These findings suggest that a high fat diet may be predisposing people to gastric cancer and that dietary changes might be a valuable intervention. **Acknowledgements:** ASIP SROPP, SC INBRE Student-Initiated Research Projects (SIRP) Program.

Poster Board 44
MUCOSAL008
Urothelial-specific Deletion of Semaphorin 3F Leads to Detrusor Underactivity
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Background: Voiding dysfunction – the inability to urinate properly -- comprises both detrusor overactivity and detrusor underactivity (DU). These conditions affect males and females of all ages and are highly prevalent with estimates of up to 40%. Published data from our group demonstrated a role for neuropilin 2 (NRP2) in the regulation of bladder smooth muscle contraction. In mice with inducible, smooth muscle-specific deletion of Nrp2, we observed an increase in evoked contraction of bladder muscle strips both in healthy mice and in mice with bladder outlet obstruction. These findings suggested that inhibiting the NRP2 axis may represent a novel strategy to treat DU. Semaphorin-3F (SEMA3F), the ligand for NRP2 is expressed in the bladder epithelium (urothelium). Exposure of primary bladder smooth muscle cells (BSMC) to recombinant SEMA3F evoked cytoskeletal rearrangement and decreased tension, consistent with a role for Sema3F-Nrp2 signaling in promoting relaxation. We hypothesized that loss of *Sema3F* in vivo would phenocopy loss of *Nrp2* resulting in increased evoked contraction. **Methods:** We used compound UPII-Cre;Sema3F$^{f/f}$ mice to elicit urothelium-specific deletion of SEMA3F in vivo. Excised bladders from male and female Sema3F$^{f/f}$ and UPII-Cre;Sema3F$^{f/f}$ mice at 10-14 wk of age were separated into mucosa and detrusor, and Sema3F expression was assessed by qRT-PCR. Bladder contraction was tested ex vivo in isometric tension analysis using full thickness and mucosa-denuded muscle strips. Urothelial permeability was determined by administration of FITC-dextran on the luminal side of mucosa mounted in Ussing chambers and measurement on the abluminal aspect by fluorimetry. **Results:** Enrichment of Sema3F in the urothelium and its loss in UPII-Cre; Sema3F$^{f/f}$ mice was confirmed by PCR. In isometric tension testing, bladder strips from male mice with urothelial deletion of Sema3F displayed a reduction in evoked contraction in response to electrical field stimulation and carbachol, compared to strips from Sema3F-intact controls. No difference in contraction was observed in tissues from Sema3F-deleted compared to Sema3F-intact female mice. Basal urothelial permeability was increased in mice with Sema3F deletion compared to non-deleted controls. **Conclusions:** Urothelium-specific deletion of Sema3F in mice led to reduced contractility of bladder muscle in male but not female mice. Loss of Sema3F was also associated with an increase in urothelial permeability. The decrease in contraction observed with Sema3F loss contrasts with the increased contraction we reported in mice lacking the Sema3F receptor Nrp2, and the reduction in cytoskeletal tension observed in BSMC exposed to Sema3F in vitro. Together, these findings identify Sema3F as a novel regulator of smooth muscle contraction that acts independently of direct effects on Nrp2. **Acknowledgements:** NIDDK R01DK104641 (RMA, DRB); Department of Veterans Affairs Medical Research Service BX001790 (MPS); Children's Urological Foundation (RMA); Vascular Biology Program at Boston Children's Hospital (DRB).

Poster Board 45
MUCOSAL009
High Fat Diet Drives Gastric Metaplasia Through Mast Cell-Driven Inflammation

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Background: Obesity has more than tripled in the United States over the past 60 years, with severe obesity nearly quadrupling. Among the key contributors to the obesity epidemic, the consumption of a High-fat diet (HFD) plays a significant role in excessive weight gain and the worsening of metabolic disorders. HFD consumption disrupts multiple physiological systems, including the cardiovascular, immune, renal, and gastrointestinal systems. While substantial research has examined the effects of a HFD on the intestine, its impact on the stomach remains underexplored- especially as highly processed, fat-rich diets become increasingly prevalent in our society. The gastric epithelium consists primarily of mucus-secreting cells, acid-producing parietal cells, and zymogenic chief cells. Under conditions of injury or parietal cell loss, chief cells can transdifferentiate into metaplastic cells. While this transformation initially serves as a protective adaptation, chronic inflammation or persistent insult can drive metaplasia progression toward dysplasia and cancer. **Hypothesis:** We hypothesize that a HFD damages the gastric epithelium, leading to an increase in mast cells, which subsequently drive inflammation and gastric metaplasia. **Methods:** To test our hypothesis, wildtype C57BL/6J mice were fed either a standard chow diet (control) or a HFD for 25 weeks. Gastric tissue was collected and analyzed to identify cellular alterations and the expression of metaplasia markers. Additionally, gastric organoids were generated from the gastric glands of wildtype C57BL/6J mice on a control diet. Gastric organoids were treated with palmitic acid, the major component of a HFD, for 14 days to assess morphological changes. **Results:** Long-term HFD consumption induced gastric metaplasia and significant alterations in gastric epithelial cell composition. H&E staining showed pronounced thickening of the gastric mucosa, loss of parietal and chief cells, and increased immune cell infiltration in HFD-fed mice compared to control mice; all hallmarks of metaplasia. Immunostaining further demonstrated a significant increase in gastric tuft cells, indicative of tuft cell hyperplasia. Mast cell infiltration, as indicated by tryptase staining, was significantly elevated in HFD mice, suggesting the presence of a pro-inflammatory microenvironment. Additionally, immunofluorescence analysis revealed upregulation of metaplasia markers CD44v9, Aquaporin 5 (AQP5), GSII, and phospho-ERK1/2 (P-ERK1/2) in mice fed a HFD. *in vitro*, palmitic acid-

treated organoids exhibited increased growth compared to DMSO-treated controls, further supporting a direct impact of HFD on gastric epithelial cells. **Conclusion:** These data demonstrate that consumption of a HFD induces gastric damage leading to gastric metaplasia and increased inflammatory cells. Our findings emphasize the critical need to understand dietary impacts on gastric health and their potential role in the progression to gastric malignancy.

Poster Board 46
MUCOSAL010
Altered Nanoparticle Uptake in P-glycoprotein Deficient Caco-2 Intestinal Cells
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Background: Inflammatory bowel disease (IBD) is a class of autoimmune diseases of unknown cause that includes Crohn's disease and ulcerative colitis. While only palliative therapies are available, patients with IBD struggle with diarrhea, abdominal pain, and other chronic symptoms. This study is interested in investigating the multidrug resistance gene (*MDR*) which encodes for the small molecule pump P-glycoprotein (P-gp). This protein is responsible for regulating and controlling drug absorption and can be found in various parts of the body including the epithelial cells of the intestines. Polymorphisms of the *MDR1* gene (encoding P-gp) have been linked directly to IBD in humans. A novel mechanism of drug delivery is through SiO_2 nanoparticles, potentially increasing drub absorption by IBD patients. The goal of this study is to characterize the ability of MDR deficient ($MDR^{-/-}$) intestinal cells to uptake nanoparticles for possible drug delivery applications. **Methods:** Control and $MDR^{-/-}$ Caco-2 intestinal cells were incubated for 4, 8, 12, or 24 hours with fluorescently labeled nanoparticles. Nanoparticle uptake was measured via flow cytometry and fluorescent microscopy. **Results:** The $MDR^{-/-}$ Caco-2 cells showed an overall lower percentage of nanoparticle positive cells than control cells with a significant difference in the 4- and 8- hour incubation time points. The $MDR^{-/-}$ Caco-2 cells showed an overall lower mean fluorescence intensity (MFI) of the nanoparticles than the control cells with a significant difference in the 8- and 12-hour incubation time points. Imaging of the cells containing the nanoparticles showed fewer $MDR^{-/-}$ cells containing nanoparticles, however, those with nanoparticles had a higher concentration of particles. **Conclusions:** The changes we detected suggest, although the $MDR^{-/-}$ cells are able to uptake nanoparticles, they exhibit less incorporation of the nanoparticles inside the cells which leads us to believe nanoparticles will be a less effective drug delivery method for IBD patients with MDR mutations.
Acknowledgements: This project was funded by SC-INBRE (MCS, and SMT), USC Upstate SARS Grants (SMT and MCS), University of South Carolina RISE Grant (SMT), The Histochemical Society Capstone Grant (MCS), and the USC Upstate Division of Natural Sciences.

Neuropathology

Poster Board 47
NEUROPATH003
Neuromodulation of Respiratory Networks in Neonatal Sepsis
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Background: Neonatal sepsis accounts for approximately 1.3 million cases leading to
203.000 deaths of newborns yearly. The neonatal immune system is a dynamic and complex living system in which immune tolerance is a key mechanism for opportunistic infections. Recently, we unraveled that PAM3CSK4 results in subtle modulation of inflammatory cytokines with respiratory life-threatening events. On the other hand, LPS initiates a robust innate immune response in respiratory neural networks without serious alterations in initiating gasping following severe hypoxia. Activation of inflammatory pathways and energy conservation from growth process might be associated with neuroprotective mechanisms of respiratory networks. Our study goal was to identify inflammatory and developmental threshold by which immune-metabolic alterations can worsen or protect respiratory neural circuits. **Methods**: CD1 mice were *i.p.* treated at PD5 or PD8 with LPS or saline (control). $Il6^{tm1Kopf}$/J and C57BL/6J mice were *i.p.* treated at PD5 with either saline or LPS. Whole- body plethysmography was performed to record the respiratory parameters. Brain tissue was obtained following 3 hours of TLR ligands administration. Total RNA was extracted, and RT-PCR was performed to evaluate gene expression levels. All analysis were performed using R Studio. **Results**: To investigate whether neuroprotective pathways were developmental state- dependent, we evaluate the hypoxic chemoreflexes in PD5 and PD8 pups. Our results show that neonatal sepsis by LPS in different ages affected equally the hypoxic chemoreflex. Next, we used several dosages of LPS in CD1 pups to elucidate the inflammatory threshold in respiratory networks. Gene expression analysis shows an increase of TNFα, IL6 and IL1b levels in the brainstem accordingly with LPS dosages. Our published findings indicate that solely LPS induces a robust expression of IL-6. Herein, we challenged IL6KO mice with LPS, in which

proinflammatory cytokines including TNFα and IL1b were modulated in a lesser extent than in C57BL/6J mice. Respiratory chemoreflexes were also modulated in neonatal sepsis without IL6 expression. **Conclusions:** Respiratory networks functions are susceptible to immune-metabolic modulation due to LPS in neonatal sepsis. The inflammatory threshold by activation of proinflammatory cytokines presents neuroprotective properties. **Acknowledgements**: This work was supported by: NIH/NHLBI R01HL132355 for JJO and FIU Start-up for MJA.

Poster Board 48
NEUROPATH004
Loss of Function in the CASK Gene Is Associated with Optic Nerve Hypoplasia and Retinal Dystrophy
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Background: The retina and optic nerve develop from the diencephalon and are part of the central nervous system. These structures are often affected in neurological conditions. Although approximately 80% of early learning may involve visual cues, the visual system has not been carefully examined in neurodevelopmental disorders that are associated with learning disabilities. Here, using microcephaly with pontine and cerebellar hypoplasia (MICPCH), which occurs due to mutations in the CASK gene, as an example, we demonstrate that the retina and optic nerve are affected in neurodevelopmental disorders. **Methods:** We used both clinical cases and analyses of mutant mouse models. Specifically, we acquired clinical data from human subjects with CASK gene variants and characterized two distinct mouse models of CASK loss-of-function. In these models, we performed imaging experiments, electroretinography, and visual behavior studies. **Results:** We demonstrate that mutations in the X-linked CASK gene can be associated with optic nerve hypoplasia (ONH), optic atrophy, and retinal dystrophy in human subjects. Furthermore, we show that while CASK heterozygous knockout female mice with a mosaic expression (~50% of cells) of CASK display ONH without any retinal dysfunction, male and female CASK hypomorphic mice (with lower expression in all cells) exhibit ONH with cone receptor dystrophy. **Conclusion:** Our study thus far indicates that (1) even with the same mutation in the CASK gene, the expression of ocular phenotypes may vary, and (2) in rodents, the pattern and level of CASK gene expression determines the nature of the ocular phenotype. Furthermore, our results confirm that neurodevelopmental disorders such as MICPCH are likely to include ocular dysfunctions. **Acknowledgment:** This work was performed with NEI grant R01 EY033391.

Poster Board 49
NEUROPATH005
Neuron-Specific CASK Loss in Mice Causes Epileptic Encephalopathy Associated with Cortical Malformation and Mitochondrial Dysfunction
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Background: Calcium/calmodulin-dependent serine protein kinase (*CASK*) is an X-linked intellectual disability gene in mammals that is ubiquitously expressed in all tissues. *CASK* heterozygous loss-of-function produces microcephaly with pontine and cerebellar hypoplasia (MICPCH), whereas *CASK* hemizygous loss-of-function produces the early infantile-onset epileptic encephalopathy (EE), which is highly devastating and fatal. The mechanisms underlying *CASK* associated pathologies remain unknown. The goal of our study is to better understand the molecular function of *CASK* and etiopathogenic mechanisms underlying *CASK*-linked EE. **Methods:** Using Cre-LoxP conditional knockout strategy, we generated a novel mouse model of EE harboring neuron-specific *CASK* deletion ($CASK^{NKO}$). The $CASK^{Wildtype}$, $CASK^{floxed}$, and $CASK^{wildtype::SynCre}$ transgenic mice were used as controls. Mice brain electrical activity were measured by video-electroencephalogram (vEEG) analysis. Immunohistochemical staining were performed to determine the expression of cerebral cortical layer-specific markers (i.e. CUX1 and TBR1) and astrocyte glial fibrillary acidic protein (GFAP). Western blot analyses were performed to determine steady-state levels of excitatory and inhibitory synapse markers, and proteins involved in mitochondrial oxidative phosphorylation and fission/fusion processes. Oxygraph analysis was performed to measure mitochondrial respiration, and transmission electron microscopy was performed to determine mitochondrial morphology and number in the mice brain. NAD^+/NADH ratio was measured by a quantitative colorimetric assay. Unbiased transcriptomic analysis was performed from the brain of $CASK^{NKO}$ mice compared to control mice. **Results:** $CASK^{NKO}$ mice exhibit severe growth retardation and early infantile-onset recurrent tonic spasms and myoclonus beginning postnatal day 8 (P8) which progressively worsens with age, and the mice die before adulthood (i.e. P25). At P18, $CASK^{NKO}$ mice exhibit decreased cerebral cortical thickness associated with increased neuronal density, and mis-expression of CUX1 and TBR1

markers compared to the control mice. *CASK*^{NKO} mice further display abnormal brain vEEG activity, altered excitatory/inhibitory neurotransmission, and marked increase in reactive astrogliosis compared to control mice. Additionally, *CASK*^{NKO} mice brain exhibit significantly decreased mitochondrial respiration, reduced number of mitochondria, increased steady-state level of Drp1, and reduced NAD^+/NADH ratio compared to control mice. The unbiased transcriptomics analysis revealed that 'mitochondrion' is the topmost gene ontology term and molecular function altered in the brain of *CASK*^{NKO} mice. **Conclusions:** Our data suggests that neuronal CASK is essential for postnatal brain development in mice and play a critical role in regulating mitochondrial function and homeostasis. **Acknowledgements:** This research work is supported by the NIH/NINDS grant award (R01NS117698) to S.S.

Ocular Pathobiology

Poster Board 50

OCULAR001

Transcriptomic Profiling of Cytokine-Induced Pathways in Human Retinal Endothelial Cells: Unraveling Angiofibrotic and Inflammatory Signaling in Neovascular AMD

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Background: Dysregulated cytokine signaling drives endothelial cell (EC) dysfunction in neovascular age-related macular degeneration (nAMD), shifting between inflammatory, angiogenic, and fibrotic states. We analyzed transcriptional changes in ECs exposed to six nAMD-associated cytokines, individually and in combination, integrating differential gene expression with gene interaction network analysis. **Methods:** Primary human retinal endothelial cells (HRECs; Cell Systems) were treated with transforming growth factor-beta 1 (TGF-β1), TGF-β2, tumor necrosis factor-alpha (TNF-α), thrombin, interleukin-6 (IL-6), or vascular endothelial growth factor (VEGF) at 10 ng/mL, either individually or as a combined cytokine 'cocktail' ($n = 6$). Untreated cells served as controls. After 24 hours, total RNA was extracted, and bulk transcriptomic sequencing was performed (Azenta Life Sciences). Differential expression analysis ($|Log_2FC| > 1$, $FDR < 0.05$) identified differentially expressed genes (DEGs) relative to controls. Pathway enrichment analysis (PEA) mapped DEGs to disease-associated pathways, while pairwise comparisons across treatment groups distinguished ubiquitously and uniquely activated pathways. To assess potential synergistic signaling effects in the multi-cytokine system, a gene interaction network was constructed for cocktail-treated cells using published gene-protein interactions and clustered via a modified Leiden algorithm. Finally, DEGs were compared to bulk RNA sequencing data from nAMD patients (GSE135922) to evaluate the clinical relevance of the model. **Results:** The pro-inflammatory cytokines TNF-α (1823 DEGs) and thrombin (1019 DEGs) induced broader transcriptional responses compared to IL-6 (6 DEGs). TGF-β2 (323 DEGs) more strongly enriched mesenchymal pathways than TGF-β1 (17 DEGs), while the pro-angiogenic VEGF (32 DEGs) triggered a limited pre-angiogenic profile. Pathway enrichment analysis (PEA) revealed a pro-fibrotic overlap among TNF-α, thrombin, and TGF-β2 treatments, driven by SNAI1-mediated activation of endothelial-mesenchymal transition (EndMT) pathways. The cytokine cocktail group exhibited an amplified transcriptional response (2559 DEGs), with 884 uniquely expressed DEGs, including the pro-angiogenic driver VEGFA. Functional clustering identified four intersecting gene networks, incorporating 265 choroidal DEGs associated with nAMD. **Conclusions:** Fibrosis-associated cytokines (TNF-α, thrombin, TGF-β2) drive a strong pathogenic response with overlapping DEGs as potential therapeutic targets. Notably, the combined cytokine treatment generates a broader DEG profile than the sum of individual effects, indicating a significant synergistic amplification of pathological pathways. This combinatorial system more accurately models the disease state, making it a powerful platform for preclinical drug testing. **Acknowledgements:** DYS is supported by the UNSW Scientia Program and Mito Foundation of Australia.

Toxicologic Pathology

Poster Board 51

TOXPATH002

Apoptotic Responses in Maternal Lung Following Prenatal Exposure to Secondhand Smoke or E-Cigarette Vapor

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Background: Apoptosis is essential for tissue homeostasis, yet environmental toxicants can disrupt its regulation. This study examines the apoptotic effects of prenatal exposure to secondhand smoke (SHS) and e-cigarette (eCig) vapor on maternal lung tissue during late gestation. **Methods:** Pregnant C57BL/6 mice were exposed to SHS or eCig vapor for four or six days. Lung tissues were collected at embryonic day 18.5 and analyzed for apoptotic markers, stress-response proteins, and growth regulators. Protein expression was quantified using dot blotting. **Results:** SHS exposure induced a sustained upregulation of pro-apoptotic markers (Bax, cytochrome c, BID) and significantly decreased anti-apoptotic proteins (Bcl-2, XIAP). The extrinsic apoptotic pathway was also activated, with Fas ligand and TNF-β upregulation. eCig vapor induced a transient apoptotic response, with milder effects on intrinsic pathways and partial retention of anti-apoptotic proteins. Both exposures increased oxidative stress markers HSP27 and HSP70. Additionally, growth factors such as IGF-1 and IGFBPs exhibited significant alterations, suggesting impaired lung tissue repair. SHS exposure elicits sustained apoptotic activation in maternal lung tissue, whereas eCig exposure triggers a more transient, yet notable, apoptotic response. **Conclusions:** These findings highlight the potential risks of both exposures during pregnancy and underscore the need for further research into their long-term consequences on maternal and fetal health. **Acknowledgements:** This work was supported by funding from the National Institutes of Health (1R15HL152257) and the Flight Attendant's Medical Research Institute (CIA2300003).

Poster Board 52
TOXPATH001
Impact of Secondhand Smoke and E-Cigarette Exposure on Placental Apoptotic and Growth-Regulatory Proteins During Mouse Pregnancy
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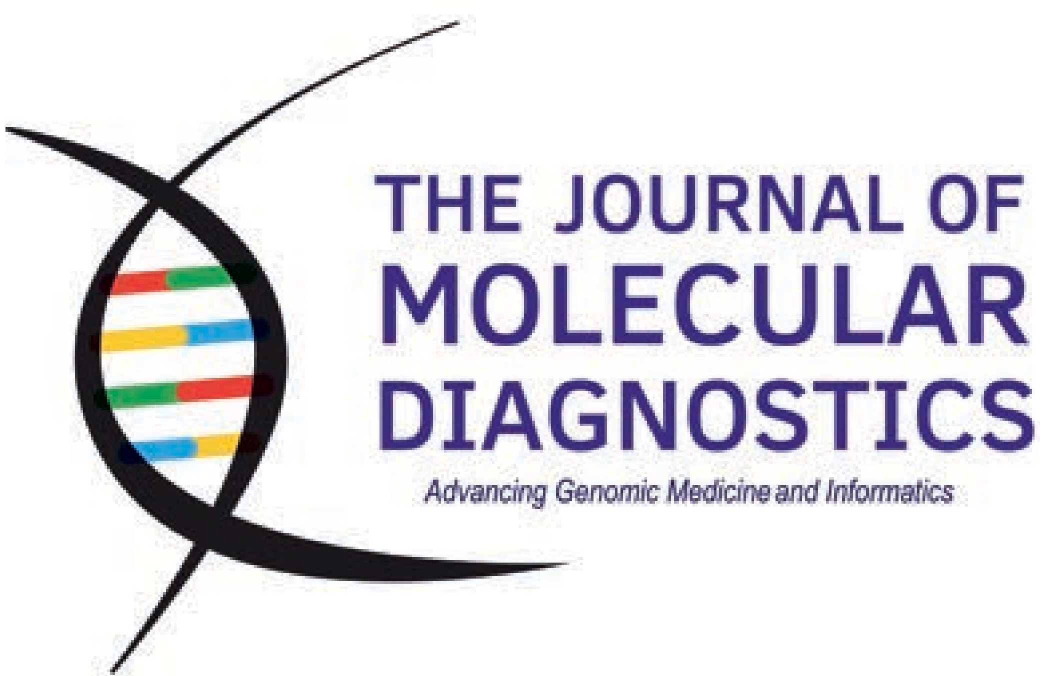
Background: Apoptosis plays a critical role in placental development, and its dysregulation is associated with pregnancy complications such as intrauterine growth restriction (IUGR) and preeclampsia (PE). Environmental exposures, including secondhand smoke (SHS) and e-cigarette aerosols (eCigs), may contribute to placental dysfunction via apoptotic pathways. This study investigates the effects of SHS and eCig exposure on placental apoptosis and growth-regulatory proteins in a murine model. **Methods:** Pregnant C57BL/6 mice were exposed to SHS or eCigs for four or six days at two gestational time points (E12.5–E18.5 and E14.5–E18.5). Placental tissues were analyzed for apoptotic markers, growth regulators, and insulin-like growth factor binding proteins (IGFBPs). Protein expression was quantified using dot blotting. SHS exposure increased pro-apoptotic markers (BAD, Fas/FasL) and decreased mitochondrial function markers (cytochrome c), indicating compromised cell survival. **Results:** Both SHS and eCig exposure reduced anti-apoptotic proteins (BCL-2, HSP27, survivin) and growth regulators (IGF-1, IGFBPs). These alterations suggest that SHS and eCigs create a pro-apoptotic environment in the placenta, potentially impairing fetal development by disrupting apoptotic and growth-regulatory pathways. Environmental exposures during pregnancy, particularly SHS and eCigs, induce placental apoptosis and alter growth-regulatory signaling. **Conclusion:** These findings highlight the need for public health strategies to minimize maternal exposure to SHS and eCigs to protect fetal development. **Acknowledgements:** This work was supported by funding from the National Institutes of Health (1R15HL152257) and the Flight Attendant's Medical Research Institute (CIA2300003).



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